HARMFUL ALGAL BLOOMS (HABS) IN LATIN AMERICA

EDITED BY: Marius Nils Müller, Jorge I. Mardones and Juan Jose Dorantes-Aranda

PUBLISHED IN: Frontiers in Marine Science

frontiers Research Topics

Frontiers eBook Copyright Statement

The copyright in the text of individual articles in this eBook is the property of their respective authors or their respective institutions or funders. The copyright in graphics and images within each article may be subject to copyright of other parties. In both cases this is subject to a license granted to Frontiers.

The compilation of articles constituting this eBook is the property of Frontiers.

Each article within this eBook, and the eBook itself, are published under the most recent version of the Creative Commons CC-BY licence. The version current at the date of publication of this eBook is CC-BY 4.0. If the CC-BY licence is updated, the licence granted by Frontiers is automatically updated to the new version.

When exercising any right under the CC-BY licence, Frontiers must be attributed as the original publisher of the article or eBook, as applicable.

Authors have the responsibility of ensuring that any graphics or other materials which are the property of others may be included in the CC-BY licence, but this should be checked before relying on the CC-BY licence to reproduce those materials. Any copyright notices relating to those materials must be complied with.

Copyright and source acknowledgement notices may not be removed and must be displayed in any copy, derivative work or partial copy which includes the elements in question.

All copyright, and all rights therein, are protected by national and international copyright laws. The above represents a summary only. For further information please read Frontiers' Conditions for Website Use and Copyright Statement, and the applicable CC-BY licence.

ISSN 1664-8714
ISBN 978-2-88963-550-4
DOI 10.3389/978-2-88963-550-4

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

HARMFUL ALGAL BLOOMS (HABS) IN LATIN AMERICA

Topic Editors:
Marius Nils Müller, Federal University of Pernambuco, Brazil
Jorge I. Mardones, Centro de Estudios de Algas Nocivas (CREAN), Instituto de Fomento Pesquero (IFOP), Chile
Juan Jose Dorantes-Aranda, Institute for Marine and Antarctic Studies, University of Tasmania, Australia

Citation: Müller, M. N., Mardones, J. I., Dorantes-Aranda, J. J., eds. (2020). Harmful Algal Blooms (HABs) in Latin America. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88963-550-4

Table of Contents

06 ***Editorial: Harmful Algal Blooms (HABs) in Latin America***
Marius N. Müller, Jorge I. Mardones and Juan J. Dorantes-Aranda

10 ***Book Review: Cyanobacteria as Environmental Determinants of Health***
Anabella Aguilera and Leda Giannuzzi

12 ***Identification of the Gene* sxtA *(Domains* sxtA1 *and* sxtA4*) in Mexican Strains of* Gymnodinium catenatum *(Dinophyceae) and Their Evolution***
Armando Mendoza-Flores, Ignacio Leyva-Valencia, Christine J. Band-Schmidt, Clara E. Galindo-Sánchez and José J. Bustillos-Guzmán

21 ***Transformation and Depuration of Paralytic Shellfish Toxins in the Geoduck Clam* Panopea globosa *From the Northern Gulf of California***
Jennifer Medina-Elizalde, Ernesto García-Mendoza, Andrew D. Turner, Yaireb Alejandra Sánchez-Bravo and Ramón Murillo-Martínez

34 ***Risk Perception of Coastal Communities and Authorities on Harmful Algal Blooms in Ecuador***
Mercy J. Borbor-Córdova, Mireya Pozo-Cajas, Alexandra Cedeno-Montesdeoca, Gabriel Mantilla Saltos, Chippie Kislik, Maria E. Espinoza-Celi, Rene Lira, Omar Ruiz-Barzola and Gladys Torres

47 ***Abundance and Distribution of the Potentially Toxic Thecate Dinoflagellate* Alexandrium tamiyavanichii *(Dinophyceae) in the Central Mexican Pacific, Using the Quantitative PCR Method***
David U. Hernández-Becerril, Winnie L. S. Lau, Kieng S. Hii, Chui P. Leaw, F. Varona-Cordero and Po T. Lim

58 ***Large-Scale Sea Turtle Mortality Events in El Salvador Attributed to Paralytic Shellfish Toxin-Producing Algae Blooms***
Oscar Amaya, Rebeca Quintanilla, Brian A. Stacy, Marie-Yasmine Dechraoui Bottein, Leanne Flewelling, Robert Hardy, Celina Dueñas and Gerardo Ruiz

68 ***Addressing the Problem of Harmful Algal Blooms in Latin America and the Caribbean- A Regional Network for Early Warning and Response***
Tomasa Cuellar-Martinez, Ana Carolina Ruiz-Fernández, Carlos Alonso-Hernández, Oscar Amaya-Monterrosa, Rebeca Quintanilla, Hector Leonel Carrillo-Ovalle, Natalia Arbeláez M., Lisbet Díaz-Asencio, Silvia M. Méndez, Maribelle Vargas, Ninoska Fabiola Chow-Wong, Lorelys Rosario Valerio-Gonzalez, Henrik Enevoldsen and Marie-Yasmine Dechraoui Bottein

75 ***Plankton Multiproxy Analyses in the Northern Patagonian Shelf, Argentina: Community Structure, Phycotoxins, and Characterization of Toxic* Alexandrium *Strains***
Valeria A. Guinder, Urban Tillmann, Bernd Krock, Ana L. Delgado, Torben Krohn, John E. Garzón Cardona, Katja Metfies, Celeste López Abbate, Ricardo Silva and Rubén Lara

96 ***The Genus* Alexandrium *(Dinophyceae, Dinophyta) in Brazilian Coastal Waters***
Mariângela Menezes, Suema Branco, Maria Cecília Miotto and Catharina Alves-de-Souza

109 ***Mass Mortality of Cultivated Northern Bluefin Tuna* Thunnus thynnus orientalis *Associated With* Chattonella *Species in Baja California, Mexico***
Ernesto García-Mendoza, Jorge Cáceres-Martínez, David Rivas, Michelle Fimbres-Martinez, Yaireb Sánchez-Bravo, Rebeca Vásquez-Yeomans and Jennifer Medina-Elizalde

125 ***Association of the Toxigenic Dinoflagellate* Alexandrium ostenfeldii *With Spirolide Accumulation in Cultured Mussels (*Mytilus galloprovincialis*) From Northwest Mexico***
Patricia Paredes-Banda, Ernesto García-Mendoza, Elizabeth Ponce-Rivas, Juan Blanco, Antonio Almazán-Becerril, Clara Galindo-Sánchez and Allan Cembella

139 ***Population Dynamics of Benthic-Epiphytic Dinoflagellates on Two Macroalgae From Coral Reef Systems of the Northern Mexican Caribbean***
Elda Damaris Irola-Sansores, Benjamín Delgado-Pech, Ernesto García-Mendoza, Erick J. Núñez-Vázquez, Aramis Olivos-Ortiz and Antonio Almazán-Becerril

153 ***Population Genetic Structure at the Northern Edge of the Distribution of* Alexandrium catenella *in the Patagonian Fjords and its Expansion Along the Open Pacific Ocean Coast***
Javier Paredes, Daniel Varela, Camila Martínez, Andrea Zúñiga, Karen Correa, Adrián Villarroel and Bianca Olivares

166 **Pyrodinium bahamense *One the Most Significant Harmful Dinoflagellate in Mexico***
Lourdes Morquecho

174 ***Microplankton Community Composition Associated With Toxic* Trichodesmium *Aggregations in the Southwest Atlantic Ocean***
Mariana Bernardi Bif, Márcio Silva de Souza, Luiza Dy Fonseca Costa and João Sarkis Yunes

185 ***Salinity-Growth Response and Ichthyotoxic Potency of the Chilean* Pseudochattonella verruculosa**
Jorge I. Mardones, Gonzalo Fuenzalida, Katherine Zenteno, Catharina Alves-de-Souza, Allisson Astuya and Juan José Dorantes-Aranda

197 ***Oceanography of Harmful Algal Blooms on the Ecuadorian Coast (1997–2017): Integrating Remote Sensing and Biological Data***
Mercy J. Borbor-Cordova, Gladys Torres, Gabriel Mantilla-Saltos, Ashley Casierra-Tomala, J. Rafael Bermúdez, Willington Renteria and Bonny Bayot

216 ***Paralytic Toxin Producing Dinoflagellates in Latin America: Ecology and Physiology***
Christine J. Band-Schmidt, Lorena M. Durán-Riveroll, José J. Bustillos-Guzmán, Ignacio Leyva-Valencia, David J. López-Cortés, Erick J. Núñez-Vázquez, Francisco E. Hernández-Sandoval and Dulce V. Ramírez-Rodríguez

255 ***Ichthyotoxicity of the Dinoflagellate* Karlodinium veneficum *in Response to Changes in Seawater pH***
Marius N. Müller, Juan José Dorantes-Aranda, Andreas Seger, Marina T. Botana, Frederico P. Brandini and Gustaaf M. Hallegraeff

261 *Massive Blooms of* Chattonella subsalsa *Biecheler (*Raphidophyceae*) in a Hypereutrophic, Tropical Estuary—Guanabara Bay, Brazil*
Tatiana V. Viana, Giovana O. Fistarol, Michelle Amario, Rafael B. Menezes, Beatriz L. R. Carneiro, Daisyane M. Chaves, Paulo I. Hargreaves, Arthur W. Silva-Lima, Jean L. Valentin, Denise R. Tenenbaum, Edilson F. Arruda, Rodolfo Paranhos and Paulo S. Salomon

275 *Spatio-Temporal Pattern of Dinoflagellates Along the Tropical Eastern Pacific Coast (Ecuador)*
Gladys Torres, Olga Carnicer, Antonio Canepa, Patricia De La Fuente, Sonia Recalde, Richard Narea, Edwin Pinto and Mercy J. Borbor-Córdova

295 *A Review on the Biodiversity and Biogeography of Toxigenic Benthic Marine Dinoflagellates of the Coasts of Latin America*
Lorena María Durán-Riveroll, Allan D. Cembella and Yuri B. Okolodkov

320 *Marine Dinoflagellate Assemblage in the Galápagos Marine Reserve*
Olga Carnicer, Patricia De La Fuente, Antonio Canepa, Inti Keith, Eduardo Rebolledo-Monsalve, Jorge Diogène and Margarita Fernández-Tejedor

333 *Effects of Salinity and Temperature on the Growth, Toxin Production, and Akinete Germination of the Cyanobacterium* Nodularia spumigena
Savênia B. Silveira and Clarisse Odebrecht

345 *The State of Knowledge of Harmful Algal Blooms of* Margalefidinium polykrikoides *(a.k.a.* Cochlodinium polykrikoides*) in Latin America*
David J. López-Cortés, Erick J. Núñez Vázquez, Juan J. Dorantes-Aranda, Christine J. Band-Schmidt, Francisco E. Hernández-Sandoval, José J. Bustillos-Guzmán, Ignacio Leyva-Valencia and Leyberth J. Fernández-Herrera

frontiers in Marine Science

EDITORIAL
published: 04 February 2020
doi: 10.3389/fmars.2020.00034

Editorial: Harmful Algal Blooms (HABs) in Latin America

Marius N. Müller[1]*, *Jorge I. Mardones*[2] *and Juan J. Dorantes-Aranda*[3]

[1] Department of Oceanography, Federal University of Pernambuco, Recife, Brazil, [2] Centro de Estudios de Algas Nocivas (CREAN), Instituto de Fomento Pesquero (IFOP), Puerto Montt, Chile, [3] Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, TAS, Australia

Keywords: field and laboratory studies, future perspectives, water quality, dinoflagellates, research community and collaboration, toxicity, monitoring, ichthyotoxicity

Editorial on the Research Topic

Harmful Algal Blooms (HABs) in Latin America

"Harmful Algal Blooms" (HABs) describe the copious presence of certain pigmented planktonic organisms that interfere with the equilibrium or balanced state of aquatic ecosystems and reservoirs in terms of ecological functions (e.g., food web disturbance, subsurface oxygen depletion) and ecosystem services benefits to humans (e.g., fishery, water quality, and tourism). The effects of harmful algal species on aquatic systems are manifold and may include the discoloration of water, the presence of toxic substances and the accumulation of high biomass leading to low oxygen or anoxic conditions due to increased bacterial respiration. Aquatic reservoirs and ecosystems are intrinsically connected to human health and well-being as they provide resources, such as drinking water and food (e.g., through fisheries and aquaculture). The biogeographical occurrence and records of HAB events have increased in frequency, distribution and severity over the past decades, which have been linked to evolved monitoring techniques, increased research efforts and global climate change (Hallegraeff, 2010). The ongoing and projected anthropogenic changes of the coastal and freshwater marine environments are altering planktonic community structures, species distribution, and migration patterns (Gobler et al., 2017). Shifts in the biogeography and bloom dynamics of HABs pose a potential risk to the health and economy of coastal communities due to the possible occurrence of HABs in regions without proper recognition, monitoring, or mitigation capabilities. Secondary metabolites produced by HABs include toxins that can be bioaccumulated through the food web and ultimately affect human health due to a variety of syndromes, such as Amnesic, Diarrhetic, Neurotoxic, Azaspiracid, and Paralytic Shellfish Poisoning (ASP, DSP, NSP, AZP, PSP, respectively), as well as Ciguatera Fish Poisoning. More algal toxins have been described in recent years and have attracted particular attention due to their potency and emergent problematic issues (Assunção et al., 2017). This includes yessotoxins, palytoxins, and amphidinols as well as harmful compounds produced by ichthyotoxic algae that still remain to be fully characterized and understood.

The monitoring and mitigation of HABs are not trivial, and as a common routine activity, abundances of harmful algal species are observed to determine any potential bloom development in association with environmental parameters. Over the past decades, HAB research has substantially advanced and improvements in prediction and mitigation have been achieved (Anderson et al., 2012). Monitoring programs have been mostly implemented in regions of known HAB occurrence and those of economic importance (e.g., aquaculture and tourism). In the light of changes in HAB biogeography due to climate change and anthropogenic activities, it is likely that the occurrence of HABs will expand to unprepared regions. HAB phenomena with severe impacts, such as persistent events in Chile and Australia and sporadic events in the USA and the Persian Gulf, have impacted

OPEN ACCESS

Edited and reviewed by:
Angel Borja,
Technological Center Expert in Marine and Food Innovation (AZTI), Spain

***Correspondence:**
Marius N. Müller
mariusnmuller@gmail.com

Specialty section:
This article was submitted to Marine Ecosystem Ecology, a section of the journal Frontiers in Marine Science

Received: *15 January 2020*
Accepted: *20 January 2020*
Published: *04 February 2020*

Citation:
Müller MN, Mardones JI and Dorantes-Aranda JJ (2020) Editorial: Harmful Algal Blooms (HABs) in Latin America. Front. Mar. Sci. 7:34. doi: 10.3389/fmars.2020.00034

coastal communities unexpectedly. This demonstrates the existence of gaps and difficulties in monitoring programs and, consequently, in the understanding and prediction of HAB dynamics to appropriately mitigate the impacts on human society as it has been achieved in regard to other natural phenomena, such as hurricanes and tsunamis. Nowadays, monitoring and mitigation of HABs should go hand in hand with the detection and discovery of toxic HAB metabolites, culturing of HAB species and communicating and integrating HAB science with society and policy makers to develop regional and local strategies.

This Research Topic was proposed to bring together HAB researchers from Latin America and to showcase current developments as well as challenges that regional scientific communities are facing. Contributions from 58 research/academic institutions distributed over 13 Latin American and 8 non-Latin American countries were received, which demonstrates a high interest and current research efforts performed by Latin American and international research communities. The articles published in this Research Topic can be grouped into reviews, observational/field studies, laboratory studies, and articles linking scientific research with the broader society and policy makers.

REVIEWS

Most reviews were focused on toxic dinoflagellates and demonstrated the major expertise and focus of Latin American researchers. Durán-Riveroll et al. reviewed the current knowledge on the distribution and the diversity of toxigenic benthic marine dinoflagellates in the tropical and subtropical Atlantic and Pacific. Morquecho focused on the importance of understanding the bloom dynamics of the dinoflagellate *Pyrodinium bahamense* and the associated impacts of Paralytic Shellfish Poisoning in the Mexican Pacific and the Gulf of Mexico. Menezes et al. summarized the occurrence of seven species of the dinoflagellate *Alexandrium* in Brazilian waters, whereby *A. tamutum* was documented for the first time and strains of *A. catenella* displayed genetic similarities with strains from Southern Chile and North America. López-Cortés et al. reviewed records and research on *Margalefidinium polykrikoides* in Latin America with regard to ecology, physiology, and toxic effects due to the production of reactive oxygen species, hemolytic and neurotoxic-like substances. Band-Schmidt et al. contributed with an overview on the ecology and physiology of paralytic shellfish toxin (PST) producing dinoflagellates, responsible for intoxications in various coastal areas of Latin America, and highlighted the distribution patterns of *Gymnodinium catenatum*, *Pyrodinium bahamense,* and several species of *Alexandrium*.

FIELD AND OBSERVATIONAL STUDIES

Plankton surveys are crucial to develop a fundamental understanding to evaluate current and future ecosystem changes and to monitor the distribution of toxigenic harmful algal species. Field and observational studies were conducted in tropical, subtropical, and temperate climate regions in coastal and continental shelf areas of Argentina, Brazil, Chile, Ecuador, El Salvador, and Mexico. Guinder et al. conducted a plankton multiproxy analysis and characterized the late winter plankton structure in terms of abundance, biomass, species composition, functional groups, and phycotoxin profiles in surface waters of the Northern Patagonian Shelf (Argentina). Phylogenetic analyses of newly isolated strains of *Alexandrium ostenfeldii* revealed a new ribotype group, suggesting a biogeographical distinction of this population. Bif et al. identified *Trichodesmium* species and their toxins during two oceanographic cruises in the Southwest Atlantic Ocean (Brazil) and gave hints that the cellular toxin production might be enhanced during aggregation at the ocean surface, inhibiting the growth of co-occurring microplanktonic organisms. Viana et al. recorded bloom events of the toxic raphidophyte *Chattonella subsalsa* in Guanabara Bay (Brazil) responsible for fish kills and highlighted the need to reduce the eutrophication levels in Guanabara Bay.

Paredes et al. illustrated the high genetic diversity of *Alexandrium catenella* responsible for high PST levels observed along the Southern Chilean fjords between 2014 and 2016. Borbor-Cordova, Torres et al. assessed the link between oceanographic variables and HABs occurrence on the Ecuadorian coast from a 20-year time series where a total of 67 HAB events were registered. Carnicer et al. provided and discussed baseline information on the spatial distribution of different dinoflagellate taxa in the Galápagos Marine Reserve (Ecuador). An oceanographic monitoring series of 4 years was evaluated by Torres et al. to study the association of El Niño/Southern Oscillation with upwelling events and dinoflagellates abundances on the Ecuadorian coast. Amaya et al. investigated the role of PST produced by the dinoflagellates *Gymnodinium catenatum*, *Alexandrium* sp., and *Pyrodinium bahamense* during large-scale sea turtle mortality events in El Salvador.

Paredes-Banda et al. associated the occurrence and accumulation of spirolids in farmed shellfish of Todos Santos Bay (Mexico) with the presence of the dinoflagellate *Alexandrium ostenfeldii*. Medina-Elizalde et al. investigated the transformation and depuration of paralytic shellfish toxins in the geoduck clam from the Northern Gulf of California and characterized the toxin profile in this shellfish species during a bloom of the dinoflagellate *Gymnodinium catenatum*. García-Mendoza et al. reported that mass mortalities of farmed tuna were associated with the presence of *Chattonella* spp. in the northwest coast of Baja California (Mexico). Irola-Sansores et al. investigated the distribution of epiphytic dinoflagellates in two coral reef systems of the Mexican Caribbean. The dominance of palytoxin producer *Ostreopsis* sp. demonstrated the potential risk that this species could create in touristic regions. Finally, the distribution and abundance of the dinoflagellate *Alexandrium tamiyavanichii* was reported for the first time in coastal and oceanic conditions of the central Mexican Pacific by Hernández-Becerril et al.

LABORATORY STUDIES

Laboratory studies are an essential step to understand the physiology and growth dynamics of harmful algal species under controlled conditions. These depend on the successful isolation and culturing of the target algal species, which require skills and good practices in HAB research. Mardones et al. shed light on

the underlying reasons of the outbreak of the dictyochophyte *Pseudochattonella verruculosa* in 2016 responsible for economic losses of US$ 800M due to farmed salmon mortalities. Results from laboratory experiments revealed the important effect of salinity on growth and ichthyotoxicity of *Pseudochattonella* species and highlighted the necessity to study the role of mucocysts in fish gill damage. Mendoza-Flores et al. reported that the acquisition and origin of saxitoxin genes in the dinoflagellate *Gymnodinium catenatum* was related to horizontal gene transfer from bacteria and that horizontal gene transfer from *Alexandrium* species toward *G. catenatum* did not occur. Silveira and Odebrecht evaluated the effects of temperature and salinity on the physiological performance and toxin production of the cyanobacteria *Nodularia spumigena* and revealed that cell growth, akinete formation, and the germination potential was negatively affected by low salinity. Müller et al. presented laboratory results on the physiology and the toxin production of the dinoflagellate *Karlodinium veneficum* in regard to changes in seawater pH and ocean acidification scenarios, indicating favorable growth conditions at elevated pH levels.

LINKING HAB RESEARCH WITH SOCIETY

The linkage between the scientific community and the society as well as policy makers is an essential aspect to develop a fast and effective information flow to secure human health and economic sustainability. Aguilera and Giannuzzi presented a book review regarding the result of the collaborative work between Argentinian researchers and the Ministry of Health raising awareness on the increased occurrence of toxigenic cyanobacteria in aquatic systems and the documented acute illnesses associated with exposure to cyanobacteria and cyanotoxins. Borbor-Córdova, Pozo-Cajas et al. assessed the knowledge, attitudes, and practices in relation to HABs in Ecuador and demonstrated that coastal communities and health authorities had limited knowledge of red tide impacts on human health. However, outreach activities, such as tailored workshops and communication between authorities and communities, could positively influence the risk perception and enhance algal bloom monitoring in the near future. Cuellar-Martinez et al. highlighted the 10-year existence of a regional HAB network of Latin American and Caribbean countries (supported by the International Atomic Energy Agency), which aims to provide training and capabilities for early HAB warning and mitigation.

FUTURE PERSPECTIVES OF HAB RESEARCH IN LATIN AMERICA

The impacts of HABs on human health are still an abstract concept for many communities in Latin American countries and additional efforts need to be implemented by the scientific community and stakeholders to reinforce the social and economic importance of HAB research. The existence of several national HAB networks and monitoring systems represent a basis for the development of additional regional HAB networks and a joint Latin American HAB program that could operate on an international basis with the existing GlobalHAB program (Berdalet et al., 2017). In Latin America there are two regional groups of the Intergovernmental Oceanographic Commission (IOC): *Algas Nocivas en el Caribe* (ANCA) and *Floraciones Algales Nocivas en Sudamérica* (FANSA), representing Central American/Caribbean and South American countries, respectively. Additionally, an independent Society for the Study of Harmful Algal Blooms (*Sociedad Mexicana para el Estudio de Florecimientos Algales Nocivos*, SOMEFAN) is present in Mexico. These groups have different backgrounds, biography and perspectives, but share a common challenge: consolidating and establishing strong HABs collaborative research activities. To date, there is no official body in Latin America identified as a leading agency for HAB research such as the IOC-UNESCO sub-commission for the Western Pacific (WESTPAC) where a Project Steering Group has been established. A leading agency or network might effectively promote and impulse research activities, integrating common efforts among countries. The main challenges to achieve these general interests are related to the limited available resources (infrastructure and equipments), the political situation within and amongst Latin American countries, the lack of trained human resources and strong policies oriented to trigger and maintain basic and applied research. The motivation and long-term research programs require a continuous and permanent IOC support and a clear integration with local governments. Meanwhile, the development of low cost monitoring programs (e.g., MicroToxMap http://microtoxmap.com/) and the increasingly availability of digital open access resources to store, share and transpose information are important developments to be adopted and potentially can advance HAB research in Latin America. We hope that this Research Topic demonstrates the advantages of regional cooperation amongst the scientific communities of Latin America to achieve common future goals.

AUTHOR CONTRIBUTIONS

MM, JM, and JD-A conceived and wrote the manuscript. All authors made direct and intellectual contribution to the work.

ACKNOWLEDGMENTS

Thanks to Dr. Leonardo Guzmán, ex-chair of the FANSA group, for constructive comments on the manuscript.

REFERENCES

Anderson, D. M., Cembella, A. D., and Hallegraeff, G. M. (2012). Progress in understanding harmful algal blooms: paradigm shifts and new technologies for research, monitoring, and management. *Annu. Rev. Mar. Sci.* 4, 143–176. doi: 10.1146/annurev-marine-120308-081121

Assunção, J., Guedes, A. C., and Malcata, F. X. (2017). Biotechnological and pharmacological applications of biotoxins and other bioactive molecules from dinoflagellates. *Mar. Drugs* 15:393. doi: 10.3390/md15120393

Berdalet, E., Kudela, R., Urban, E., Enevoldsen, H., Banas, N. S., Bresnan, E., et al. (2017). GlobalHAB: a new program to promote international research, observations, and modeling of harmful algal blooms in aquatic systems. *Oceanography* 30, 70–81. doi: 10.5670/oceanog.2017.111

Gobler, C. J., Doherty, O. M., Hattenrath-Lehmann, T. K., Griffith, A. W., Kang, Y., and Litaker, R. W. (2017). Ocean warming since 1982 has expanded the niche of toxic algal blooms in the North Atlantic and North Pacific oceans. *Proc. Natl. Acad. Sci. U.S.A.* 114, 4975–4980. doi: 10.1073/pnas.1619575114.

Hallegraeff, G. M. (2010). Ocean climate change, phytoplankton community responses, and harmful algal blooms: a formidable predictive challenge. *J. Phycol.* 46, 220–235. doi: 10.1111/j.1529-8817.2010.00815.x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Müller, Mardones and Dorantes-Aranda. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Marine Science

BOOK REVIEW
published: 18 July 2018
doi: 10.3389/fmars.2018.00242

Book Review: Cyanobacteria as Environmental Determinants of Health

Anabella Aguilera[1]* *and Leda Giannuzzi*[2]

[1] Instituto de Investigaciones en Biodiversidad y Biotecnología (INBIOTEC-CONICET) and Fundación para Investigaciones Biológicas Aplicadas (FIBA), Mar del Plata, Argentina, [2] Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA-CONICET), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina

Keywords: Argentina, cyanotoxins, cyanobacterial blooms, environmental epidemiology, risk assessment and risk management

A Book Review on
Cianobacterias Como Determinantes Ambientales de la Salud

Giannuzzi L., Petcheneshsky T.,Hansen M. (Argentina: Ministerio de Salud de laNación. Dirección Nacional de Determinantes de la Salud e Investigación), 2017, 258 Pages, ISBN: 978-950-38-0255-7.

OPEN ACCESS

Edited by:
Marius Nils Müller, Universidade Federal de Pernambuco, Brazil

Reviewed by:
Rosalba Alonso-Rodríguez, Universidad Nacional Autónoma de México, Mexico

****Correspondence:***
Anabella Aguilera
anabella.aguilera@gmail.com

Specialty section:
This article was submitted to Marine Ecosystem Ecology, a section of the journal Frontiers in Marine Science

Received: *22 May 2018*
Accepted: *25 June 2018*
Published: *18 July 2018*

Citation:
Aguilera A and Giannuzzi L (2018) Book Review: Cyanobacteria as Environmental Determinants of Health. Front. Mar. Sci. 5:242. doi: 10.3389/fmars.2018.00242

Toxic cyanobacterial blooms represent a water quality issue worldwide which incidence and severity are predicted to increase due to climate change and eutrophication (O'Neil et al., 2012). Argentina is not an exception to this trend. Cyanobacterial proliferations have increased in the last two decades as a consequence of water quality changes due to human activities (Otaño et al., 2012; Aguilera et al., 2018). Unfortunately, Argentina lacks specific regulations concerning cyanobacteria and cyanotoxins in freshwater bodies, and information on cyanotoxins occurrence is still scant (Aguilera et al., 2018).

This book is the result of the collaborative work between Argentinean researchers and the Ministry of Health who constituted the "Working Group on Health Effects of Cyanobacteria in Water" intended to raise the awareness concerning the increased occurrence of toxigenic cyanobacteria and the threats they pose (Provision 2/2011, Secretariat for Health Relations and Research). The synergy and collaborative interactions within this group have been key steps to promote the establishment of national guidelines for cyanobacterial blooms in drinking and recreational waters.

This second edition is an expanded version that contains updates on topics already described in the first edition launched in 2011. The book contains 14 chapters written by 19 Argentinean researchers and specialists with extensive experience in Cyanobacteria, who complemented the text with own results and experiences. It begins with updated information on cyanobacterial taxonomy and ecology, including the description and photographs of harmful cyanobacteria found in Argentina. The book reviews molecular tools currently available for monitoring toxigenic cyanobacteria. This includes a brief introduction of the Polymerase chain reaction (PCR) technique and its use for cyanobacterial identification and the detection of toxigenic cyanobacterial populations or environmental samples. The application of quantitative PCR (qPCR) and DNA chips (diagnostic microarrays) are also described.

Another important aspect of the book is management of harmful cyanobacteria and risk assessment. In this sense, it offers an overview of the factors that trigger bloom formation and the physical, chemical and biologic methods to control them. It also describes restoration measures for eutrophic water bodies and technologies used for nutrient reduction or removal. Within risk management, it presents the sequence of actions that can be used to provide a graduated response to the onset and progress of a cyanobacterial bloom. Alert Levels Framework is presented as a decision tree, therefore assisting decision making in water treatment plant operators and managers.

The book provides detailed coverage of documented acute illnesses associated with exposure to cyanobacteria and cyanotoxins in animals and humans. Additionally, it includes a chapter on the use of cyanobacteria in dietary supplements reviewing available studies about the presence of cyanotoxins in different commercially available products containing cyanobacteria.

Lastly, the last part reviews all the actions carried out by the Ministry of Health regarding the epidemiology of cyanobacteria and their toxins in Argentina. Epidemiological data are still limited in Argentina. There are few clinical data describing adverse health effects from exposure to cyanobacteria and cyanotoxins, making the clinical differential diagnosis difficult. In this sense, the Ministry initiated a framework for multidisciplinary interaction and cooperation among specialists in human, animal and environmental health. In cities strongly affected by cyanobacterial blooms, efforts were underway to train primary care personnel, veterinarians and those who work on or near cyanobacteria-impacted water bodies (e.g., lifeguards, environmental professionals) to identify cyanobacterial blooms, and collect and report animal and human signs and symptoms from exposure to cyanobacteria. The Ministry also introduced the concept of dogs as sentinels, as it is used in other countries as the first indication of an ongoing cyanobacterial bloom (Hilborn and Beasley, 2015). The tool kit of materials (posters to raise awareness among the public, health records for the health personnel) as well as public health promotion activities and partnerships carried out by The Ministry of Health are presented in the last chapter of the book.

In sum, this handbook is an important resource for researchers, water manager professionals, and also specialists working in human or animal health in Latin America. It can be of interest to the wider resource management community assessing the health of freshwater ecosystems in the region. It also constitutes a reference book at a level and depth for advance graduate and research students who want to approach their research on toxic cyanobacteria.

Cyanobacterial blooms are an environmental public health issue that needs continuing attention at local and national level. Moreover, attention at international level must also be considered when countries have a share in river basins, as in the case of Latin America. We prospect this book will promote parallel initiatives in Latin American countries, and also encourage further interchange among researchers, water managers, and health authorities within our continent to take actions and work collaboratively. Finally, this book review constitutes a nice opportunity to present and spread the work done in Argentina regarding this environmental and health issue. Hopefully, it will promote scientific interchange with the non-Latin American community as well.

Link to download the handbook in PDF (in Spanish): http://www.msal.gob.ar/images/stories/bes/graficos/0000000334cnt-Ciano_2017.pdf

AUTHOR CONTRIBUTIONS

AA and LG wrote the book review and approve the manuscript.

REFERENCES

Aguilera, A., Haakonsson, S., Martin, M. V., Salerno, G. L., and Echenique, R. O. (2018). Bloom-forming cyanobacteria and cyanotoxins in Argentina: a growing health and environmental concern. *Limnologica* 69, 103–114. doi: 10.1016/j.limno.2017.10.006

Hilborn, E. D., and Beasley, V. R. (2015). One Health and Cyanobacteria in freshwater systems: animal illnesses and deaths are sentinel events for human health risks. *Toxins* 7, 1374–1395. doi: 10.3390/toxins7041374

O'Neil, J. M., Davis, T. W., Burford, M. A., and Gobler, C. J. (2012). The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. *Harmful Algae* 14, 313–334. doi: 10.1016/j.hal.2011.10.027

Otaño, S., Salerno, G. L., Ruiz, M., Aguilera, A., and Echenique, R. O. (2012). "Argentina: Cyanobacteria and cyanotoxins: Identification, toxicology, monitoring and risk assessment" in *Current Approaches to Cyanotoxin Risk Assessment, Risk Management and Regulations in Different Countries*, ed I. Chorus (Berlin: Umweltbundesamt), 16–20.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Aguilera and Giannuzzi. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Marine Science

ORIGINAL RESEARCH
published: 29 August 2018
doi: 10.3389/fmars.2018.00289

Identification of the Gene *sxtA* (Domains *sxtA1* and *sxtA4*) in Mexican Strains of *Gymnodinium catenatum* (Dinophyceae) and Their Evolution

Armando Mendoza-Flores[1]*, *Ignacio Leyva-Valencia*[2], *Christine J. Band-Schmidt*[1], *Clara E. Galindo-Sánchez*[3] and *José J. Bustillos-Guzmán*[4]

[1] Departamento de Plancton y Ecología Marina, Centro Interdisciplinario de Ciencias Marinas, Instituto Politécnico Nacional, La Paz, Mexico, [2] CONACyT, Departamento de Plancton y Ecología Marina, Centro Interdisciplinario de Ciencias Marinas, Instituto Politécnico Nacional, La Paz, Mexico, [3] Departamento de Biotecnología Marina, Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, Mexico, [4] Centro de Investigaciones Biológicas del Noroeste, La Paz, Mexico

OPEN ACCESS

Edited by:
Marius Nils Müller, Universidade Federal de Pernambuco, Brazil

Reviewed by:
Shauna Murray, University of Technology Sydney, Australia
Paulo Sergio Salomon, Universidade Federal do Rio de Janeiro, Brazil

***Correspondence:**
Armando Mendoza-Flores
armando.mf6@gmail.com;
amendozaf1200@alumno.ipn.mx

Specialty section:
This article was submitted to Marine Ecosystem Ecology, a section of the journal Frontiers in Marine Science

Received: *29 April 2018*
Accepted: *27 July 2018*
Published: *29 August 2018*

Citation:
Mendoza-Flores A, Leyva-Valencia I, Band-Schmidt CJ, Galindo-Sánchez CE and Bustillos-Guzmán JJ (2018) Identification of the Gene sxtA (Domains sxtA1 and sxtA4) in Mexican Strains of Gymnodinium catenatum (Dinophyceae) and Their Evolution. Front. Mar. Sci. 5:289. doi: 10.3389/fmars.2018.00289

Saxitoxin (STX) and its analogs are a broad group of natural neurotoxic alkaloids, commonly known as paralytic shellfish toxins. *SxtA* is the initial gene in the biosynthesis of saxitoxin. It has been proposed that the genes for STX biosynthesis had a bacterial origin and were acquired in the dinoflagellates by a horizontal gene transfer (HGT). In *Gymnodinium catenatum,* the origin of the STX genes is not well established. In this paper, we sequenced *sxtA* gene (domains *sxtA1* and *sxtA4*) and determined the gene copy number in the genome in four Mexican strains of *G. catenatum*. We compare them with sequences of *G. catenatum, Pyrodinium bahamense,* and *Alexandrium* spp. from other geographic regions, and non-toxic producing dinoflagellates. Amplifications were performed for domains *sxtA1* and *sxtA4* from strains of *G. catenatum* and the phylogenetic analyses was done by maximum likelihood and Bayesian inference. The copy number determination was carried out using qPCR. The phylogenetic tree of domain *sxtA4* showed the formation of two clades where *G. catenatum* sequences separated from the *Alexandrium/Pyrodinium* clade. The domain *sxtA1* formed a higher number of clades than *sxtA4.* Sequences of *G. catenatum* were grouped together with sequences of *Alexandrium*. Dinoflagellates sequences that do not produce saxitoxin formed a separate clade. The gene copy number was 64 ± 30 and 110 ± 50 copies of *sxtA1* and *sxtA4* respectively. The identification of the gene *sxtA* of *G. catenatum* shows that the sequences are similar to those of *Alexandrium* species with low variations between species. These results may indicate that the acquisition of the gene *sxtA* was an early HGT event in the evolution of dinoflagellates. The possible loss of the ability to produce STX in some species suggests that the HGT from *Alexandrium* species toward *G. catenatum* is not possible.

Keywords: dinoflagellate, *Gymnodinium catenatum*, horizontal gene transfer, paralytic shellfish toxins, gene copy number

INTRODUCTION

Saxitoxin (STX) and its analogs are a broad group of natural neurotoxic alkaloids, commonly known as paralytic shellfish toxins (PSTs). PSTs are the causative agents of paralytic shellfish poisoning (PSP) and are mostly associated with marine dinoflagellates (eukaryotes) and freshwater cyanobacteria (prokaryotes), which form extensive blooms around the world (Wiese et al., 2010). The biosynthetic pathway and genes responsible for STX synthesis have been characterized in the cyanobacteria *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju T3. This biosynthesis pathway is encoded by a fragment larger than 35 kb, comparative sequences analysis assigns 30 catalytic functions that correspond to 26 proteins (Kellmann and Neilan, 2007; Kellmann et al., 2008). Subsequently, the STX gene cluster was characterized in the cyanobacteria *Anabaena circinalis* Rabenhorst ex Bornet & Flahault, *Aphanizomenon* sp. Morren ex Bornet & Flahault, *Rhaphidiopsis brookii* Hill and *Lyngbya wollei* Farlow ex Gomont (Mihali et al., 2009, 2011; Moustafa et al., 2009; Murray et al., 2011).

The origin of the STX genes in cyanobacteria involved multiple events of horizontal gene transfer (HGT) from different sources, followed presumably by the coordination of the expression of foreign and native genes in the common ancestor. The possible ancestral source for some STX genes was a proteobacteria; suggesting that the key PSP-toxin biosynthesis gene may have evolved in a prokaryotic organism (Kellmann et al., 2008; Moustafa et al., 2009).

SxtA is the unique starting gene of STX-synthesis in cyanobacteria. This gene has a polyketide synthase (PKS)-like structure characterized by four catalytic domains with activities of a *S*-adenosyl-methionine- (SAM) dependent methyltransferase (*sxtA1),* GCN5-related *N*-acetyltransferase (*sxtA2),* acyl carrier protein (*sxtA3*), and a class II aminotransferase (*sxtA4*) (Kellmann et al., 2008).

Some dinoflagellate species of the genus *Alexandrium* Halim, as well as *Pyrodinium bahamense* Plate and *Gymnodinium catenatum* Graham, are known for producing PST (Scholin et al., 1995; Blackburn et al., 2001). Homologs of the genes putatively associated for the STX-synthesis have been identified in *A. tamarense* (Lebour) Balech Group IV, *A. fundyense* Balech, and *A. minutum* Halim (Stüken et al., 2011; Hackett et al., 2013). The transcripts of *A. tamarense* and *A. minutum* show that the dinoflagellate transcripts of *sxtA* have the same domain structure as the cyanobacterial *sxtA* genes. In contrast to the bacterial homologs, the dinoflagellate transcripts are monocistronic, have a higher guanine–cytosine (GC) content, occur in multiple copies, contain typical dinoflagellate spliced-leader sequences, and eukaryotic polyA-tails (Stüken et al., 2011).

The genes responsible for the STX-synthesis were not exchanged directly between cyanobacteria and dinoflagellates. The strong monophyly of *sxtA*-related proteins from STX^+ dinoflagellates, and separately from cyanobacteria, supports the hypothesis that precursors of these *sxtA* genes were acquired independently by each linage (Hackett et al., 2013). Based on the *sxtA* data, the origin of the STX gene cluster within the dinoflagellates may have occurred between an ancestral STX^+ bacterium and the common ancestor of *Alexandrium* and *Pyrodinium* (Orr et al., 2013).

Gymnodinium catenatum, a chain-forming dinoflagellate is the only species that can produce PST within the genus (Attaran-Fariman et al., 2007; Negri et al., 2007; Hallegraef et al., 2012; Gu et al., 2013). The origin of SXT genes in this species is not well established, but the distribution of the *sxtA* and *sxtG* genes suggest that *G. catenatum* acquired STX genes independently from a secondary dinoflagellate-dinoflagellate transfer (Orr et al., 2013). Another hypothesis, proposing that a single horizontal transfer event occurred early in dinoflagellate evolution, is the most parsimonious explanation for the origin of STX in dinoflagellates (Murray et al., 2015).

In this paper, we analyse the identification, the genomic copies number and the evolution of the *sxtA* gene in *G. catenatum* using Mexican strains and comparing them with sequences of *G. catenatum, P. bahamense,* and *Alexandrium* species from other geographic regions, as well other non-toxic producing dinoflagellates.

MATERIALS AND METHODS

Strains and Culture Maintenance

The strains of *G. catenatum* in our study were isolated from several localities along the Mexican Pacific and the Gulf of California (**Figure 1**, **Table 1**). The strains were grown in modified GSe media (Bustillos-Guzmán et al., 2015) at 24 ± 1°C under a 12:12 h light:dark photoperiod and a photon irradiance of 150 μE $m^{-2}s^{-1}$ from white cool fluorescent lights.

DNA Extraction and PCR

For the DNA extraction, 30 mL of each strain were harvested during the exponential growth phase by centrifugation at 1,000 × g for 10 min; the supernatant was removed by decantation. The pellet was frozen until extraction of DNA, using the CTAB method (Band-Schmidt et al., 2012). Quantity and quality of DNA were determined using nanodrop instrument (NanoDrop, Thermo Fisher Scientific, Waltham, MA) and by agarose gel electrophoresis.

The genomic DNA was amplified by PCR in a PCR thermal-cycler system (MJ Mini, Bio-Rad Laboratories, Hercules, CA). The PCR reactions to amplify *sxtA1* and *sxtA4* were carried out in a final volume of 25 μl, the reaction mix consisted of 1 x PCR buffer, 0.5% DMSO, 1 mM $MgCl_2$, 0.5 μM of each primer (**Table 2**), 200 μM dNTP's, and 1 U *Taq* DNA polymerase (Invitrogen, Carlsbad, CA). The *sxtA1* was amplified as follows: 1 cycle of 5 min at 94°C; 35 cycles of 30 s at 94°C, 30 s at 61°C, and 1 min at 72°C, and 1 cycle of 7 min at 72°C. The *sxtA4* was amplified as follows: 1 cycle of 5 min at 94°C; 35 cycles of 30 s at 94°C, 30 s at 58°C, and 1 min at 72°C, and 1 cycle of 7 min at 72°C. The fragments were visualized on an agarose gel by electrophoresis.

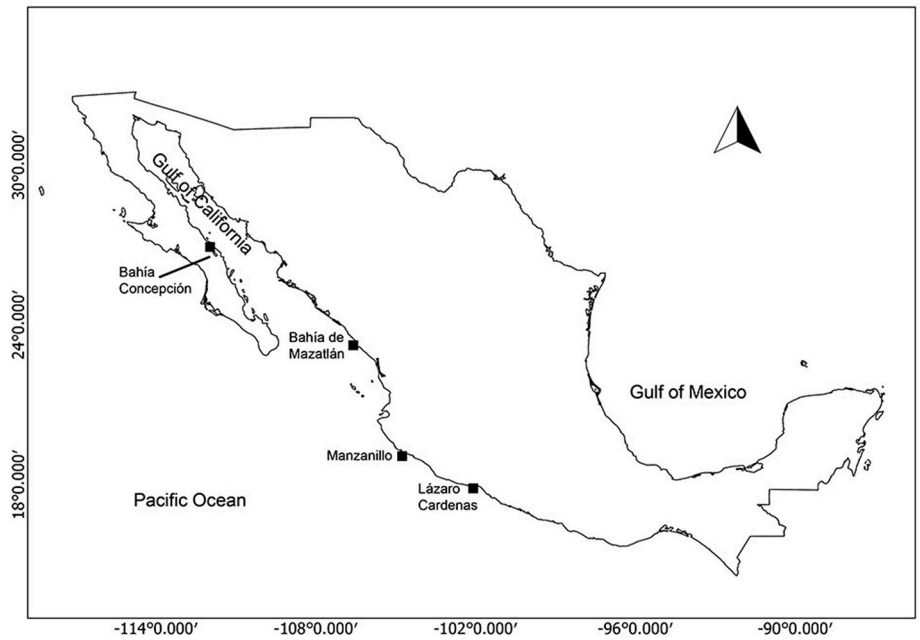


FIGURE 1 | Isolation localities of *Gymnodinium catenatum* strains in the Gulf of California and the Mexican Pacific.

TABLE 1 | Strains, place and year of isolation, and GenBank accession number of the Mexican *Gymnodinium catenatum*.

Strain	Place and date of isolation	Isolator	GenBank accession number	
			sxtA1	***sxtA4***
GCMV-7*	Bahía de Mazatlán, Sinaloa, 2007	C. Band	KY575974	KY575969
LC-62	Lázaro Cardenas, Michoacán, 2005	M. Rodríguez	KY575972	KY575968
G7 new	Manzanillo, Colima, 2010	S. Quijano	KY575971	KY575969
GCCV-6*	Bahía Concepción, B.C.S., 2000	C. Band	KY575973	

**Provided by the Colección de Dinoflagelados Marinos of CIBNOR (CODIMAR).*

TABLE 2 | List of primers of each domain, name, sequence and the reference, were used in the study.

Domain	Primer	Sequence 5′–3′	References
sxtA1	Sxt001F	TGCAGCGMTGCTACTCCTACTAC	Stüken et al., 2011
	Sxt002R	GGTCGTGGTCYAGGAAGGAG	Stüken et al., 2011
sxtA4	Sxt007F	ATGCTCAACATGGGAGTCATCC	Stüken et al., 2011
	Sxt008R	GGGTCCAGTAGATGTTGACGATG	Stüken et al., 2011
*sxtA4*qPCR	sxtA4Gcat	CGTACGCTTGAAGCACAATG	This study
	sxtA4Gcat	TGCGAGTCGTCAACGAGTAT	This study
*sxtA1*qPCR	sxtA1Gcat	AGCACCAGACGTTCTTCACT	This study
	sxtA1Gcat	CTTCACGTGCTCGTAGATGC	This study

Sequencing and Phylogenetic Analyses

PCR products were purified by precipitating with ethanol and sent for sequencing in Macrogen (Seoul, South Korea) using an automatic DNA sequencer (ABI 3730 × 1).

For the phylogenetic analysis of the domain *sxtA1* the EST from different species of dinoflagellates deposited in the Marine Eukaryote Transcriptome Sequencing Project were used. To obtain the domain from these sequences BLAST searches were done using the software BLAST+ 2.7.1 (National Center for Biotechnology Information, USA).

Local databases were created using the peptides sequences and comparing them with *Alexandrium* protein sequence as a query, for the searches blastp was used, hits with *e*-value$<1e^{-3}$ were included in the phylogenetic analyses. Sequences of toxic and non-toxic *Alexandrium* species deposited in GenBank were also included in the analyses.

For the phylogenetic analyses of the *sxtA4* domain, only sequences of *Alexandrium* species and *Pyrodinium bahamense* deposited in GenBank were included (**Table 3**). In both cases (*sxtA1* and *sxtA4*) the sequences were compared with cyanobacterial sequences of the gen *sxtA*.

The sequences were examined and edited with Sequencher 4.1.4 software (Gene Codes, Ann Arbor, MI, USA). The sequences of domain *sxtA1* of *G. catenatum, P. bahamense* and

TABLE 3 | Strains and GenBank accession numbers of species used in phylogenetic analyses for the *sxtA1* and *sxtA4* domains.

Species	Strain designation	GenBank accession number		Isolation place
		sxtA1	*sxtA4*	
Alexandrium fundyense	CCMP-1979		JF343354	Bay of Fundy, Canada
Alexandrium fundyense	CCMP-1719	JF343371	JF343337	Portsmouth, USA
Alexandrium fundyense	CCMP-1979	JF343309	JF343352	Bay of Fundy, Canada
Alexandrium fundyense	CCMP-1719		JF343345	Portsmouth, USA
*Alexandrium pacificum**	ACSH-02		JF343260	Syndey, Australia
*Alexandrium pacificum**	ACCC-01	JF343243	JF343259	NSW, Australia
*Alexandrium pacificum**	CCMP-1493	JF343283	JF343329	Hong Kong, China
*Alexandrium pacificum**	ACTRA-02	JF343246	JF343261	Tasmania, Australia
Alexandrium pacificum	OF-101		KF985179	
Alexandrium pacificum	ATTL-01		KF985178	Thau Lagoon, France
Alexandrium pacificum	ACQH-01		KF985182	Washington, USA
Alexandrium tamarense	CCMP-1771	JF343285	JF343331	Plymouth, England
Alexandrium pacificum^{+}	ACSH-02		KM104250	Sydney, Australia
Alexandrium pacificum^{+}	CS-315	KM104305	KM104289	Victoria, Australia
Alexandrium australiense^{+}	ATBB-01	JF343291	JF343333	Tasmania, Australia
Alexandrium minutum	AMAD-16	JF343278	JF343328	Port River, Australia
Alexenadrium minutum	CCMP-1888	JF343307	JF343348	Laguna Obidos, Portugal
Alexandrium minutum	CCMP-113	JF353269	JF343313	Vigo, Spain
Alexandrium minutum	ALSP-02		JF343318	Vigo, Spain
Alexandrium ostenfeldii	AOF-0927	KC835400	KC835401	Aland, Baltic Sea
Alexandrium ostenfeldii	NCH-85	KC835499	KC835402	Skagerrak, North Sea
Alexandrium margalefii	CS-322	KM104228		
Alexandrium fraterculus	CAWD52	KM104312		Coromandel, New Zeland
Alexandrium pseudogoniaulax	CAWD54	KM104226		Tamaki Strait, New Zealand
Gymnodinium catenatum	GCTRA-02	JF343257	JF343266	Australia
Gymnodinium catenatum	CS-395	JF343253		Australia
Pyrodinium bahamense			GBX01000001	
Cylindrospermopsis raciborski	T3		EU629178	
Anabaena circinalis	AWQC118	ACF94633	EU629177	
Aphanizomenon flos-aquae	NH-5	ACF94632	EU629175	
Lyngbya wollei			EU629174	

**Before named Alexandrium catenella.* $^{+}$*Before named Alexandrium tamarense.*

Alexandrium was translated to amino acid sequences using the program CLC Sequence Viewer 7 (CLC Bio, Qiagen, Denmark). The dinoflagellate amino acid sequences were aligned with MAFFTv7.313 (Yamada et al., 2016) with the L-INS-I model using the default parameters. Poorly aligned positions of amino acid alignments were removed with Gblocks set to the least stringent trimming options.

The best fit model for the amino acid sequences was selected using MEGA6 (Tamura et al., 2013); that WAG model was the optimal evolutionary model. Maximum Likelihood analyses were performed with RAxMLGUI (Silvestro and Michalak, 2012), PROTWAG model was used, with 100 bootstrap replicates. Bayesian inference was performed with the software MrBayes 3.2.6 (Huelsenbeck and Ronquist, 2001) using the same model; one million generations were run until the standard deviation of split sequences was less than 0.01.

The nucleotide sequences of domain *sxtA4* were aligned with MAFFTv7.313 with the G-INS-I model using the default parameters. Poorly aligned positions of nucleotide alignments were removed with Gblocks. Maximum Likelihood analyses were performed with RAxMLGUI (Silvestro and Michalak, 2012). The GTR model with gamma distribution was used for the analysis with 500 bootstrap replicates. The Bayesian Inference was performed using GTR model; one million generations were run until the deviation standard was lower than 0.01. Trees were sampled every 200 generations with a burning of 1000 trees.

Copy Number Determination of the Domains *sxtA1* and *sxtA4*

For the determination of the copy number *G. catenatum* (strain 62L) we cultivated the strain under the culture conditions previously described, using batch cultures in triplicate in 250 mL

Erlenmeyer flasks with 150 mL of media. For DNA extraction 100 mL were harvested by centrifugation in the lag phase, 50 mL in the early exponential phase and 30 mL in the late exponential and stationary phase; in each phase a 2 mL sample was taken to determine the cell abundance using a 1 mL Sedgewick–Rafter chamber, the counts were done in an inverted microscope Carl Zeiss Axio Vert.A1. For cell counts cells were fixed with lugol.

Primers for qPCR were designed from conserved regions of *G. catenatum* to amplify partial sequences *sxtA1* (150 bp) and *sxtA4* (180 pb) using the software Primer 3 (Untergasser et al., 2012). The qPCR reactions were performed in triplicate in 10 μL reactions mixtures containing, 0.5 μM of each primer, 2.5 mM $MgCl_2$, 1x PCR buffer, 200 μM dNTPs, 1U Taq Platinum (Invitrogen), and 1 × EvaGreen dye. Amplifications were carried out in a CFX96 Touch Real-Time PCR Detection System (Bio-Rad), with an initial denaturing step at 95°C for 4 minutes and 45 cycles of 95°C for 15 s, 56 or 55°C (to *sxtA1* and *sxtA4* primers, respectively) for 30 s. A melting-curve analysis was performed at the end of each cycle to confirm amplification specificity.

For both domains standard curves were constructed, from a 10-fold dilution series using fresh PCR product of each domain, ranging from 5 to 5×10^{-5} ng. The molecules of PCR product were determined: $(A \times 6.022 \times 10^{23}) \times (660 \times B)^{-1}$, where A is the concentration of the PCR product, 6.022×10^{23} Avogadro's number, 660: average molecular weight per base pair and B is the length of the PCR product.

RESULTS

Phylogenetic Analysis Domain *stxA1*

The sequences length of domain *sxtA1* of *G. catenatum* were among 525 and 541 bp, with an agreement between predicted and observed PCR amplifications. The GC content of the sequences was 64%. The search performed in BLAST, shows that the amplified sequences of *G. catenatum* correspond to sequences of the same species and domain (98% identities and 0.0 *e*-value). The second best match was related to *Alexandrium fundyense* and gene *sxtA* (91% identities and 0.0 *e*-value).

The phylogenetic inference of domain *sxtA1* showed the separation of the cyanobacteria group, named cyanobacteria clade. Dinoflagellates were included in a separate group, named dinoflagellate clade. With this clade two well-defined branches were observed, one branch grouping dinoflagellates from different genera and some non-PSP producer species of *Alexandrium* (*A. ostenfeldii, A. monilatum,* and *A. fratercolum*) and the second branch including toxic and non-PSP producer species of *Alexandrium,* as well strains of *G. catenatum* (**Figure 2**).

Within the dinoflagellate clade two subclades were formed. One contained sequences of *G. catenatum* strains from Australia and Mexico, without any differentiation as a result of the low differences between *G. catenatum* strains (0.2%), and another subclade formed by *Alexandrium* spp. sequences. The sequences used in the phylogenetic analysis of *Alexandrium* generally were distributed without species- or strain-related patterns. The pairwise distances between *Alexandrium* subclade and *Gymnodinium* subclade were between 7.1 and 10.7%.

Phylogenetic Analysis Domain *sxtA4*

PCR amplification of the domain *sxtA4* of *G. catenatum* strains resulted in one product of approximately 658 bp; there was agreement between predicted and observed PCR amplifications with a GC content of the sequences of 60%. For this domain, sequences from the three dinoflagellate genera/species that produce STX (*Alexandrium, G. catenatum,* and *P. bahamense*) were used. BLAST searches indicated that the amplified sequences of Mexican strains of *G. catenatum* aligned in the first place with the sequence of the *sxtA4* domain of a strain of *G. catenatum* (99% identity); the second alignment in BLAST corresponded to the species *A. fundyense* (90% identity).

The cyanobacterial sequences formed a well-supported clade separate from the dinoflagellate clade (**Figure 3A**). The phylogenetic tree constructed by maximum-likelihood and Bayesian inference showed that *G. catenatum* sequences form a fully supported cluster (100 bootstrap value and 1.0 posterior probabilities), separated from *Alexandrium* and *Pyrodinium* species. In this case, within the *Gymnodinium* clade one Mexican strain was grouped with the Australian strain and the other two Mexican strains were grouped in a second branch (**Figure 3B**). The differences within *Gymnodinium* strains $was 3%.

The only available sequence of *P. bahamense* was placed together with *Alexandrium* species, forming their own cluster (100-bootstrap value and 1.0 posterior probabilities), which was designated as the *Alexandrium/Pyrodinium* cluster. Similar to the *sxtA1* domain, the sequences of *Alexandrium* do not show a species- or strains-related pattern. The differences between the sequences of *Alexandrium* and *G. catenatum* were of 13%.

Copy Number Determination

Between 75 and 32 genomic copies of *sxtA1,* and 143–74 genomic copies of *sxtA4* in *G. catenatum* were found in triplicate batch cultures of strain 62L collected in different phases of the growth curve, based on the qPCR assay (**Figure 4**). There was no difference in the number of genomic copies among the different growth phases of the culture.

DISCUSSION

Horizontal Gene Transfer (HGT) is a common process in eukaryotes and plays an important role in the genome evolution in these organisms (Andersson, 2005; Keeling and Palmer, 2008). In dinoflagellates, HGT has been reported in various species, especially in genes involved in photosynthesis and the Calvin Cycle. The source of these genes differs, being transferred mainly between eukaryotes (Takishita et al., 2003, 2008; Nosenko et al., 2006; Patron et al., 2006; Minge et al., 2010). In these cases the HGT correspond to tertiary endosymbiosis where the endosymbionts transferred the genes to the genome of the host.

Murray et al. (2015) propose that the most parsimonious explanation for the origin of STX in dinoflagellates is a single horizontal transfer event that occurred early in dinoflagellate evolution. The presence of paralogs of the *sxtA1* domain in species that do not produce STX may support this hypothesis. If HGT occurred in an early stage in the evolution of dinoflagellates,

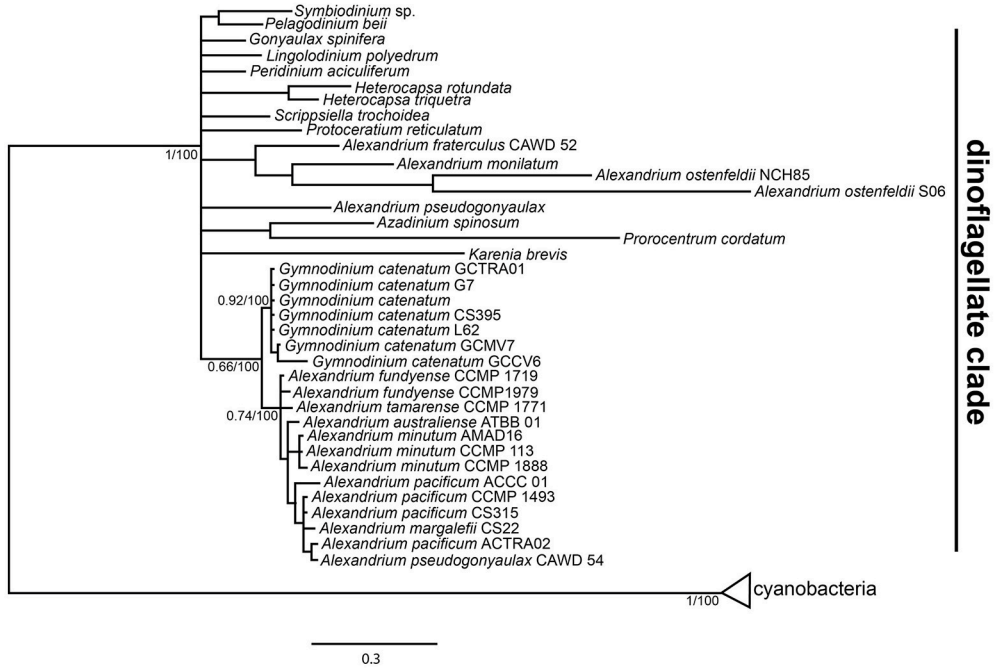


FIGURE 2 | Phylogenetic tree of the domain *sxtA1* from amino acids sequences of dinoflagellates and cyanobacteria. The tree is reconstructed with Bayesian Inference, the numbers in the internal nodes represent the posterior probabilities/boostrap values.

the horizontal transfer from *Alexandrium* to *G. catenatum* proposed by Orr et al. (2013) may not have been possible.

Ingestion of prey or phagocytosis has been described as the mechanism of foreign gene acquisition in eukaryotes (Doolittle, 1998). If *Alexandrium* was a prey of *G. catenatum* and transferred its genes, it would be expected that *G. catenatum* was included within the same clade with *Alexandrium* and *Pyrodinium* species. Since *G. catenatum* in both domains form a well-supported clade, that separates from *Alexandrium* and *Pyrodinium* species, this could indicate that the HGT from *Alexandrium* to *Gymnodinium* probably did not take place. It is possible that HGT occurred during early evolution of dinoflagellates, with loss of genes in other species of the genus. Moreover, *G. catenatum* can only feed on small phytoplankton species $<12\mu$m (Jeong et al., 2005). Feeding of *G. catenatum* on *Alexandrium* spp. has hitherto not been reported.

The genus *Alexandrium* originated around the late Cretaceous 77 MYA (John et al., 2003), while *G. catenatum*, may have evolved around of 150–140 MYA from *Gymnodinium* microreticulate complex (Bolch, 1999). The presence of paralogs of the *sxtA1* domain in two species of the order Gymnodiniales (*Karenia brevis* and *Pelagodinium beii*), suggest that HGT occurred before the divergence of Gymnodiniales and Gonyaulacales, with the subsequent loss in the capacity of PST biosynthesis in other dinoflagellates in the order Gymnodiniales; especially the loss of the domain *sxtA4,* which is present only in species that can produce PST.

In cyanobacteria, STX production genes originated from multiple HGT. The *sxtA* gene has a chimeric origin with the fusion of two different sources in one gene (Kellmann et al., 2008; Moustafa et al., 2009). The presence of a single domain of the *sxtA* gene in the dinoflagellates that do not produce STX can be explained by the loss of the domain that is found in the STX-producing species or that there were multiple transfers and in this case the transfer of the *sxtA4* domain in non-toxic dinoflagellates has not occurred.

Within the *G. catenatum* strains, the Mexican and Australian sequences group together, but the evolutionary distance between the strains of both regions was greater in the *sxtA4* domain than the *sxtA1* domain. One possible evolutionary scenario is that both domains have different mutation rates. Between *Alexandrium* and *G. catenatum* a similar pattern of evolutionary distance was observed.

The difference in the sequences within the *G. catenatum* strain in each domain may be explained since these domains are present in multiple copies inside the genome, and this modification corresponds to the variance between the copies without a relationship within their geographic origin. One characteristic of the dinoflagellates is that they have a large genome and most of the genes families are present in multiple copies (Bachvaroff and Place, 2008; Lee et al., 2014) and they normally occur in 30–5000 copies per genome, indicating that a high gene copy number is widespread in dinoflagellates (Hou and Lin, 2009). In *Alexandrium* species, there are differences in the sequences in clones of the same strain, which may result from the presence of multiple copies of the same gene (Stüken et al., 2011; Wiese et al., 2014; Murray et al., 2015). This is not only observed in STX genes, but also by the presence of many copies of the actin gene inside

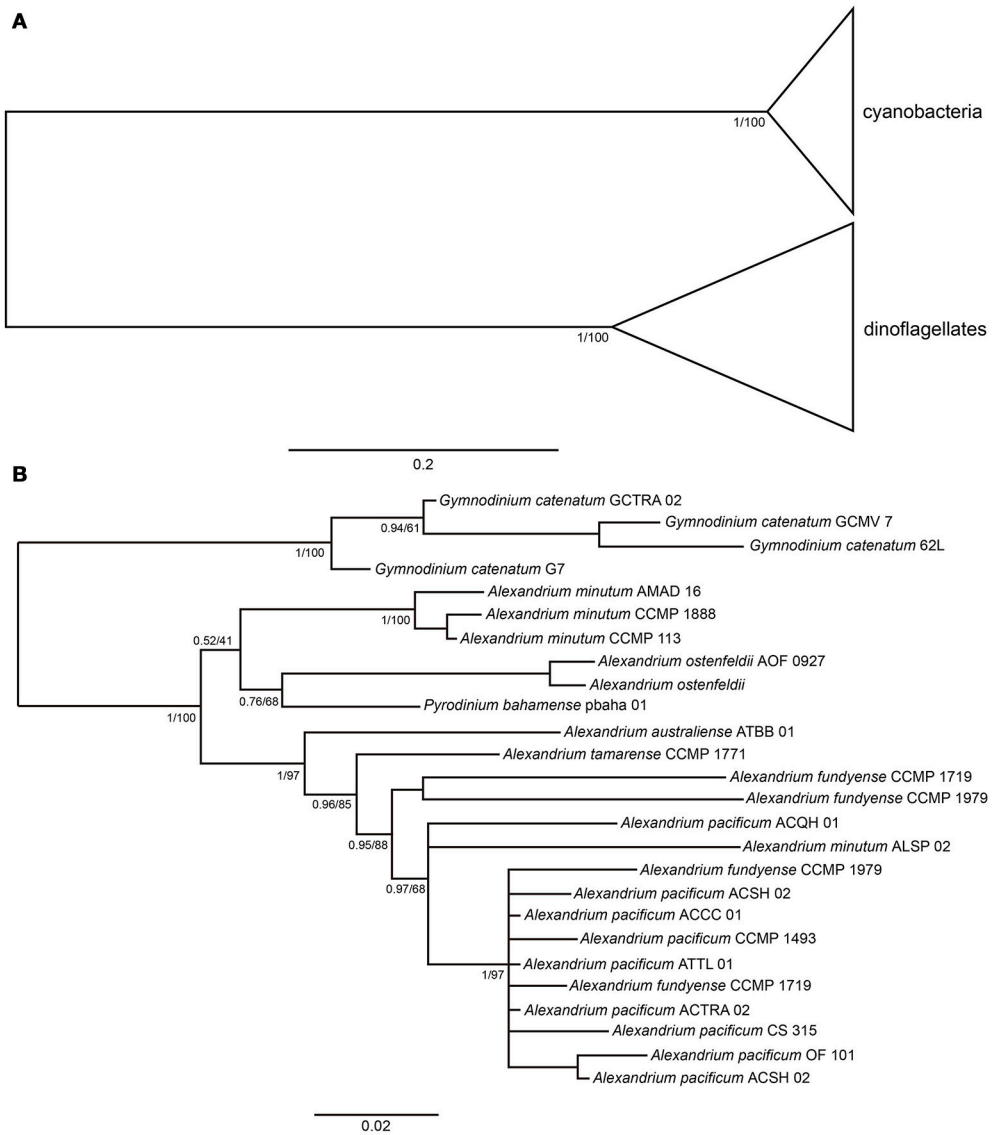


FIGURE 3 | (A) Phylogenetic tree of *sxtA4* domain of cyanobacteria and dinoflagellates, the node labels indicate the posterior probabilities and bootstrap values. **(B)** Phylogenetic tree of *sxtA4* domain of dinoflagellates, the tree was reconstructed by Bayesian Inference, the node labels indicate the posterior probabilities and bootstrap values.

the genome of *Amphidinium* and the changes between copies (Bachvaroff and Place, 2008).

The genomic copy number of the domain *sxtA4* of *G. catenatum* is lower than the reported for *A. catenella* from 100 to 280 cell^{-1} (Murray et al., 2011; Stüken et al., 2011); and higher than the values reported for *A. minutum* and *A. ostenfeldii* with 1.5–10.8 and 1–15 copies cell^{-1} respectively (Stüken et al., 2015; Savela et al., 2016). There is no information about the genomic copy number of the domain *sxtA1* in species of the genus *Alexandrium,* but in this study the gene copy number of *G. catenatum* of domain *sxtA1* is lower than number for the *sxtA4* domain. Stüken et al. (2015) proposed that gene copy number are not related to the genome size in *A. minutum* when comparing many strains of the species. Within the *Alexandrium* species, *A. catenella* and *A. ostenfeldii* have a similar genome size; 100 pg cell^{-1} and 115 pg cell^{-1} respectively (Figueroa et al., 2010), where the difference in the *sxA4* copy number is great. Within dinoflagellates the genome size exhibits a positive correlation with cell size (Lin, 2011). The size of *G. catenatum* varies from 31 to 41 µm long and from 27 to 33 µm wide (Blackburn et al., 1989), while *A. catenella* size varies from 21 to 23.5 µm long and from 23 to 25 µm wide (Kim et al., 2002). Although the genome size of *G. catenatum* is unknow; it can be assumed that *G. catenatum* has a higher genome than *A. catenella*. The copy number in *sxtA4* domain is higher than the copy number in *G. catenatum,* therefore, it could be suggested that

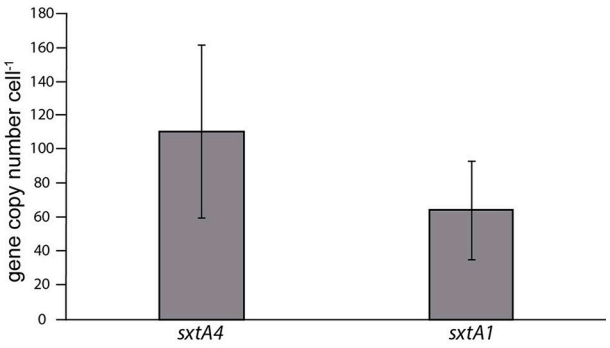


FIGURE 4 | Average gene copy number cell^{-1} of domains *sxtA4* and *sxtA1* in *G. catenatum* L62.

the genome size is not related to the *sxtA4* copy number in dinoflagellates.

Here it is shown for the first time the gene copy number of the domain *sxtA4* of *G. catenatum*, as well as for the domain *sxtA1*, and a variation in the domain *sxtA4* with respect to *Alexandrium* species. Murray et al. (2015) mention that following the acquisition of the genes trough HGT, a duplication process of the genes occurred. However, this duplication process was not the same within dinoflagellates that produce STX when the genes were acquired, and this could be the reason of the difference in gene copy numbers within dinoflagellates, and the selection pressure can act in various ways over the STX genes.

This study establishes that HGT of the gene *sxtA* did not occur from *Alexandrium* to *G. catenatum,* and that the HGT in *G. catenatum* was an early evolutionary event that probably occurred before this species diverged. The posterior loss of the capacity to produce STX in other members of the microreticulate complex, like in the *Alexandrium* genus, resulted in a the unique species of the genus *Gymnodinium* that has the capacity to produce STX.

If the HGT occurred in an ancestor of *G. catenatum* and *Alexandrium,* it is important to observe the presence of the paralogs of the *sxtA1* domain in more species of the order Gymnodiniales; especially in the *Gymnodinium* microreticulate complex, in order to gain a better understanding of how the transfer and losses of STX genes has been within the complex, and how the evolution of these genes occurred in dinoflagellates.

AUTHOR CONTRIBUTIONS

AM-F carried out all experimental work, acquired, analyzed, and interpreted data and drafted the manuscript. IL-V, CB-S participated in drafting the manuscript and analyzed and interpretation of phylogenetic data and supervised the overall progress of this project. CG-S participated in the experimental design of the qPCR analysis and drafting the manuscript. JB-G contributed with strains and commented and approved the manuscript.

ACKNOWLEDGMENTS

This research was funded by Instituto Politécnico Nacional (IPN grants SIP 2018-0662), and Consejo Nacional de Ciencia y Tecnología (CONACyT grant 248468, FORDECyT grant 260040). AM-F. is a recipient of fellowship from CONACyT (grant 268184) and BEIFI - IPN, CB-S. is a COFFA – IPN and EDI fellow. I. Fogel and D. W. Johnson for the English language editing.

REFERENCES

Andersson, J. O. (2005). Lateral gene transfer in eukaryotes. *Cell. Mol. Life Sci.* 62, 1182–1197. doi: 10.1007/s00018-005-4539-z

Attaran-Fariman, G., de Salas, M. F., Negri, A. P., and Bloch, C. J. S. (2007). Morphology and phylogeny of *Gymnodinium trapeziforme* sp. nov. (Dinophyceae): a new dinoflagellate from the southeast coast of Iran that forms microreticulate resting cysts. *Phycologia* 46, 644–656. doi: 10.2216/07-05.1

Bachvaroff, T. R., and Place, R. (2008). From stop to start: tandem gene arrangement, copy number and *trans*-splicing sites in the dinoflagellate *Amphidinium caterae*. *PLoS ONE* 3:e2929. doi: 10.1371/journal.pone.0002929

Band-Schmidt, C. J., Martínez-López, A., Bustillos-Guzmán, J. J., Carreón-Palau, L., Morquecho, L., Olguín-Monroy, N., et al. (2012). Morphology, biochemistry, and growth of raphidophyte strains from the Gulf of California. *Hydrobiologia* 693, 81–97. doi: 10.1007/s10750-012-1088-y

Blackburn, S. I., Bolch, C. J. S., Haskard, K. A., and Hallegraeff, G. M. (2001). Reproductive compatibility among four global populations of the toxic dinoflagellate *Gymnodinium catenatum* (Diniohyceae). *Phycologia* 40, 78–87. doi: 10.2216/i0031-8884-40-1-78.1

Blackburn, S. I., Hallegraeff, G. M., and Bolch, C. J. (1989). Vegetative reproduction and sexual life cycle of the toxic dinoflagellate *Gymnodinium catenatum* from Tasmania, Australia. *J. Phycol.* 25, 577–590.

Bolch, C. J. S. (1999). *Evolution, Species Resolution and Molecular Population Genetics of the Gymnodinium Catenatum Toxic Dinoflagellate Species Complex: Tracing Global Dispersal and Population Dynamics*. Doctoral thesis, University of Tasmania.

Bustillos-Guzmán, J. J., Band-Schmidt, C. J., Durán-Riveroll, L. M., Hernández-Sandoval, F. E., López-Cortés, D. J., Núñez-Vázquez, E. J., et al. (2015). Paralytic toxin profile of the marine dinoflagellate *Gymnodinium catenatum* Graham from the Mexican Pacific as revealed by LC-MS/MS. *Food. Addit. Contam. A* 32, 381–394. doi: 10.1080/19440049.2014.1000978

Doolittle, W. F. (1998). You are what you eat: a gene transfer ratchet could account for bacterial genes in eukaryotic nuclear genomes. *Trends. Genet.* 14, 307–311.

Figueroa, R. I., Garcés, E., and Bravo, I. (2010). The use of flow cytometry for species identification and life-cycle studies in dinoflagellates. *Deep Sea Res. II Top. stud. Oceanogr.* 57, 301–307. doi: 10.1016/j.dsr2.2009.09.008

Gu, H., Liu, T., Vale, P., and Luo, Z. (2013). Morphology, phylogeny and toxin profiles of *Gymnodinium inusitatum* sp. nov., *Gymnodinum catenatum* and Gymnodinium microreticulatum *(Dinophyceae)* from the Yellow Sea, China. *Harm. Algae* 28, 97–107. doi: 10.1016/j.hal.2013.06.001

Hackett, J. D., Wisecaver, J. H., Brosnahan, M. L., Kulis, D. M., Anderson, D. M., Bhattacharya, D., et al. (2013). Evolution of saxitoxin synthesis in cyanobacteria and dinoflagellates. *Mol. Biol. Evol.* 30, 70–78. doi: 10.1093/molbev/mss142

Hallegraef, G. M., Blackburn, S. I., Doblin, M. A., and Bolch, C. J. S. (2012). Global toxicology, ecophysiology and population relationship of the chainforming PST dinoflagellate *Gymnodinium catenatum*. *Harm. Algae* 14, 130–143. doi: 10.1016/j.hal.2011.10.018

Hou, Y., and Lin, S. (2009). Distinct gene number-genome size relationships for eukaryotes and non-eukaryotes: gene content estimation for dinoflagellate genomes. *PLoS ONE* 4:e6978 doi: 10.1371/journal.pone.0006978

Huelsenbeck, J. P., and Ronquist, F. (2001). MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17, 754–755. doi: 10.1093/bioinformatics/17.8.754

Jeong, H. J., Yoo, Y. Y., Park, J. Y., Song, J. Y., Kim, S. T., Lee, S. H., et al. (2005). Feeding by phototrophic red-tide dinoflagellates: five species newly revealed and six species previously known to be mixotrophic. *Aquat. Microb. Ecol.* 40, 133–150. doi: 10.3354/ame040133

John, U., Fensome, R. A., and Medlin, L. K. (2003). The application of a molecular clock based on molecular sequences and the fossil record to explain biogeographic distributions within the *Alexandrium tamarense* "species complex" (Dinophyceae). *Mol. Biol. Evol.* 20, 1015–1027. doi: 10.1093/molbev/msg105

Keeling, P. J., and Palmer, J. D. (2008). Horizontal gene transfer in eukaryotic evolution. *Nat. Rev. Genet.* 9, 605–618. doi: 10.1038/nrg2386

Kellmann, R., Mihali, T. K., Jeon, Y. J., Pickford, R., Pomati, F., and Neilan, B. A. (2008). Biosynthetic intermediate analysis and functional homology reveal a saxitoxin gene cluster in cyanobacteria. *Appl. Environ. Microb.* 74, 4044–4053. doi: 10.1128/AEM.00353-08

Kellmann, R., and Neilan, B. A. (2007). Biochemical characterization of paralytic shellfish toxin biosynthesis *in vitro*. *J. Phycol.* 43, 497–508. doi: 10.1111/j.1529-8817.2007.00351.x

Kim, K. Y., Yoshida, M., Fukuyo, Y., and Kim, C. H. (2002). Morphological observation of *Alexandrium tamarense* (Lebour) Balech, *A. catenella* (Whedon et Kofoid) Balech and related morphotype (Dinophyceae) in Korea. *Algae* 17, 11–19. doi: 10.4490/ALGAE.2002.17.1.011

Lee, R., Lai, H., Banoo-Malik, S. B., Saldarriaga, J. F., Keeling, P. J., and Slamovits, C. H. (2014). Analysis of EST data of the marine protist *Oxyrrhis marina*, an emerging model for alveolate biology and evolution. *BMC Genomics* 15:122. doi: 10.1186/1471-2164-15-122

Lin, S. (2011). Genomic understanding of dinoflagellates. *Res. Microbiol.* 162, 551–569. doi: 10.1016/j.resmic.2011.04.006

Mihali, T. K., Carmichael, W. W., and Neilan, B. A. (2011). A putative gene cluster from a *Lynbya wollei* bloom that encodes paralytic shellfish toxin biosynthesis. *PLoS ONE* 6:e14657. doi: 10.1371/journal.pone.0014657

Mihali, T. K., Kellmann, R., and Neilan, B. A. (2009). Characterization of the paralytic shellfish toxin biosynthesis gene clusters in *Anabaena circinalis* AWQC131C and *Aphanizomenon* sp. NH-5. *BMC Biochem.* 10:8. doi: 10.1186/1471-2091-10-8

Minge, M. A., Shalchian-Tabrizi, K., Torresen, O. K., Takishita, K., Probert, I., Inagaki, Y., et al. (2010). A phylogenetic mosaic plastid proteome and unusual plastid-targeting signals in the green-colored dinoflagellate *Lepidodinium chlorophorum*. *BMC Evol. Biol.* 10:191. doi: 10.1186/1471-2148-10-191

Moustafa, A., Loram, J. E., Hackett, J. D., Anderson, D. M., Plumley, F. G., and Bhattacharya, D. (2009). Origin of saxitoxin biosynthetic genes in cyanobacteria. *PLoS ONE* 4:e5758. doi: 10.1371/journal.pone.0005758

Murray, S. A., Diwan, R., Orr, R. J., Kohli, G. S., and John, U. (2015). Gene duplication, loss and the selection in the evolution of saxitoxin biosynthesis in alveolates. *Mol. Phylogenet. Evol.* 92, 165–180. doi: 10.1016/j.ympev.2015.06.017

Murray, S. A., Mihali, T. K., and Neilan, B. A. (2011). Extraordinary conservation, gene loss, and positive selection in the evolution of an ancient neurotoxin. *Mol. Biol. Evol.* 28, 1173–1182. doi: 10.1093/molbev/msq295

Negri, A. P., Bolch, C. S., Geier, S., Green, D. H., Park, T. G., and Blackburn, S. J. (2007). Widespread presence of hydrophobic paralytic shellfish toxins in *Gymnodinium catenatum*. *Harm. Algae* 6, 774–780. doi: 10.1016/j.hal.2007.04.001

Nosenko, T., Lidie, K. L., Van Dolah, F. M., Lindquist, E., Cheng, J. F., US Department of Energy-Joint Genome Institute, et al. (2006). Chimeric plastid proteome in the Florida "red tide" dinoflagellate *Karenia brevis*. *Mol. Biol. Evol.* 23, 2026–2038. doi: 10.1093/molbev/msl074

Orr, R. J., Stüken, A., Murray, S. A., and Jakobsen, K. S. (2013). Evolution and distribution of saxitoxin biosynthesis in dinoflagellates. *Mar. Drugs* 11, 2814–2828. doi: 10.3390/md11082814

Patron, N. J., Waller, R. F., and Keeling, P. J. (2006). A tertiary plastid uses genes from two endosymbionts. *J. Mol. Biol.* 357, 1373–1382. doi: 10.1016/j.jmb.2006.01.084

Savela, H., Harju, K., Spoof, L., Lindehoff, E., Meriluoto, J., Vehniäinen, M., et al. (2016). Quantity of the dinoflagellate toxin production in *Alexandrium ostenfeldii* blooms. *Harm. Algae* 52, 1–10. doi: 10.1016/j.hal.2015.10.018

Scholin, C. A., Hallegraeff, G. M., and Anderson, D. M. (1995). Molecular evolution of the *Alexandrium tamarense* "species complex" (Dinophyceae): dispersal in the North American and West Pacific regions. *Phycologia* 34, 472–485.

Silvestro, D., and Michalak, I. (2012). raxmlGUI: a graphical front-end for RAxML. *Org. Divers. Evol.* 12, 335–337. doi: 10.1007/s13127-011-0056-0

Stüken, A., Orr, R. J., Kellman, R., Murray, S. A., Neilan, B. A., and Jakobsen, K. S. (2011). Discovery of nuclear-encoded genes for the neurotoxin saxitoxin in dinoflagellates. *PLoS ONE* 6:e20096. doi: 10.1371/journal.pone.0020096

Stüken, A., Riobó, P., Franco, J., Jakobsen, K. S., Guillou, L., and Figueroa, R. I. (2015). Paralityc shellfish toxin content is related to genomic *sxtA4* copy number in *Alexandrium minutum* strains. *Front. Microbiol.* 6:404. doi: 10.3389/fmicb.2015.00404

Takishita, K., Ishida, K., and Maruyama, T. (2003). An enigmatic GAPDH gene in the symbiotic dinoflagellate genus *Symbiodinium* and its related species (the order Suessiales): possible lateral gene transfer between two eukaryotic algae, dinoflagellate and euglenophyte. *Protist* 154, 443–454. doi: 10.1078/143446103322454176

Takishita, K., Kawachi, M., Noël, M. H., Matsumoto, T., Kakizoe, N., Watanabe, M. M., et al. (2008). Origins of plastid and glyceraldehyde-3-phosphate dehydrogenase genes in the green-colored dinoflagellate *Lepidodinium chlorophorum*. *Gene.* 410, 26–36. doi: 10.1016/j.gene.2007.11.008

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* 30, 2725–2729. doi: 10.1093/molbev/mst197

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., et al. (2012). Primer3 – new capabilities and interfaces. *Nucleic Acids Res.* 40:e115. doi: 10.1093/nar/gks596

Wiese, M., D'angostino, P. M., Mihali, T. K., Moffitt, M. C., and Neilan, B. A. (2010). Neurotoxic alkaloids: saxitoxin and its analogs. *Mar. Drugs* 8, 2185–2211. doi: 10.3390/md8072185

Wiese, M., Murray, S. A., Alvin, A., and Neilan, B. A. (2014). Gene expression and molecular evolution of *sxtA4* in a saxitoxin producing dinoflagellate *Alexandrium catenella*. *Toxicon* 92, 102–112. doi: 10.1016/j.toxicon.2014.09.015

Yamada, K. D., Tomii, K., and Katoh, K. (2016). Application of the sequence alignment program to large data – reexamination of the usefulness of chained guide trees. *Bioinformatics* 32, 3246–3251. doi: 10.1093/bioinformatics/btw412

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Mendoza-Flores, Leyva-Valencia, Band-Schmidt, Galindo-Sánchez and Bustillos-Guzmán. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Marine Science

ORIGINAL RESEARCH
published: 24 September 2018
doi: 10.3389/fmars.2018.00335

Transformation and Depuration of Paralytic Shellfish Toxins in the Geoduck Clam *Panopea globosa* From the Northern Gulf of California

Jennifer Medina-Elizalde[1], Ernesto García-Mendoza[2]*, Andrew D. Turner[3], Yaireb Alejandra Sánchez-Bravo[2] and Ramón Murillo-Martínez[2]*

[1] Departamento de Biotecnología Marina, Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, Mexico, [2] Departamento de Oceanografía Biológica, Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, Mexico, [3] Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth, United Kingdom

OPEN ACCESS

Edited by:
Marius Nils Müller, Universidade Federal de Pernambuco, Brazil

Reviewed by:
Paulo João Vieira Vale, Instituto Português do Mar e da Atmosfera (IPMA), Portugal
Luiza Dy Fonseca Costa, Fundação Universidade Federal do Rio Grande, Brazil

***Correspondence:**
Ernesto García-Mendoza
ergarcia@cicese.mx
Jennifer Medina-Elizalde
jennifer.medina.elizalde@gmail.com

Specialty section:
This article was submitted to Marine Biogeochemistry, a section of the journal Frontiers in Marine Science

Received: *02 July 2018*
Accepted: *29 August 2018*
Published: *24 September 2018*

Citation:
Medina-Elizalde J, García-Mendoza E, Turner AD, Sánchez-Bravo YA and Murillo-Martínez R (2018) Transformation and Depuration of Paralytic Shellfish Toxins in the Geoduck Clam Panopea globosa From the Northern Gulf of California. Front. Mar. Sci. 5:335. doi: 10.3389/fmars.2018.00335

In January 2015, a harmful algae bloom (HAB) of the dinoflagellate *Gymnodinium catenatum* occurred in the Northern Gulf of California (NGC). This species produces paralytic shellfish toxins (PSTs), a group of potent neurotoxins. The harvesting and commercialization of geoduck *Panopea globosa* are important economic activities in this region and were prohibited for several months due to the accumulation of PSTs in clam tissues. We analyzed PSTs concentrations in *P. globosa* collected on a weekly basis during 2015 near San Felipe, Baja California. The aim of the study was to evaluate the transformation and depuration characteristics of PSTs in different geoduck tissues. The PST content was evaluated in the visceral mass and in the siphon by high-performance liquid chromatography with post-column oxidation (HPLC-PCOX). Additionally, 10 selected samples were analyzed by hydrophilic interaction chromatography coupled to tandem mass spectrometry (HILIC-MS/MS). Toxicity in all siphon samples was lower than the regulatory limit (RL) for PSTs of 800 μg STX eq kg^{-1}. In contrast, the maximum toxicity of 16,740 μg STX eq kg^{-1} detected in the visceral mass exceeded 21 times the RL and it took 210 days to reach values below 800 μg STX eq kg^{-1}. Therefore, *P. globosa* can be considered a slow detoxifier bivalve with a depuration rate of 4.3% day^{-1} (calculated by an exponential decay model; R^2 = 0.80). The N-sulfocarbamoyl toxins C1 and 2 were the most abundant analogs in the siphon and viscera samples collected close to the HAB occurrence. The concentration of these analogs decreased and GTX5 and more toxic analogs such as dcGTX2 and dcSTX were detected. M-type analogs were detected by HILIC-MS/MS and represented up to 75% of total PSTs in some samples. M-type analogs contributed to 48% of toxicity estimated in the sample. We report for the first time the depuration rate, PSTs profile, and its change over time in *P. globlosa*. This information is essential to characterize the metabolism of toxins in this economically important bivalve but also to develop management plans for fisheries if the organism is going to be recurrently exposed to PSTs producing blooms, as seems the case for the NGC.

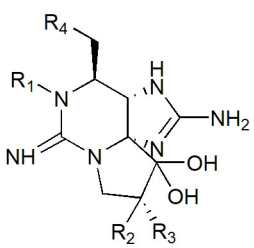
Keywords: *Gymnodinium catenatum*, HPLC-PCOX, HILIC-MS/MS, harmful algae bloom, saxitoxin

INTRODUCTION

Paralytic shellfish toxins (PSTs) are a group of at least 50 water-soluble neurotoxic alkaloids analogs to saxitoxin (Wiese et al., 2010). Dinoflagellates of the genera *Gymnodinium*, *Alexandrium*, and *Pyrodinium* are the primary producers of PSTs in marine environments (Jaime et al., 2007). PSTs attach reversibly to the voltage-dependent sodium channel and block the pores of the channels. This prevents the transmission of the nerve impulses resulting in neuromuscular paralysis (Cestèle and Catterall, 2000; Bricelj et al., 2005). PSTs vary in their potency or biological activity: the carbamate (saxitoxin STX, neosaxitoxin NEO, and gonyautoxins GTX1–4) are the most potent toxins since they have a higher affinity to sodium channels. N-sulfocarbamoyl toxins (B1 and 2 and C1–4) are the least potent ones, while decarbamoyl toxins (dcGTX1–4, dcSTX, and dcNEO) exhibit intermediate specific toxicities (Bricelj and Shumway, 1998). **Figure 1** shows the structures of the primary toxin analogs and are grouped according to the molecular substitutions.

Bivalves, crustaceans, some gastropods, and planktivorous fishes that feed on PSTs producing organisms can accumulate these toxins in their tissues and incorporate them into the food chain. Consumption of contaminated shellfish with PSTs, mainly bivalves, can lead to paralytic shellfish poisoning (PSP) in humans. This syndrome can, at sufficient high toxin concentrations, cause death from cardio respiratory arrest (Botana, 2000). In Mexico, PSP is the most important toxic syndrome related to harmful algae blooms (HABs) of marine species and the only one associated with human fatalities (Lewitus et al., 2012). In Mexico, PSP has affected at least 460 individuals with 32 recognized fatalities (Band-Schmidt et al., 2010; Bustillos-Guzmán et al., 2016; Santiago-Morales, 2016). The first recognized PSP outbreak occurred in 1979 in the coast of Sonora to Jalisco (**Figure 2**) and was associated with a bloom of *Gymnodinium catenatum* (Mee et al., 1986). The intoxication of 19 people occurred, including three deaths, as well as an extensive fish die-off (De la Garza, 1983; Mee et al., 1986). Other outbreaks have been documented along the Mexican Pacific coast (Bustillos-Guzmán et al., 2016). The bivalves responsible for the intoxications were not identified in the majority of the outbreaks. However, PSTs have been detected in shellfish in most of these events. In the 1979 outbreak, the rocky oyster *Crassostrea iridiscens* accumulated 10,700–17,200 μg STX eq kg^{-1} and the clam *Donax* sp. accumulated 76,400 μg STX eq kg^{-1} (Mee et al., 1986). In November 1989, three people died and 99 others became intoxicated in the Gulf of Tehuantepec region (Oaxaca coast, Mexican tropical Pacific, **Figure 2**) and the accumulation of 8,110 μg STX eq kg^{-1} in oyster was documented (Saldate-Castañeda et al., 1991). Also along the Oaxaca coast, three people died and 17 were intoxicated between August 2001 and February 2002. Associated with this outbreak, up to 14,560 μg STX eq kg^{-1} were detected in mussels (Ramírez-Camarena et al., 2004; Band-Schmidt et al., 2010; Santiago-Morales, 2016).

In general, the species responsible for the accumulation of the PSTs during the outbreaks are *G. catenatum* in the northern coasts of the Mexican Pacific Ocean and *Pyrodinium bahamense* var. *compressum* in the south (Lewitus et al., 2012; Bustillos-Guzmán et al., 2016; Santiago-Morales, 2016). Most HABs of these species occur between February and May when the water temperature is between 17–25°C (Manrique and Molina, 1997; Gárate-Lizárraga et al., 2004, 2006; Lewitus et al., 2012). In January 2015, an intense and extensive HAB of *G. catenatum* occurred in the north of the Gulf of California (GC; **Figure 2**). This HAB affected the economy, the ecology, and public health of the region. Most notably, the HAB was associated with seabirds and marine mammal die-offs (Garcia-Mendoza et al., in preparation), also resulting in the intoxication of at least five people at Los Angeles Bay after the ingestion of wild bivalves (Comisión Federal para la Protección contra Riesgos Sanitarios, 2015). As a direct consequence of this bloom, the extraction and commercialization of some shellfish species was prohibited



R1	R2	R3	Carbamate toxins	N-Sulfocarbamoyl toxins	Decarbamoyl toxins	Others analogues M toxins		
H	H	H	STX	B1 (GTX5)	dcSTX			
OH	H	H	NEO	B2 (GTX6)	dcNEO			
OH	H	OSO_3^-	GTX1	C3	dcGTX1			
H	H	OSO_3^-	GTX2	C1	dcGTX2			
H	OSO_3^-	H	GTX3	C2	dcGTX3			
OH	OSO_3^-	H	GTX4	C4	dcGTX4			
H	OH	H				M1	M2	
H	OH	OH				M3	M4	
Not structurally characterized								M5
			R4 $OCONH_2$	R4 $OCONHCH_3$	R4 OH	R4 $OCONH_2$	R4 $OCONHCH_3$	

FIGURE 1 | Structure of the paralytic shellfish primary toxin analogs, grouped according to the molecular substitutions.

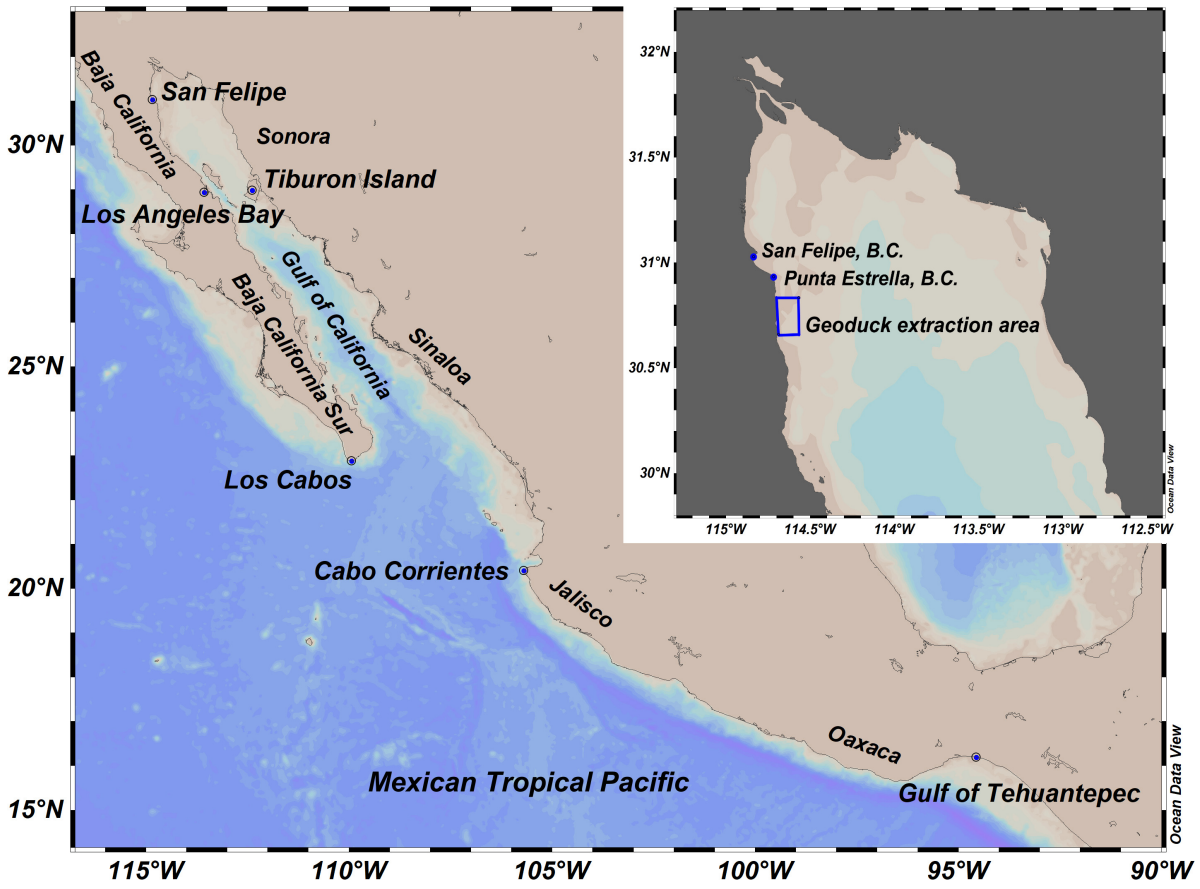


FIGURE 2 | Gulf of California and Mexican Tropical Pacific where outbreaks of paralytic shellfish toxins have occurred. The northern part of the Gulf of California and the location of Atenea en el Mar company federal approved area for harvesting of *Panopea globosa* in San Felipe, BC, are presented in the inset of the map.

almost all year. Harvesting of *Panopea globosa* was particularly affected.

Panopea globosa is an endemic geoduck clam from the northwest coast of Mexico. Its distribution extends from the upper GC to Magdalena Bay on the Pacific side of the Baja California peninsula (Aragón-Noriega et al., 2012). Geoducks clams are large infaunal bivalves, their shells can be larger than 25 cm, the siphon may exceed 1 m in length and can reach 2 kg of weight (Goodwin and Pease, 1987). These organisms live buried in the sediment ranging from the intertidal zone to a depth of 110 m (Bureau et al., 2002). *P. globosa* is one of the most important economic resources of the GC. San Felipe is the main port of arrival for these bivalves with a mean production of 848 t year^{-1} from 2005 to 2010 that represent approximately 10 million USD per year (Secretaría de Pesca de Baja California, 2016). The market for Mexican geoducks is the United States and Asian countries which demands live adult organisms (Aragón-Noriega et al., 2012). The sanitary ban implemented in 2015 significantly affected the industry in the Northern Gulf of California (NGC). The extraction of the clams was prohibited for 5 consecutive months and after June 2015, closures occurred intermittently for another 5 months due to the detection of PSTs concentrations in visceral mass above the regulatory limit (RL) of 800 μg STX eq kg^{-1} (Comisión Federal para la Protección contra Riesgos Sanitarios, 2015).

Accumulation of PSTs in geoduck species has been only characterized in *Panopea generosa* (=*Panopea abrupta*). The intra- and inter-population toxin variability was described in organisms from two localities of the Washington coast (Curtis et al., 2000). PSTs concentrations were highly variable among locations and between the sampling depths of the organisms. Most important, the amount of toxins accumulated in viscera was much higher than in the siphon and its concentration was above the RL for the entire 5 months of the study (Curtis et al., 2000). There was no information about the presence of the phytoplankton organism producing the PSTs. However, the data indicated that *P. generosa* is a slow depurating organism, with PSTs being retained in the tissues for long periods of time. There is no information about the PSTs metabolism in *P. globosa*. Therefore, the aim of this present study was to describe the transformation and depuration of PSTs in viscera and the siphon of the geoduck *P. globosa*. This information is important for the characterizations of PSTs metabolic processes in bivalves and it is necessary for the management of this important regional fishery in case of HABs producing PSTs.

MATERIALS AND METHODS

Study Area and Sample Collection

The GC, located in the northwest of Mexico, is a narrow shelf sea of approximately 1,100 km length and 150 km width (Gutiérrez et al., 2004). The GC is a productive semienclosed sea that supports several industrial and small-scale fisheries (Cudney-Bueno et al., 2009). The GC is situated between the Baja California Peninsula (West) and the Sonora and Sinaloa coasts (East). The Northern border is limited by the Colorado River base level and the Southern border is delimited by an imaginary line from the tip of the Baja California Peninsula to Cabo Corrientes, Jalisco, Mexico (Espinosa-Carreón and Valdéz-Olguín, 2007). The Northern Gulf goes from Colorado River to the south of Tiburon Island (**Figure 2**).

There are approximately 20 *Panopea* harvesting areas in the NGC. The laboratory FICOTOX of Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California (CICESE) has been monitoring the phytoplankton community intermittently since 2011 in surface water samples collected from a harvesting area located south from San Felipe, Baja California, Mexico (30°39′30″–30°50′00″ N and 114°34′00″–114°41′15″ W; **Figure 2**). This area is a federal concession to the company Atenea en el Mar for geoduck extraction. After the detection of the bloom, water samples were collected every week from January to June of 2015. Also, geoduck clams were collected every week in this area after the detection of the bloom.

The phytoplankton community was evaluated in water samples collected at the surface and from 10 m depth vertical net hauls; 250 mL of water were placed in dark plastic bottles (Nalgene type) and fixed with 1–2 mL of a concentrated lugol-acetate solution. This solution is recommended for evaluation of the phytoplankton community with the Utermöhl technique (Sournia, 1978). Lugol-acetate increases the settling velocity of microalgae, and can be used for the majority of phytoplankton species without damaging the cells. Also, has low toxicity to humans compared with other fixatives (Andersen and Throndsen, 2004). The Utermöhl technique (Sournia, 1978) was used to identify and to evaluate phytoplankton cell abundance; 10–25 mL of the sample were sedimented for at least 24 h and the complete surface of the sedimentation chamber was analyzed with a LEICA DMI3000B (Leica Microsystems, Germany) inverted microscope. The abundance of *G. catenatum* (in cells L^{-1}) was calculated according to the sedimented volume. A minimum of 500 cells were counted to estimate the relative abundance of *G. catenatum* to total phytoplankton cells (in %) present in samples collected from net hauls.

Geoduck clams collected by scuba diving at 12–20 m depth were maintained in cooler boxes and kept at 12–16°C until delivery to the laboratory. Samples were transported to the laboratory FICOTOX in less than 24 h after the collection. Associated with internal paperwork problems of the company some organisms could not be delivered to the laboratory after field sampling. Organisms collected on June 7 and 29, July 2 and 25, and August 7 and 8 were kept alive in the company tanks without food at 18°C before delivering the samples to the laboratory. These samples arrived at the laboratory no more than 8 days after collection from the field.

Once in the laboratory, organisms were dissected. The toxin content was evaluated both in the visceral mass that contains all the geoduck organs and in the siphon, the muscular part of the organism. To obtain a representative sample and according to recommendations of the national sanitary regulation for PSTs monitoring in geoduck (Secretaría de Salud, 2016), each sample consisted of three individuals. The tissues of the three dissected organisms were pooled together, homogenized in a blender and kept frozen at −20°C until its analysis by high-performance liquid chromatography with post-column oxidation (HPLC-PCOX) according to Van de Riet et al. (2011). To extract the toxins, 5 g of each tissue homogenate (consisting of three siphons or three visceral masses) were mixed with 5 mL of 0.1 M HCl and boiled for 5 min. After cooling to room temperature, samples were centrifuged at 3,000 *g* for 10 min and an aliquot of 500 μL of the supernatant was recovered and deproteinized with 35 μL of trichloroacetic acid (TCA) 30% solution. The pH was adjusted to 3 before and after the boiling step with 5 M NaOH or 5 M HCl. The pH was also adjusted after the addition of the TCA with 25 μL of 0.1 M NaOH. The aliquot was subsequently filtered through a 45 μm pore size nylon membrane filter before injection onto the HPLC-PCOX system.

Quantification of PSTs by HPLC-PCOX

HPLC-PCOX was conducted as described by Van de Riet et al. (2011). The analysis was performed using an Agilent Technologies 1100 model HPLC (Agilent, United States) calibrated with eight certified reference standards obtained from the Institute of Biotoxin Metrology, National Research Council Canada (NRC, Halifax, Nova Scotia, Canada). For C toxins (C1 and 2) 5 μL of each sample was injected onto an Agilent Zorbax SB-C8 (250 mm × 4.6 mm, 5 μm) HPLC column. For GTX toxins (GTX1 and 4, GTX2 and 3, dcGTX2 and 3, GTX5) and STX toxins (NeoSTX, STX, dcSTX) 10 μL of each sample were injected onto an Agilent Zorbax Bonus-RP (150 mm × 4.6 mm, 3.5 μm) column. After the chromatographic separation, samples were oxidized online in a Vector PCX derivatization instrument (Pickering Labs Inc., United States) and detected by fluorescence. Excitation was at 330 nm and emission detection at 395 nm. When the concentration of PSTs in samples was higher than the calibration curve concentration range, extracts were diluted with a 0.1 M HCl (pH 3) solution. The dilution factor was considered in the calculation of PSTs concentration.

Total toxicity of the sample was calculated by the multiplication of the concentration of each toxin, the molecular weight of STX diHCl and the toxicity equivalent factor (TEF) of each analog according to Oshima (1995; **Table 1**).

Quantification of PSTs by HILIC-MS/MS

Ten selected samples collected at different dates were also analyzed by hydrophilic interaction liquid chromatography coupled to tandem mass spectrometry (HILIC-MS/MS). After

the centrifugation step, the sample was lyophilized and sent for analysis to the Centre for Environment, Fisheries, and Aquaculture Science (Cefas). Here, the samples were subjected to semiautomated solid phase (SPE) clean-up using Supelclean ENVI-Carb 250 mg 3 mL^{-1} SPE cartridges (Sigma–Aldrich, St. Louis, MO, United States). Sample extracts were eluted and collected through the addition of 2 mL 20% acetonitrile (MeCN) + 0.25% acetic acid at 3 mL min^{-1}. SPE eluents were vortex-mixed prior to dilution of 100 μL aliquots in 700 μL Verex polypropylene autosampler vials (Phenomenex, Manchester, United Kingdom) with 300 μL MeCN.

HILIC-MS/MS was conducted according to Boundy et al. (2015) using a 1.7 μm, 2.1 mm × 150 mm Waters (Manchester, United Kingdom) Acquity BEH Amide UPLC column in conjunction with a Waters VanGuard BEH Amide guard cartridge; 2 μL were injected into a Waters Acquity UPLC I-Class coupled to a Waters Xevo TQ-S tandem quadrupole mass spectrometer (MS/MS). The HILIC-MS/MS method was calibrated with nine STX analogs with certified reference standards obtained from NRC (C1 and 2, dcGTX2 and 3, GTX2 and 3, GTX1 and 4, GTX5, dcSTX, dcNEO, NEO, STX) plus four reference materials (C3 and 4, GTX6, doSTX, and tetrodotoxin TTX) from Cawthron Natural Compounds (CNC, New Zealand) and CIFGA laboratories (Lugo, Spain). Levels of toxins with no reference standards, such as dcGTX1 and 4 and M-toxins, were semiquantified using the response factor of the nearest structural analog with a certified standard and applying a relative response factor (RRF; Boundy et al., 2015; (1.8 for dcGTX1, 1.93 for dcGTX4, and 1 for M toxins; Turner et al., 2015). In the absence of relative toxicity data for M-toxins, TEFs were taken from structurally similar PSTs analogs (**Table 1**).

Data Analysis

SigmaPlot 11.0 (Systat Software Inc.) was used to fit an exponential decay function to the change of PSTs in time. Also, the software was used to fit a linear model and an exponential rise to a maximal regression model to the PSTs present in the siphon in relation to the concentration of these toxin in the visceral mass.

TABLE 1 | Toxin equivalent factors (TEFs; Oshima, 1995) applied for quantitation and semiquantitation of PST analogs.

Analog	TEF	Analog	TEF	Analog	TEF
C1	0.01	GTX2	0.4	dcNEO	0.4
C2	0.1	GTX3	0.6	STX	1
C3	0.02	GTX1	0.99	NEO	0.9
C4	0.1	GTX4	0.7	M1	0.1[c]
dcGTX2	0.15	GTX5	0.06	M2	0.3
dcGTX3	0.4	GTX6	0.1	M3	0.1[c]
dcGTX1	0.5[a]	doSTX	0.05[b]	M4	0.3[d]
dcGTX4	0.5[a]	dcSTX	0.5	M5	0.1[c]

[a]*dcGTX1 and dcGTX4 based on assumed toxicity equivalency factors (Sullivan et al., 1983).* [b]*doSTX toxicity equivalency factor (Harwood et al., 2014).* [c]*Toxicity factors derived from EFSA GTX5 and GTX6.* [d]*Toxicity factors derived from EFSA 11OH-STX.*

RESULTS

Gymnodinium catenatum Abundance

The phytoplankton community has been monitored by the laboratory FICOTOX intermittently from 2011 in surface water samples collected every two weeks or every month in the *P. globosa* extraction area located south from San Felipe. In general, *G. catenatum* has been present from December to April and surface abundances has been no higher than 10×10^3 cells L^{-1} before 2015 (data not shown). On January 14, 2015, the abundance of this dinoflagellate reached 152×10^3 cells L^{-1} (**Figure 3A**). From this date, surface and vertical net tow water samples were analyzed on a weekly basis. One week after, the maximum abundance was detected the presence of *G. catenatum* decreased to 9.3×10^3 cells L^{-1}. Detection of this species at the water surface continued in the following weeks and a second abundance peak was registered on February 24 (18.5×10^3 cells L^{-1}). After this date, this species disappeared and was detected again in April and May at abundances lower than of 3.8×10^3 cells L^{-1} (**Figure 3A**).

The abundance of *G. catenatum* evaluated in vertical net tow samples clearly showed the presence of the bloom in the area (**Figure 3B**). The relative abundance of this species (abundance of *G. catenatum* to total phytoplankton cells in the net sample) was 55% on January 14 and decreased in the following weeks. The relative abundance increased in February and reached a maximum abundance of 87%. *G. catenatum* was detected in net tow samples until March 10. This species disappeared after this date and was detected again on May 1 with a relative abundance of 62%. *G. catenatum* appeared in net tow samples on May 12 for the last time with relative abundance of 27% (**Figure 3B**).

Toxicity in the Siphon and Visceral Mass of *P. globosa*

The visceral mass (viscera ball) contains the gut, the digestive gland, gonads, heart, kidney, and gills. Although this part of the clams is rarely consumed, the visceral mass is the target tissue to evaluate the concentration of PSTs in geoduck clams. The muscle (siphon) is the part of the organism that is preferentially consumed. PST concentrations together with their transformation and depuration were evaluated in both tissues. Toxin concentrations were determined in 23 siphon and 40 viscera ball samples collected in 2015. Also, toxin concentrations in nine samples collected in 2017 were considered in the analysis of the relation between the siphon and viscera toxicity (see below).

Paralytic shellfish toxins were present in the siphon, although concentrations were lower than the RL of 800 μg STX eq kg^{-1} in all samples (**Figure 3C**). The highest PSTs concentration (429 μg STX eq kg^{-1}) was detected on January 27 and decreased from this day. The lowest concentration was detected after five months (60 μg STX eq kg^{-1}) from the quantification of the maximum PSTs accumulation (**Figure 3C**). Therefore, after July, no more samples of siphon were processed. An exponential decay model was fitted to the data describing the reduction in toxicity level over time. The determination coefficient (R^2) was 0.82 and the

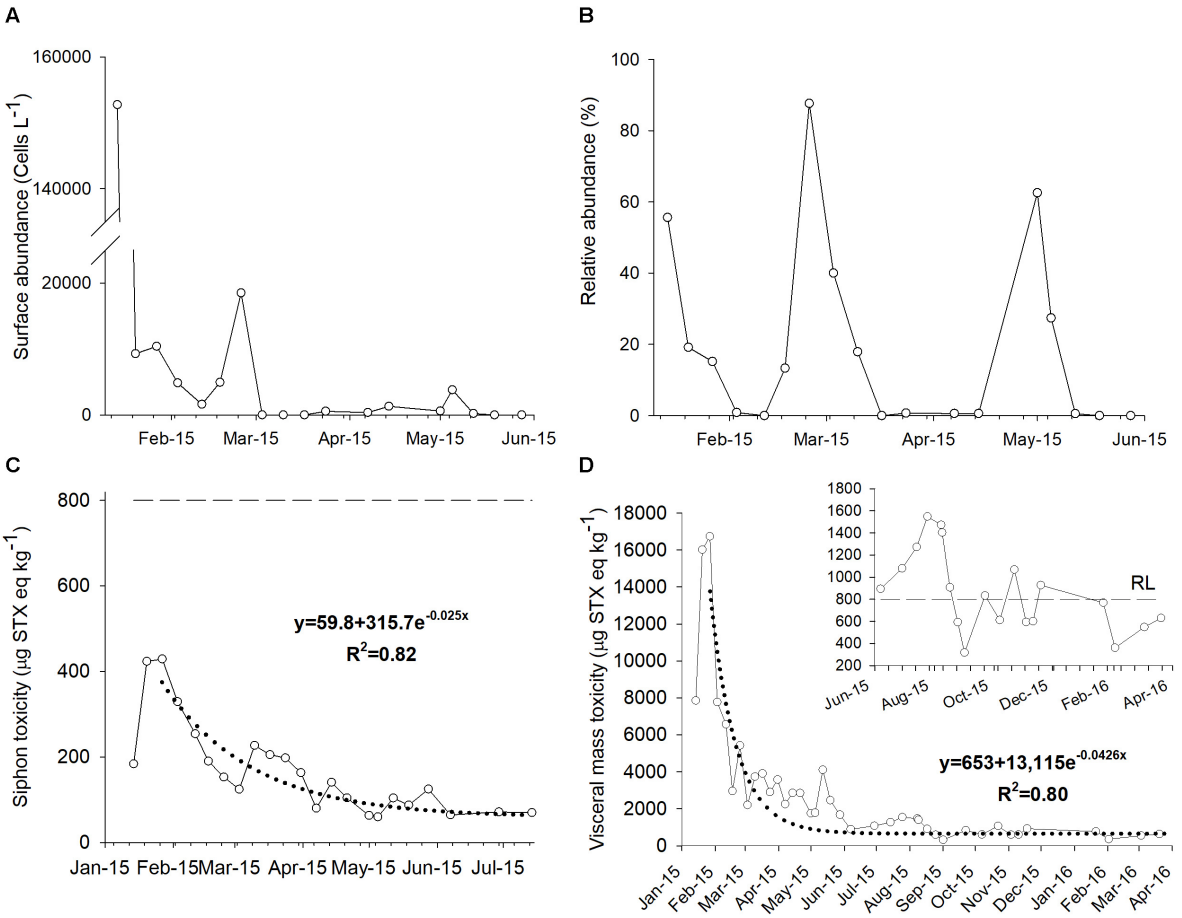


FIGURE 3 | Surface **(A)** and relative abundance **(B)** of *Gymnodinium catenatum* during 2015 in the northern region of the Gulf of California. Toxicity associated with paralytic shellfish toxins in the siphon **(C)** and visceral mass **(D)** of geoduck samples collected from January 2015 to April of 2016. Dotted line shows the fitted of exponential decay function to the data and the dashed line is the regulatory limit (RL) of 800 µg STX eq kg^{-1}.

calculated depurating rate of PSTs in the siphon was 2.5% of toxin loss per day (**Figure 3C**).

Panopea globosa accumulated PSTs mainly in the visceral mass. Maximum PSTs concentration within these tissues reached 16,740 µg STX eq kg^{-1} in January. This concentration represented 21 times the RL (**Figure 3D**). The toxicity was higher than this limit for approximately eight months. A sample with a concentration lower than 800 µg STX eq kg^{-1} was detected on August 25, 210 days after the detection of the maximum concentration. However, the toxicity increased again in the following weeks and fluctuated around the RL during the rest of 2015. The last sample processed for the study was collected in March 2016 and presented a PSTs concentration of 629 µg STX eq kg^{-1}.

An exponential decay model was also fitted to the change of PSTs in viscera over time (R^2 = 0.80). The maximum PSTs concentration detected on January 27 was the first data used in the analysis (**Figure 3D**). The calculated depuration rate was 4.3% of toxin loss per day. According to this, the PSTs concentration should have reached the RL 106 days after the maximum concentration was detected.

Paralytic shellfish toxins in the siphon were related to the concentration of these toxins in the visceral mass. This relation is shown in **Figure 4**. PSTs concentrations detected in viscera and siphon after another HAB that occurred in January 2107 were also considered in this analysis. On January 24 of 2017, *G. catenatum* surface abundance reached 283 × 10^3 cells L^{-1} in the *P. globosa* extraction area. The relative abundance of *G. catenatum* in net tow samples was 87% on this date.

After the 2017 HAB, concentrations above 800 µg STX eq kg^{-1} were detected in three siphon samples (**Figure 4**). An exponential rise to a maximal value function explains 78% (R^2 = 0.78) of the variance between PSTs present in the siphon in relation to the presence of these toxin in the viscera. However, a high PSTs concentration detected in the siphon (1,063 µg STX eq kg^{-1}) clearly influenced the relation and increased R^2 when the exponential function is considered. This concentration in the siphon was detected when viscera presented 49,166 µg STX eq kg^{-1}. Regression analysis considering different models (linear, exponential, polynomial) were performed without considering these high concentrations of PSTs (outlier). PSTs in the siphon were linearly related (best fit) to the concentration detected in

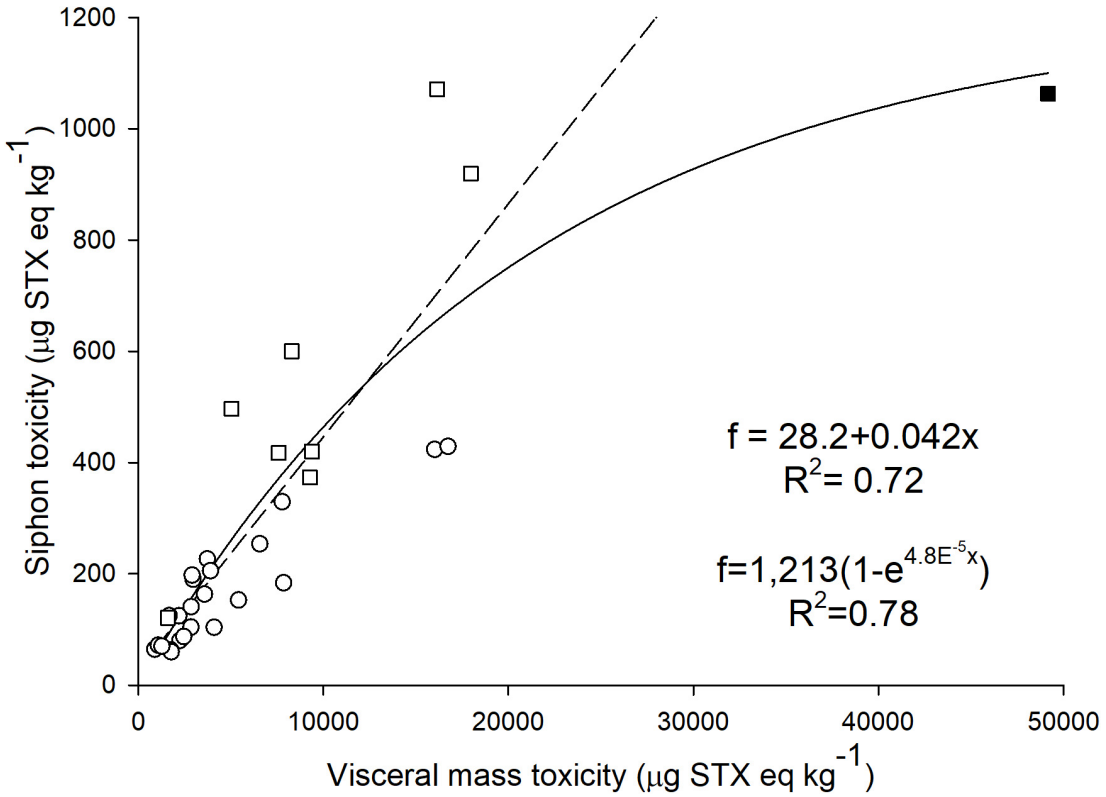


FIGURE 4 | Relation between calculated toxicity of the siphon and visceral mass in geoduck samples collected during 2015 (circles) and 2017 (squares). The filled square shows the highest paralytic shellfish toxin concentration detected in viscera in 2017. This point was omitted (outlier) when a linear regression analysis was applied (R^2 = 0.72, dashed line; see text). An exponential rise to a maximal function was used for the regression analysis considering all data (R^2 = 0.78, solid line).

viscera below 20,000 μg STX eq kg^{-1} (R^2 = 0.72; **Figure 4**). According to this model, toxicity in viscera was 23.8 times higher than the one detected in the siphon. Importantly, the toxicity in the siphon will exceed the RL when toxicity in visceral mass reaches 18,424 μg STX eq kg^{-1}.

Paralytic Shellfish Toxins in the Siphon and Visceral Mass of *P. globosa*

To characterize the accumulation and biotransformation process of PSTs, it is essential to evaluate the analogs present in the samples. Therefore, we evaluated the concentrations of 12 PSTs analogs in both the siphon and visceral mass samples (**Figure 5**). Both tissues contained the major analogs C1 and 2, GTX2 and 3, GTX5, dcGTX2 and 3, and dcSTX. We detected trace amounts of GTX4, Neo, and STX in some viscera samples, while STX and Neo were not detected in any of the siphon samples.

The variation in the molar concentration of each toxin and its contribution to total toxicity in the siphon and viscera during the monitoring period is shown in **Figure 5**. In the siphon, the molar content was 16 nmol g^{-1} in February and decreased to 1 nmol g^{-1} in June (**Figure 5A**). C1 and 2 were the prevalent toxins in all the samples throughout the monitoring period. These analogs represented 95% of the total PSTs content in the siphon samples during February and decreased to 28% at the end of the sampling period (**Figure 5C**). The reduction of C1 and 2 was accompanied with an increase of GTX5, dcGTX2, and dcSTX to a maximum combined relative concentration of 60% (0.6 nmol g^{-1}; **Figure 5C**). The contribution to total toxicity in terms of STX equivalents of each analog is shown in **Figure 5E**. Type C toxins contributed up to 73% to the toxicity in January. With the reduction of C1and2 in the siphon, a significant increase of dcSTX contribution to total toxicity was detected at the end of the sampling period (68% in June, **Figure 5E**).

Toxin content in the visceral mass was 457 nmol g^{-1} in January 2015 and decreased to 3.6 nmol g^{-1} in February 2016 (**Figure 5B**). Similar to the siphon samples, C1 and 2 were the dominant analogs (**Figure 5D**) in February 2015 since they represented 90% of the molar content (384 nmol g^{-1}). In contrast with the siphon, there was a sharp reduction of the concentration of these toxins with time in viscera (**Figure 5D**). However, an increase of C toxins with respect to previous concentrations was detected two times in May. PSTs molar concentration of C toxins increased from 18% on April 24 to 33% on May 1 and from 22% on May 12 to 44% on May 19 (**Figure 5D**). The reduction of C toxins was accompanied with the appearance of GTX5 and the more potent toxins, dcSTX and dcGTX2 (**Figure 5F**). After February, dcSTX was the analog that contributed the most to total toxicity. The highest dcSTX concentration (338 nmol g^{-1}) was detected on

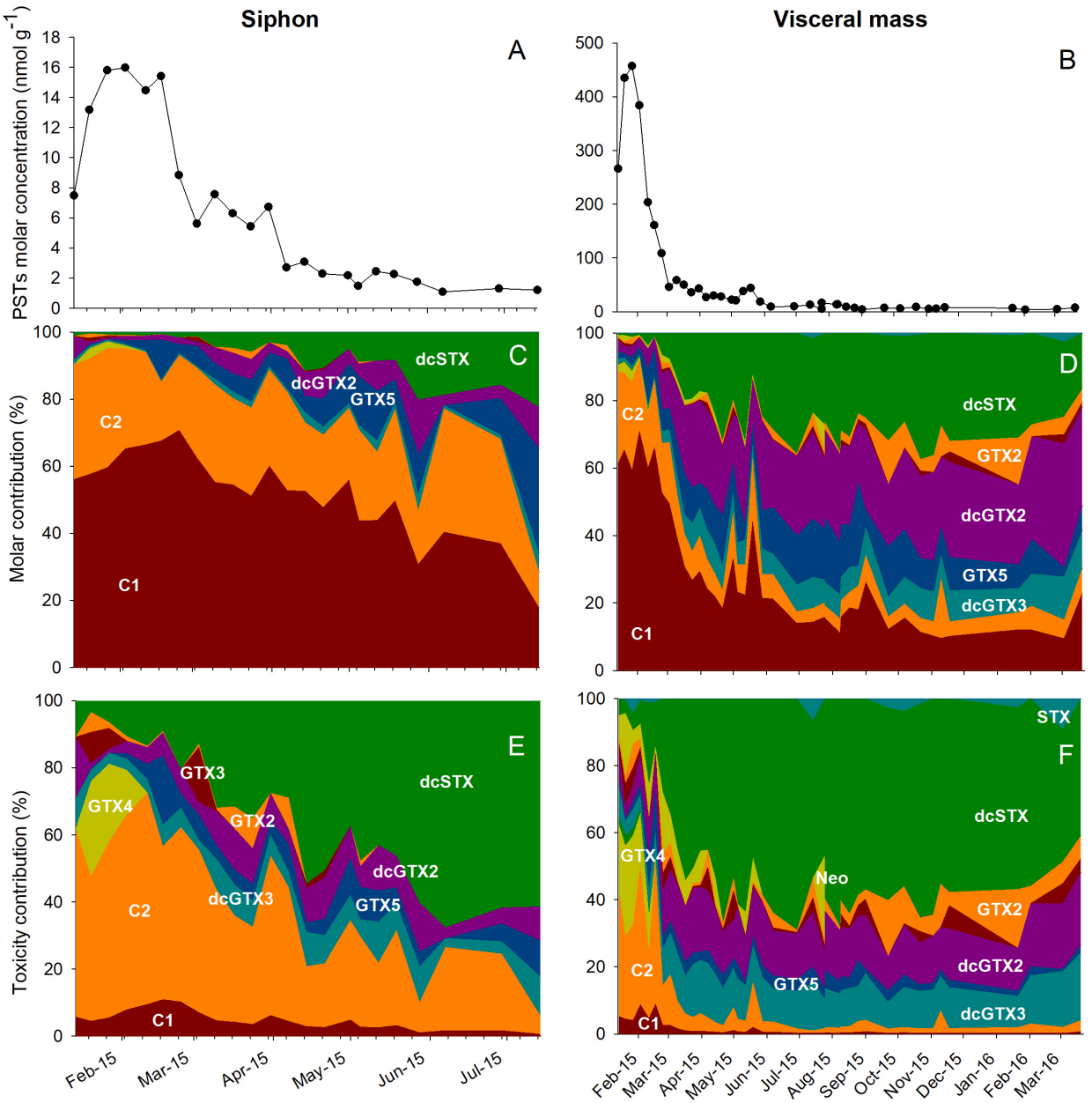


FIGURE 5 | Paralytic toxins in the siphon **(A,C,E)** and in the visceral mass **(B,D,F)** of geoduck samples collected from January 2015 to April of 2016. The paralytic toxins molar concentration (nmol g^{-1}) is presented in **A** and **B**. The molar contribution (in %, **C,D**) and toxicity contribution (in %, **E,F**) of different paralytic toxins analogs detected during the sampling period is also shown. The toxicity of each analog was calculated according to the toxin equivalent factors shown in **Table 1**.

May 12 and represented 63% of the 2,610 μg STX eq kg^{-1} calculated on this date. At the end of the monitoring period, low STX concentrations were detected (maximum concentration of 1.2 nmol g^{-1}) that represented the 9% of the toxicity calculated in March 2016 (50 μg STX eq kg^{-1}).

There is a limitation on the detection and quantification of PSTs analogs by HPLC-PCOX. The detection of PSTs by this method was only directed to the commercially toxin certified reference materials available when the method was developed. Therefore, to evaluate the presence of other analogs, 10 selected viscera samples were also analyzed by HILIC-MS/MS. This methodology allowed the detection of 12 other analogs not detected by HPLC-PCOX, including type M toxins. The five M analogs described so far were all detected in the tissue samples. M5 was the most abundant M-toxin, followed by M1 and M3. M-toxins represented between 30% (230 nmol g^{-1}, January 20) and 75% (28 nmol g^{-1}, June 29) of total PSTs detected by HILIC-MS/MS (**Table 2**). There was not a clear pattern in time of the contribution of M-toxins to total PSTs. Considering the TEFs shown in **Table 1**, the concentration of these toxins represented

TABLE 2 | Contribution of M-toxins to molar concentration (nmol g^{-1}) of PST and total toxicity (μg STXeq kg^{-1}) calculated by HILIC-MS/MS analysis in geoduck visceral mass samples.

Sample date	nmol g^{-1}		Toxicity (μg STXeq kg^{-1})	
	Total	M-toxins	Total	M-toxins
1/14/2015	497.7	131.6 (52.9%)	1,8633.7	10, 970.8 (58.9%)
1/20/2015	768.9	118 (30.7%)	29,448.9	9, 842.4 (33.4%)
1/27/2015	1,086.9	241.4 (44.4%)	41,027	20, 119 (49%)
1/17/2015	154.6	34.6 (44.8%)	6,026.6	2, 884.1 (47.9%)
3/17/2015	58.1	15.3 (52.8%)	3236.5	1, 279.6 (39.5%)
5/1/2015	45.9	13.2 (57.7%)	3844.7	1, 103.6 (28.7%)
6/29/2015	37.8	14.1 (74.8%)	2450.2	1, 179 (48.1%)
8/17/2015	51.6	13.3 (52.1%)	2813.1	1, 109.6 (39.4%)
8/25/2015	11.2	3.3 (58.5%)	885.9	274.7 (31%)
11/10/2015	12.0	2.8 (45.9%)	1023.7	230.5 (22.5%)

9,718 μg STXeq kg^{-1} on January 20 (33% of total toxicity) and 1,838 μg STXeq kg^{-1} on June 29 (48% of total toxicity, **Table 2**).

DISCUSSION

Here, we report PSTs profiles in the viscera and the siphon tissues of *P. globosa*. This is the first characterization of the transformation and depuration of different analogs in a geoduck clam. The accumulation of PSTs in *P. globosa* was associated with a HAB of *G. catenatum* that was present in the NGC during the first months of 2015. HABs of *G. catenatum* have been reported in different areas of the GC (Band-Schmidt et al., 2010), but they have not been registered in the NGC since there was not a continuous monitoring program in the region. Monitoring of PSTs in organisms extracted from areas located south from San Felipe initiated in 2010 and the first sanitary ban for the extraction of *P. globosa* was implemented in the same year. As a consequence, FICOTOX research laboratory has been monitoring the phytoplankton community in the area since 2011. We have documented that *G. catenatum* is a conspicuous species of the phytoplankton community from December to April with abundances lower than 10×10^3 cells L^{-1} (Murillo-Marínez, 2011). However, in 2015, the abundance reached 152×10^3 cells L^{-1} in samples collected at the surface of the water column. The presence of *G. catenatum* was related to maximum toxicity of 16,740 μg STXeq kg^{-1} (27 the RL) in viscera of *P. globosa*. In 2017, another bloom was registered in the region and maximum registered abundance of the dinoflagellate (283×10^3 cells L^{-1} in surface water samples) and associated accumulation of PSTs (49,166 μg STX eq kg^{-1} in viscera samples) in *P. globosa* increased importantly.

Although the relationship between the two variables is complex, the abundance of *G. catenatum* seems not particularly high when considering the PSTs concentrations and total sample toxicity quantified in the clams. Higher abundances of this species have been reported during HABs in other regions. In the GC, for example, *G. catenatum* blooms have been associated with wild and cultured animals die-offs, economical loses (Núñez-Vázquez et al., 2011) and caused human intoxications and fatalities (Morey-Gaines, 1982; De la Garza, 1983; Mee et al., 1985; Band-Schmidt et al., 2010). Abundances registered during these HABs were in the order of 1×10^6 cells L^{-1} (Morey-Gaines, 1982; De la Garza, 1983; Mee et al., 1985; Band-Schmidt et al., 2010). *G. catenatum* has also been reported in high abundances in others parts of the world. In Spain, abundances of 2×10^6 cells L^{-1} were reported in 1986 causing the accumulation of up to 26,400 μg STX eq kg^{-1} in mussels (Anderson et al., 1989). In Portugal, the dinoflagellate has occurred since 1986, with blooms in autumn of 1986, 1990, 1992, 1994, 1995, and annually from 2005 to 2012 (Vale et al., 2008; Silva et al., 2015) with abundances as high as 22×10^4 cells L^{-1} in 2007 (Rodrigues et al., 2012). Intermittent and multiannual scale blooms of *G. catenatum* are devastating to mussel aquaculture production in Galicia and to the harvesting of natural shellfish banks on the Galician and Portuguese coasts. Toxin levels of up to 60,000 μg STX eq. kg^{-1} in mussel meat were reported in 2005 and 2010 (Pazos et al., 2006; Vale et al., 2008), leading to prolonged harvesting closures lasting until the following spring (Trainer et al., 2010). *G. catenatum* surface abundance did not reflect the magnitude of the HAB event in the NGC since this species can be distributed subsuperficially. The high relative abundances detected in net tow samples of the NGC in this study indicate this subsurface distribution.

The high levels of toxin accumulation and the time required for toxin depuration in the clams was related to the long duration of the HAB. The presence of *G. catenatum*, in January, part of February and from March to May caused that PSTs in viscera remained above the RL for almost all 2015. The characterization of PSTs concentration changes in terms of STX eq is essential for regulation purposes. The calculated depuration rate of *P. globosa* was 4.3% of STX eq loss per day. Therefore, after the accumulation of 16,740 μg STX eq kg^{-1} in viscera, total PSTs should have reached the RL 106 days after this maximum level of toxicity. However, the RL was reached 104 days later than the date estimated by the exponential decay model. PSTs concentrations were found to decrease in time but there were periods when the toxicity increased compared to previous measurements (**Figures 3C,D**). This increase in toxicity was related to the increase in the abundance of *G. catenatum* in the water column that represented a continuous source of PSTs for geoduck clams. This consequently represents a bias in the calculation of the depuration rate in *P. globosa*. However, even with an under-estimation of the rate, it is evident that this species is a low depuration organism since it accumulates toxins for several months in their visceral tissues. Bricelj and Shumway (1998) classified bivalves that need several months to years to detoxify below the RL and present detoxification rates between 0.1 and 4% of STX eq loss per day, as a low depuration species. In contrast, a fast depurator bivalve has an elimination rate between 6 and 17% of toxin loss per day, and reach the RL between 1 and 10 weeks, depending on the amount of toxin assimilated (Bricelj and Shumway, 1998). *Panopea* species have low depurating physiology since *P. generosa* was also found to retain toxicity for long periods of time (Curtis et al., 2000). Another low depuration clam species is *Saxidomus giganteus* with an average

of 0.7% of STX eq loss per day in the whole tissues (Quayle, 1969; Madenwald, 1985; Price et al., 1991), *Spisula solidissima* depurates at 1.9%, measured in whole animals (Shumway et al., 1988), *Placopecten magellanicus* at 0.6% when pools of digestive tissues, gills, and mantle are measured (Shumway et al., 1988), and *Patinopecten yessoensis* with an average of 2.6% in digestive tissues (Nishihama, 1980; Ogata et al., 1982; Tazawa et al., 1988).

C1 and C2 were the most abundant analogs in the siphon and viscera samples collected close to the HAB occurrence. These are the main analogs present in *G. catenatum* and represented 95% of the PSTs detected in phytoplankton samples during the bloom, dcGTX3, dcNeo, and GTX2 and 3 comprised the remaining 5%, (Bustillos-Guzmán et al., 2016; García-Mendoza et al., in preparation). Following the incorporation of toxins into the clam tissues through feeding activities, toxins transformation occurred principally in the viscera, where the low potency toxins C1 and 2 were converted into GTX5 and the more toxic analogs dcGTX2 and dcSTX. The presence of high toxic analogs and the increase in its relative contribution to the PSTs content in viscera caused that the toxicities remained above the RL for several months. Since there is a high number of analogs and different reactions involved in their transformation (Cembella et al., 1994; Oshima, 1995; Guéguen et al., 2011; Turner et al., 2012b, 2013), it is difficult to describe all the biotransformation steps and conversion rates that occurred in *P. globosa* after toxin bioaccumulation. However, we identified the principal biotransformation reactions in this species (**Figure 6**). Epimerization occurred between the epimeric pairs C1 and 2 and dcGTX2 and 3. C1 was then converted into GTX5 by desulfuration in R3 position or into dcGTX2 by desulfo-carboxylation in R4. C1 can also be transformed into dcSTX if both reactions occur in parallel. However, according to the time of appearance of PSTs analogs in *P. globosa*, it is more likely that formation of GTX5 is an intermediate step for the formation of dcSTX by desulfo-carboxylation in the R4 position. These biotransformation reactions explain the observation of the reduction of C1 and 2 which was accompanied with an increase in GTX5, dcGTX2, and dcSTX to a maximum combined relative concentration of 59%. Even when enzymatic conversion of PSTs analogs to decarbamoyl (dc) derivatives is uncommon among bivalves (Bricelj and Shumway, 1998) and has been only demonstrated in the Pacific clam *Protothaca staminea* (Sullivan et al., 1983), the surf clam *Spisula solida* (Turner et al., 2013) and

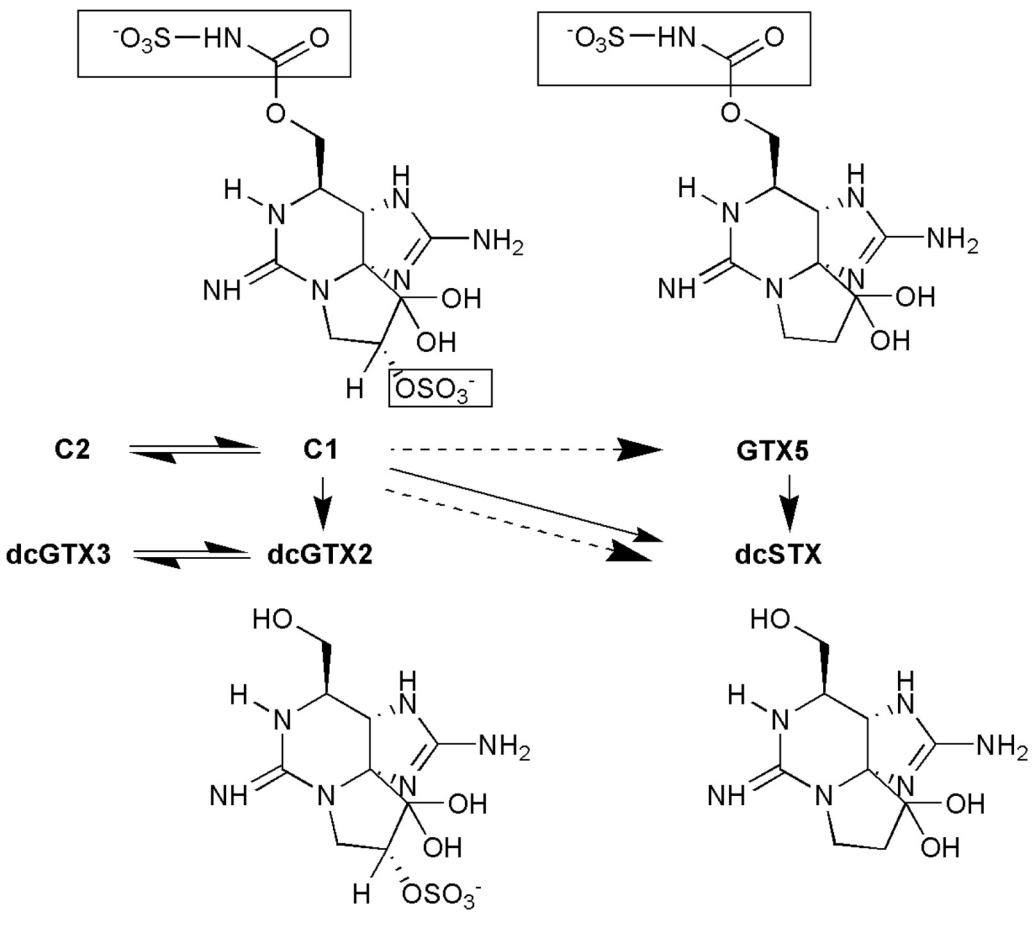


FIGURE 6 | Diagram of assumed biotransformations that occurred in viscera of geoduck *Panopea globosa* contaminated with *Gymnodinium catenatum* paralytic shellfish toxins. The part of the molecule that is modified to become in another analog is highlighted in a square. Solid arrow: desulfo-carboxylation, dashed arrow: desulfurization, double arrow: epimerization.

in the Japanese clams *Mactra chinensis* and *Peronida venulosa* (Oshima, 1995), we identified in this study the production of dcGTX2 and 3 and dcSTX by N-sulfocarbamoyl toxins hydrolysis.

The characterization of the PSTs conversion steps and conversion rates becomes more complicated if the M-toxins are considered. Five M analogs were present in *P. globosa* tissues and represented a significant proportion (between 30–75%) of the PSTs detected in the samples. This is the first time that M-toxins are reported in geoduck clams. These analogs were described in 2008 in blue mussels (*Mytilus edulis* and *Mytilus trossulus*) from eastern Canada during a HAB of *Alexandrium tamarense* (Dell'Aversano et al., 2008). M-toxins are most probably metabolites or degradation products formed in shellfish since they have not been detected in microalgae (Dell'Aversano et al., 2008). Five M-toxins analogs have been described. Vale (2010) found that M1 is the most abundant in species that retain PSTs toxins for longer periods. M1 toxin has been reported in mussels (*Mytilus galloprovinciallis*), cockles (*Cerastoderma edule*), and clams from estuarine (*Ruditapes decussatus*) and oceanic habitats (*Donax trunculus* and *Ensis* spp.) collected in Portugal during a HAB of *G. catenatum* (Vale, 2010). M1 could contribute to an important fraction of the PSTs profile (up to 70% of total GTX5) and has been proposed that it is originated from the metabolism of GTX5 (Vale, 2010). In the case of *P. globosa*, M5 was the most abundant M-toxin. Probably, there is a rapid transformation of some other M-toxins into M5 in this species. Metabolism and biotransformation of this type of toxins has not yet been elucidated.

The results of the present work have important implications for both regulation and management. Evaluation of the phytoplankton community in the NGC should be performed more intensively during winter to monitor the appearance of harmful species. The presence of *G. catenatum* should be evaluated not only in surface samples but also in water samples throughout appropriate depths within the water column, or vertical net hauls, tube integrated or hose integrated samples must be analyzed. Risk abundance indexes should also be developed to evaluate the possible accumulation of PSTs in geoduck clams above the RL.

Panopea globosa accumulated approximately 24 times more toxins in the viscera than in the siphon. Curtis et al. (2000) reported that accumulation of PSTs in viscera of *P. generosa* was 30 times higher than in the siphon. At the beginning of the fishery in the 1970s, PSTs were not considered by the Washington State Department of Health as a public health risk because the geoduck viscera were presumed to be discarded. However, it was later discovered that some members of a Native American tribe and of Asian immigrant communities in the United States consumed the viscera in soup (Curtis et al., 2000). Therefore, the sanitary programs of United States included the viscera as the target tissue for PSTs analyzes. This approach was adopted recently in Mexico to be in accordance with the United States regulation (Secretaría de Salud, 2016). The visceral ball is easily separated from the siphon and mantle without risk of contamination. Consequently, it is considered that consumption of the siphon is safe since muscle tissue does not accumulate high concentration of PSTs (Curtis et al., 2000). Geoduck Clam Biotoxin Monitoring Plan of Alaska (Alaska Department of Environmental Conservation, 2017) considers evisceration and use of the siphon in some cases when unacceptable levels of PSTs are detected in viscera. We found that the accumulation of toxins in muscular tissue was proportional to the concentration of PSTs in the viscera. This indicates that a fix proportion of the toxins present in the viscera are translocated to the muscle. The siphon will exceed the RL when visceral mass accumulates 18,424 μg STX eq kg^{-1}. More data with high PSTs concentrations in the siphon are needed to describe a more robust statistical relation, but it is clear that this tissue can present toxicity above the RL, as actually occurred in the HAB of 2017. In this year, PSTs concentrations in viscera were almost three times higher than in 2015 and the PSTs concentration in the siphon exceeded the RL on three dates. These results have important regulatory implications since the siphon could present a concentration above the RL if there is a high accumulation of PSTs in viscera.

The mouse bioassay (MBA; Anon, 2005) was the only approved method for PSTs detection in shellfish for regulatory purposes. Recently, European Community, Canada (Turner et al., 2012a), and United States (U.S. Food and Drug Administration, 2015) sanitary legislations included HPLC-based methodologies as alternative methods to monitor these toxins. The presence of M-type toxins in shellfish represents a monitoring challenge since the MBA will be eliminated from sanitary monitoring programs. There are no certified reference standards of these toxins and its quantification and toxicity estimation have been performed based on structure-activity relations with other saxitoxin analogs (Dell'Aversano et al., 2008). In the case of *P. globosa*, we estimated that M toxins represented up to 49% of calculated total toxicity in some samples. Furthermore, the contribution of M-toxins to total toxicity was not dependent on the depuration process in the clam. The relative concentration of these analogs did not vary with time after the clams were exposed to *G. catenatum*. Importantly, *P. globosa* represents a potential source of these toxins for isolation due to high concentrations found in viscera. The results obtained in the present work are important to elucidate biotransformation pathways in the metabolism of PSTs in an economically important bivalve. In addition, they are important to develop management plans for the *P. globosa* fishery since PST producing blooms have become recurrent (last documented bloom occurred in January 2018) in the last years in the NGC.

AUTHOR CONTRIBUTIONS

JM-E processed geoduck samples for PSTs determination, analyzed and interpreted the data, checked the data for accuracy and integrity, and wrote the manuscript. EG-M interpreted and reviewed the data for accuracy and integrity, wrote and edited the manuscript. AT performed HILIC-MS/MS analysis, revised and corrected the manuscript. YS-B and RM-M performed phytoplankton analysis and interpreted the associated data.

FUNDING

This work was supported by CONACyT scholarship 242882 – CV 353239; FORDECYT – CONACyT project number 260040-2015; Red Temática sobre Florecimientos Algales Nocivos, CONACyT (RedFAN), 2015–2017 projects; and Cefas, Seedcorn internal funding (contract code DP345).

ACKNOWLEDGMENTS

We thank Atenea en el Mar company for providing geoduck and water samples and Dr. Juan Blanco for the guide and invaluable comments that helped to improve the work. We also thank Dr. Marcela Ovalle Marroquin and Dr. David Rivas for the review of the final draft of the manuscript.

REFERENCES

Alaska Department of Environmental Conservation (2017). *Geoduck Clam Biotoxin Monitoring Plan. Division of Environmental Health - Food Safety and Sanitation Program*. Alaska: Alaska Department of Environmental Conservation, 7.

Andersen, P., and Throndsen, J. (2004). "Estimating cell numbers," in *Manual on Harmful Marine Microalgae*, eds G. M. Hallegraeff, D. M. Anderson, and A. D. Cembella (Paris: UNESCO), 99–130.

Anderson, D. M., Sullivan, J. J., and Reguera, B. (1989). Paralytic shellfish poisoning in northwest Spain: the toxicity of the dinoflagellate *Gymnodinuim catenatum*. *Toxicon* 27, 665–674. doi: 10.1016/0041-0101(89)90017-2

Anon, S. (2005). "AOAC official method 959.08. Paralytic Shellfish Poison. Biological method. Final action," in *AOAC Official Methods for Analysis, Chapter 49: Natural Toxins*, 18th Edn, ed. M. W. Truckses (Gaithersburg, MD: AOAC International), 79–80.

Aragón-Noriega, E. A., Alcántara-Razo, E., Calderon-Aguilera, L. E., and Sánchez-Fourcade, R. (2012). Status of geoduck clam fisheries in Mexico. *J. Shellfish Res.* 31, 733–738. doi: 10.2983/035.031.0317

Band-Schmidt, C. J., Bustillos-Guzmán, J. J., Gárate-Lizárraga, I., López-Cortés, D. J., Núñez-Vázquez, E. J., and Hernández-Sandoval, F. E. (2010). Ecological and physiological studies of *Gymnodinium catenatum* in the Mexican Pacific: a review. *Mar. Drugs* 8, 1935–1961. doi: 10.3390/md8061935

Botana, L. M. (2000). *Seafood and Freshwater Toxins: Pharmacology, Physiology and Detection*. New York, NY: CRC Press, 816.

Boundy, M. J., Selwood, A. I., Harwood, D. T., McNabb, P. S., and Turner, A. D. (2015). Development of a sensitive and selective liquid chromatography–mass spectrometry method for high throughput analysis of paralytic shellfish toxins using graphitised carbon solid phase extraction *J. Chromatogr. A* 1387, 1–12. doi: 10.1016/j.chroma.2015.01.086

Bricelj, V. M., Connell, L., Konoki, K., MacQuarrie, S. P., Scheuer, T., Catterall, W. A., et al. (2005). Na+ channel mutation leading to saxitoxin resistance in clams increases risk of PSP. *Nature* 434, 763–767. doi: 10.1038/nature03415

Bricelj, V. M., and Shumway, S. E. (1998). paralytic shellfish toxins in bivalve molluscs: occurrence, transfer kinetics, and biotransformation. *Rev. Fish. Sci.* 6, 315–383. doi: 10.1080/10641269891314294

Bureau, D., Rajas, W., Surry, N. W., Rand, C. M., Dovey, G., and Campbell, A. (2002). Age, size structure and growth parameters of geoducks (*Panopea abrupta*, Conrad 1849) from 34 locations in British Columbia sampled between 1993 and 2000. *Can. Tech. Rep. Fish. Aquat. Sci.* 2413:84.

Bustillos-Guzmán, J. J., Leyva-Valencia, I., Hernández-Sandoval, F. E., Band-Schmidt, C. J., López-Cortés, D. J., and Núñez-Vázquez, E. J. (2016). "Ficotoxinas en aguas del Golfo de California: una revisión," in *Florecimientos Algales Nocivos en México*, eds E. García-Mendoza, S. I. Quijano-Scheggia, A. Olivos-Ortíz, and E. J. Núñez-Vazquez (Ensenada: CICESE), 162–179.

Cembella, A. D., Shumway, S. E., and Larocque, R. (1994). Sequestering and putative biotransformation of paralytic shellfish toxins by the sea scallop *Placopecten magellanicus*: seasonal and spatial scales in natural populations. *J. Exp. Mar. Biol. Ecol.* 180, 1–22. doi: 10.1016/0022-0981(94)90075-2

Cestèle, S., and Catterall, W. A. (2000). Molecular mechanisms of neurotoxin action on voltage-gated sodium channels. *Biochimie* 82, 883–892. doi: 10.1016/S0300-9084(00)01174-3

Comisión Federal para la Protección contra Riesgos Sanitarios (2015). *Presencia de marea roja en México durante 2015. Secretaría de Salud*. Available at: http://www.cofepris.gob.mx/ [accessed November 25, 2016].

Cudney-Bueno, R., Lavín, M. F., Marinone, S. G., Raimondi, P. T., and Shaw, W. (2009). Rapid effects of marine reserves via larval dispersal. *PLoS One* 4:e4140. doi: 10.1371/journal.pone.0004140

Curtis, K. M., Trainer, V. L., and Shumway, S. E. (2000). Paralytic shellfish toxins in geoduck clams (*Panopea abupta*): variability, anatomical distribution and comparison of two toxin detection methods. *J. Shellfish Res.* 19, 313–319. doi: 10.1080/10641269891314294

De la Garza, A. J. (1983). Intoxicación alimentaria por ingestión de mariscos contaminados. *Sal. Pub. Mex.* 25, 145–150. doi: 10.3305/nh.2015.32.2.9205

Dell'Aversano, C., Walter, J. A., Burton, I. W., Stirling, D. J., Fattorusso, E., and Quilliam, M. A. (2008). Isolation and structure elucidation of new and unusual saxitoxin analogues from mussels. *J. Nat. Prod.* 71, 1518–1523. doi: 10.1021/np800066r

Espinosa-Carreón, T. L., and Valdéz-Olguín, J. E. (2007). Variabilidad interanual de clorofila en el Golfo de California. *Ecol. Apl.* 6, 83–92.

Gárate-Lizárraga, I., Bustillos-Guzmán, J. J., Alonso-Rodríguez, R., and Luckas, B. (2004). Comparative paralytic shellfish toxin profiles in two marine bivalves during outbreaks of *Gymnodinium catenatum* (Dinophyceae) in the Gulf of California. *Mar. Poll. Bull.* 48, 378–402. doi: 10.1016/j.marpolbul.2003.10.032

Gárate-Lizárraga, I., Bustillos-Guzmán, J. J., López-Cortés, D. J., Hernández-Sandoval, F., Erler, K., and Luckas, B. (2006). Paralytic shellfish toxin profiles in net phytoplankton samples from Bahía Concepción, Gulf of California, Mexico. *Mar. Poll. Bull.* 52, 800–815. doi: 10.1016/j.marpolbul.2006.03.003

Goodwin, C. L., and Pease, B. C. (1987). *The distribution of geoduck (Panopea abrupta) size, density and quality in relation to habitat characteristics such as geographic area, water depth, sediment type and associated flora and fauna in Puget Sound, Washington*. Technical Report no. 102. Olympia, WA: Washington Department of Fisheries.

Guéguen, M., Baron, R., Bardouil, M., Truquet, P., Haberkorn, H., Lassus, P., et al. (2011). Modelling of paralytic shellfish toxin biotransformations in the course of *Crassostrea gigas* detoxification kinetics. *Ecol. Model.* 222, 3394–3402. doi: 10.1016/j.ecolmodel.2011.07.007

Gutiérrez, O. Q., Marinone, S. G., and Parés-Sierra, A. (2004). Lagrangian surface circulation in the Gulf of California from a 3D numerical model. *Deep Sea Res.* 51, 659–672. doi: 10.1016/j.dsr2.2004.05.016

Harwood, D. T., Selwood, A. I., Van Ginkel, R., Waugh, C., McNabb, P. S., Munday, R., et al. (2014). Paralytic shellfish toxins, including deoxydecarbamoyl-STX, in wild-caught Tasmanian abalone (*Haliotis rubra*). *Toxicon* 90, 213–225. doi: 10.1016/j.toxicon.2014.08.058

Jaime, E., Gerdts, G., and Luckas, B. (2007). In vitro transformation of PSP toxins by different shellfish tissues. *Harmful Algae* 6, 308–316. doi: 10.1016/j.hal.2006.04.003

Lewitus, A. J., Horner, R. A., Caron, D. A., García-Mendoza, E., Hickey, B. M., Hunter, M., et al. (2012). Harmful algal blooms along the North American west coast region: history, trends, causes, and impacts. *Harmful Algae* 19, 133–159. doi: 10.1016/j.hal.2012.06.009

Madenwald, N. D. (1985). "Effect of water temperature on the loss of paralytic shellfish poison from the butter clam *Saxidomus giganteus*," in *Toxic Dinoflagellates*, eds D. M. Anderson, A. W. White, and D. G. Baden (New York: Elsevier-North Holland), 479–484.

Manrique, F. A., and Molina, R. E. (1997). Presencia de mareas rojas en la Bahía de Bacochibampo, Guaymas, Sonora, México. *Hidrobiológica* 7, 81–86.

Mee, L. D., Espinosa, M., and Díaz, G. (1986). Paralytic shellfish poisoning with a *Gymnodinium catenatum* red tide on the Pacific coast of Mexico. *Mar. Environ. Res.* 19, 77–92.

Mee, L. D., Ramírez-Flores, A., Flores-Verdugo, F., and González-Frías, F. (1985). Coastal upwelling and fertility of the Southern Gulf of California: impact of the 1982–83 ENSO event. *Trop. Ocean Atmos. Newsl.* 31, 9–10.

Morey-Gaines, G. (1982). *Gymnodinium catenatum* Graham (Dinophyceae): morphology and affinities with armoured forms. *Phycologia* 21, 154–163.

Murillo-Marínez, R. (2011). *Caracterización de Fitoplancton Tóxico y Acumulación de Toxinas de tipo Paralizante en la Almeja Panopea globosa (Dall 1898) en la Franja Costera de San Felipe-Puertecitos, B. C.* Bachelor's degree thesis, Facultad de Ciencias de la Universidad Autónoma de Baja California, Ensenada.

Nishihama, Y. (1980). *Seasonal abundance of Protogonyaulax sp. causing paralytic shellfish poisoning in Funka Bay, Hokkaido, Japan,* 1978–1980. Anchorage: Proc. North Pacific Aquaculture Symp, 319–372.

Núñez-Vázquez, E. J., Gárate Lizárraga, I., Band-Schmidt, C. J., Cordero-Tapia, A., Hernández -Sandoval, F. E., and Heredia-Tapia, A. (2011). Impact of harmful algal blooms on wild and cultured animals in the Gulf of California. *J. Environ. Biol.* 32, 413–423.

Ogata, T., Kodama, M., Fukuyo, Y., Inoue, T., Kamiya, H., Matsuura, F., et al. (1982). The occurrence of *Protogonyaulax* spp. in Ofunato Bay, in association with the toxification of the scallop *Patinopecten yessoensis*. *Bull. Jpn. Soc. Sci. Fish*. 48, 563–566.

Oshima, Y. (1995). "Chemical and enzymatic transformation of paralytic shellfish toxins in marine organisms," in *Harmful Marine Algal Blooms*, eds P. Lassus, G. Arzul, E. Erard-Le Denn, P. Gentien, and C. Marcaillou-Le Baut (Paris: Lavoisier Publishing ILP), 475–480.

Pazos, Y., Moroño, A., Triñanes, J., Doval, M., Montero, P., Vilarinho, M. G., et al. (2006). "Early detection and intensive monitoring during an unusual toxic bloom of *Gymnodinium catenatum* advected into the Galician Rías (NW Spain," in *Proceedings of the 12th International Conference on Harmful Algae*, Copenhagen,

Price, D. W., Kizer, K. W., and Hansgen, K. H. (1991). California's paralytic shellfish poisoning prevention program, 1927–89. *J. Shellfish Res.* 10, 119–145.

Quayle, D. (1969). *Paralytic shellfish poisoning in British Columbia.* Nanaimo: Fisheries Research Board of Canada Biological Station, 68.

Ramírez-Camarena, C., Martínez-García, A., Juárez-Ruíz, N., Rojas-Crisóstomo, R., and Rámírez-García, H. (2004). "Impactos de Pyrodinium bahamense var. compressum durante el florecimiento algal nocivo 2001-2002, en la Costa Suroeste de México," in *Proceedings of the VI International Meeting of Planktology: XIII Reunión Nacional SOMPAC*, (Nayarit: ITMar Núm), 62.

Rodrigues, S. M., De Carvalho, M., Mestre, T., Ferreira, J. J., Coelho, M., Peralta, R., et al. (2012). Paralytic shellfish poisoning due to ingestion of *Gymnodinium catenatum* contaminated cockles – Application of the AOAC HPLC official method. *Toxicon* 59, 558–566. doi: 10.1016/j.toxicon.2012.01.004

Saldate-Castañeda, O., Vázquez-Castellanos, J. L., Galván, J., Sánches-Anguiano, A., and Nazar, A. (1991). Poisoning from paralytic shellfish toxins in Oaxaca. *México Sal. Pub. Mex.* 33, 240–247.

Santiago-Morales, S. I. (2016). "Florecimientos algales nocivos en la costa de Oaxaca," in *Florecimientos Algales Nocivos en México*, eds E. García-Mendoza, S. I. Quijano-Scheggia, A. Olivos-Ortíz, and E. J. Núñez-Vazquez (Ensenada: CICESE), 242–255.

Secretaría de Pesca de Baja California (2016). *Carta Estatal Pesquera de Baja California 2000-2015. Secretaría de pesca.* Available at: http://www.sepescabc.gob.mx/x/salaDePrensa/difusionAcciones/docs/CartaEstatalPesquera.pdf [accessed January 15, 2018].

Secretaría de Salud. (2016). *Guía para el laboratorio de análisis de agua de mar y moluscos bivalvos. Programa Mexicano de Sanidad de Moluscos Bivalvos.* México: Secretaría de Salud, 94.

Shumway, S. E., Sherman-Caswell, S., and Hurst, J. W. Jr. (1988). Paralytic shellfish poisoning in Maine: monitoring a monster. *J. Shellfish Res.* 7, 643–652.

Silva, T., Caeiro, M. F., Reis Costa, P., and Amorim, A. (2015). *Gymnodinium catenatum* Graham isolated from the Portuguese coast: toxin content and genetic characterization. *Harmful Algae* 48, 94–104. doi: 10.1016/j.hal.2015.07.008

Sournia, A. (1978). *Phytoplankton Manual*, Vol. 6. Paris: UNESCO Press, 1–337.

Sullivan, J. J., Iwaoka, W, T., and Liston J. (1983). Enzymatic transformation of PSP toxins in the littleneck clam (Protothacastaminea). *Biochem. Biophys. Res. Commun.* 114, 465–472. doi: 10.1016/0006-291X(83)90803-3

Tazawa, T., Ito, T., Ishige, M., and Satoh, N. (1988). Seasonal variations of paralytic shellfish poison in scallops from Funka Bay. *Rep. Hokkaido Inst. Public Health* 38, 63–65.

Trainer, V. L., Pitcher, G. C., Reguera, B., and Smayda, T. J. (2010). The distribution and impacts of harmful algal bloom species in eastern boundary upwelling systems. *Prog. Oceanogr.* 85, 33–52. doi: 10.1016/j.pocean.2010.02.003

Turner, A. D., Dhanji-Rapkova, M., Algoet, M., Suarez-Isla, B. A., Cordova, M., Caceres, C., et al. (2012a). Investigations into matrix components affecting the performance of the official bioassay reference method for quantitation of paralytic shellfish poisoning toxins in oysters. *Toxicon* 59, 215–230. doi: 10.1016/j.toxicon.2011.11.013

Turner, A. D., Lewis, A. M., Hatfield, R. G., Galloway, A. W., and Higman, W. A. (2012b). Transformation of paralytic shellfish poisoning toxins in *Crassostrea gigas* and *Pecten maximus* reference materials. *Toxicon* 60, 1117–1134. doi: 10.1016/j.toxicon.2012.07.013

Turner, A. D., Lewis, A. M., O'Neill, A., and Hatfield, R. G. (2013). Transformation of paralytic shellfish poisoning toxins in UK surf clams (Spisula solida) for targeted production of reference materials. *Toxicon* 65, 41–58. doi: 10.1016/j.toxicon.2013.01.008

Turner, A. D., McNabb, P. S., Harwood, D. T., Selwood, A. I., and Boundy, M. J. (2015). Single-laboratory validation of a multitoxin ultra-performance LC-hydrophilic interaction LC-MS/MS method for quantitation of paralytic shellfish toxins in bivalve shellfish. *J. AOAC Int.* 98, 609–621. doi: 10.5740/jaoacint.14-275

U.S. Food and Drug Administration (2015). *National Shellfish Sanitation Program (NSSP), Guide for the Control of Molluscan Shellfish: 2015 Revision*. Washington, DC: Government Printing Office, 445.

Vale, P. (2010). Metabolites of saxitoxin analogues in bivalves contaminated by *Gymnodinium catenatum*. *Toxicon* 55, 162–165. doi: 10.1016/j.toxicon.2009.07.010

Vale, P., Botelho, M. J., Rodrigues, S. M., Gomes, S. S., and Sampayo, M. A. M. (2008). Two decades of marine biotoxin monitoring in bivalves from Portugal (1986–2006): a review of exposure assessment. *Harmful Algae* 7, 11–25. doi: 10.1016/j.hal.2007.05.002

Van de Riet, J., Gibbs, R. S., Mugg ah, P. M., Rourke, W. A., MacNeil, J. D., and Quilliam, M. A. (2011). Liquid chromatography post-column oxidation (PCOX) method for the determination of paralytic shellfish toxins in mussels, clams, oysters, and scallops: collaborative study. *J. AOAC Int.* 94, 1154–1176.

Wiese, M., D'Agostino, P. M., Mihali, T. K., Moffitt, M. C., and Neilan, B. A. (2010). Neurotoxic alkaloids: saxitoxin and its analogs. *Mar. Drugs* 8, 2186–2211. doi: 10.3390/md8072185

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Medina-Elizalde, García-Mendoza, Turner, Sánchez-Bravo and Murillo-Martínez. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Marine Science

ORIGINAL RESEARCH
published: 11 October 2018
doi: 10.3389/fmars.2018.00365

Risk Perception of Coastal Communities and Authorities on Harmful Algal Blooms in Ecuador

Mercy J. Borbor-Córdova[1], Mireya Pozo-Cajas[2], Alexandra Cedeno-Montesdeoca[3], Gabriel Mantilla Saltos[3], Chippie Kislik[4], Maria E. Espinoza-Celi[1], Rene Lira[5], Omar Ruiz-Barzola[6] and Gladys Torres[7]*

[1] Facultad de Ingeniería Marítima, Ciencias Biológicas, Oceánicas y Recursos Naturales, Escuela Superior Politécnica del Litoral, ESPOL, Guayaquil, Ecuador, [2] Facultad de Ciencias Naturales y Matemáticas, Universidad de Guayaquil, Guayaquil, Ecuador, [3] Facultad de Ciencias Naturales y Matemáticas, Escuela Superior Politécnica del Litoral, ESPOL, Guayaquil, Ecuador, [4] Department of Environmental Science, Policy, and Management, University of California, Berkeley, Berkeley, CA, United States, [5] Centro de Biofísica y Bioquímica, Instituto Venezolano de Investigaciones Científicas (IVIC), Maracaibo, Venezuela, [6] Facultad de Ciencias de la Vida, Escuela Superior Politécnica del Litoral, ESPOL, Guayaquil, Ecuador, [7] Biologia, Instituto Oceanografico de la Armada del Ecuador, INOCAR, Guayaquil, Ecuador

OPEN ACCESS

Edited by:
Jorge I. Mardones, Centro de Estudios de Algas Nocivas (CREAN), Instituto de Fomento Pesquero (IFOP), Chile

Reviewed by:
Luis Alberto Henríquez, Instituto de Fomento Pesquero (IFOP), Chile
Angel Pérez-Ruzafa, Universidad de Murcia, Spain

***Correspondence:**
Mercy J. Borbor-Córdova
meborbor@espol.edu.ec

Specialty section:
This article was submitted to Marine Ecosystem Ecology, a section of the journal Frontiers in Marine Science

Received: *19 May 2018*
Accepted: *21 September 2018*
Published: *11 October 2018*

Citation:
Borbor-Córdova MJ, Pozo-Cajas M, Cedeno-Montesdeoca A, Mantilla Saltos G, Kislik C, Espinoza-Celi ME, Lira R, Ruiz-Barzola O and Torres G (2018) Risk Perception of Coastal Communities and Authorities on Harmful Algal Blooms in Ecuador. Front. Mar. Sci. 5:365. doi: 10.3389/fmars.2018.00365

The ocean is intrinsically linked to human health as it provides food and wellbeing, yet shifts in its dynamics can pose climate-ecological risks, such as harmful algal blooms (HABs) that can impact the health and economy of coastal communities. For decades, Ecuadorian coastal communities have witnessed seasonal algal blooms, events that are driven by factors including complex ocean–climate interactions, nutrient availability, and ecological variables. However, little is known about the risk perceived by coastal populations regarding such events. Understanding how specific groups of people in specific places perceive HABs risks is critical for communicating, promoting, and regulating public health measures. This study assessed the knowledge, attitudes, and practices of fishermen, restaurant owners, and coastal authorities in relation to HABs, or 'red tide' events, in coastal Ecuador. Methods utilized in this study include a non-probabilistic sampling approach for the two studied populations: coastal communities comprised of fishermen and restaurant owners (N_1 = 181), and authorities comprised of coastal officials in the sectors of health, and environment and risk management (N_2 = 20). Using contingency tables, chi-square test, Cramer's V correlation statistic, and multiple correspondence analysis, this study compared the responses of these two groups, coastal communities and authorities, to determine whether principal activity, or livelihood, affected risk perception in each group. This project implemented four workshops to interact with coastal stakeholders and more deeply understand risk perception within studied populations. Results demonstrated that principal activity indeed influenced risk perception of red tides, and that fishermen, restaurant owners, and health authorities had limited knowledge and low risk perception of red tide impacts on human health. Recommendations produced from this research include tailored workshops and improved communication between authorities and coastal communities to enhance algal bloom monitoring and coastal management during future red tide events.

Keywords: coastal authorities, fishermen, human health, knowledge, attitudes and practices (KAP), red tides

INTRODUCTION

The ocean is intrinsically linked to human health by providing food and wellbeing, but can also pose climate-ecological risks such as harmful algal blooms (HABs), which are considered natural events that can flourish in response to warm sea surface temperatures, thermocline shoaling, coastal upwelling, and other factors not yet well understood (Moore et al., 2008; Fleming et al., 2014; McCabe et al., 2016). However, climate variability such as El Niño-Southern Oscillation (ENSO), along with climate change and the expansion of nutrient enrichment, increase the frequency of algal blooms with toxins that impact the livelihood and health of coastal communities (Backer and McGillicuddy, 2006; Heisler et al., 2008; Moore et al., 2008; Hallegraeff, 2010; Fleming et al., 2014; Glibert et al., 2014). This was evidenced by the anomalously warmer conditions in the eastern Pacific Ocean in 2015–2016, from Alaska to Chile (McCabe et al., 2016; National Oceanic and Atmospheric Administration [NOAA], 2016; Guzman et al., 2017). During the so-called 'Blob,' Pseudo-nitzschia blooms off the shores of Washington, Oregon, and California reached record-breaking production of the toxin domoic acid, and prompted coastal managers to close beaches, discard thousands of clams, and apply communication strategies to prevent human health impacts (McCabe et al., 2016). During this era, the coast of southern Chile experienced anomalous climate-ocean conditions that triggered harmful flagellate blooms, causing massive deaths of salmon from aquaculture farms and raising public health concerns (Clément et al., 2017).

Harmful algal blooms have been recognized as global threats to human health, requiring international cooperation, interdisciplinary approaches, and local-context strategies for effective management (Moore et al., 2008; Bauer et al., 2010; Fleming et al., 2014). Previous social research on HABs have recognized the importance of risk perception approaches in shifting the way the public thinks and behaves during HAB events. Risk perception is a complex process by which individuals acquire and interpret information and knowledge through the contexts of lived experiences, personality, and culture (Slovic, 1987, 2000; Boholm, 1998; Finkel, 2008; Brisson et al., 2017). Thus, understanding how specific groups of people, in specific places, perceive HAB risks is critical for communicating, promoting, and regulating public health measures. HAB studies conducted in Florida applied a broader framing and theoretical framework that included risk perception, social amplification of risk, and place-specific context, and developed strategies for HAB risk communication, planning, and outreach to the general public (Kuhar et al., 2009; Nierenberg et al., 2010; Kirkpatrick et al., 2014). In Washington State, a cross-cultural study analyzed the health risk perception of domoic acid ingestion through razor clam consumption within groups of coastal Native American nations and recreational razor clam harvesters. An ethnographic study on Quebec, Canada, applied a risk perception approach to gain a better understanding of the attitudes of citizens toward cyanobacteria and public health measures (Brisson et al., 2017). These studies have demonstrated that it is critical to incorporate the knowledge, awareness, and actions of coastal communities into response practices to prevent negative economic, ecological, and human health impacts (Nierenberg et al., 2010; Kirkpatrick et al., 2014; Taylor et al., 2014). In this research, we applied the knowledge, attitude, and practices (KAP) framework in the context of red tide risk perception to gauge this information within groups of coastal communities. This research framework is widely used to study human behavioral changes in response to a problem or disease (World Health Organization [WHO], 2008; Wan, 2014; Rav-Marathe et al., 2016), and we leveraged KAP surveys to understand place-based information that can contribute to local HAB policy formation.

Within the eco-toxicological realm of HABs, prior studies have identified five syndromes linked to these events, including ciguatera poisoning, paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP), amnesic shellfish poisoning (ASP), and diarrheic shellfish poisoning (DSP) (García et al., 2005; Grattan et al., 2016). There is evidence that those who come into contact with toxic algae may experience skin irritation, gastroenteritis, respiratory problems, and even liver failure (Rose et al., 2001; Fleming et al., 2002; Kuhar et al., 2009). At this time, no routine clinical tests exist to identify these symptoms, and there are no known antidotes for these illnesses (Grattan et al., 2016). Thus, it is extremely difficult to ascertain the effects, duration, and remedies to HAB-related syndromes.

Along coastal Ecuador, communities have witnessed 'red tides' for decades (Torres, 2013, 2015, 2017). However, there is little awareness on the links between HABs and the potential production of toxins in the algae and accumulation in shellfish. Ecuadorian researchers have recorded around 150 red tide events from 1968 to 2015, resulting in 30 reported mortalities of fish, shrimp, and shrimp larvae. The most notable red tides occurred in 1985, 2003, and 2007, with seasonal peaks in March, April, and May. The Gulf of Guayaquil was recorded to have the greatest number of events within the existing Ecuadorian red tides records. Out of 71 species identified during the red tides events, 33 were potentially toxic. Within this group, 23 dinoflagellates potentially toxic included: *Gymnodinium* sp., *Margalefidinium catenatum* (Okamura, 1916) F. Gómez, Richlen and D. M. Anderson, 2017, *Cochlodinium* sp., *Prorocentrum maximum* (Gourret) Schiller, 1937, *Prorocentrum micans* Ehrenberg, 1834, *Tripos furca* (Ehrenberg) F. Gómez, 2013, *Scrippsiella trochoidea* (Stein) Loeblich III, 1976, *Prorocentrum cordatum* (Ostenfeld) J. D. Dodge, 1975, *Karenia brevis* (C. C. Davis) Gert Hansen and Ø. Moestrup, 2000, *Dinophysis caudata* Saville-Kent, 1881, three diatom pennate including *Pseudo-nitzschia* sp., *Pseudo-nitzschia pungens* (Grunow ex Cleve) G. R. Hasle, 1993, *Pseudo-nitzschia delicatissima* (Cleve) Heiden, 1928, four Cyanobacterias that are *Trichodesmium erythraeum* Ehrenberg ex Gomont, 1892, *Raphidiopsis curvata, Nodularia* sp., *Oscillatoria limnetica* var. *acicularis* Nygaard, 1950. Others groups registered were *Prymnesiophyte, Raphidophyte,* and *Haptophyta* that are *Phaeocystis* sp., *Chattonella subsalsa* B. Biecheler, 1936, *Cocolitus* sp. (Jiménez, 1989, 1993, 1996; Torres and Palacios, 2007; Torres, 2013; Torres, 2017, unpublished data; WoRMS Editorial Board, 2018).

Several of the aforementioned algal species have been proven to negatively impact human health, including *Pseudo-nitzschia*,

which has been associated with ASP, *Gymnodinium*, which is associated with PSP, *Dynophisis*, associated with DSP, and recently *Ostreopsis* cf. *ovata,* associated with the production of a palytoxin-like condition (Torres, 2013, 2015, 2017; Carnicer et al., 2016). Despite the relevance to public health concerns, there are no established monitoring programs of HABs that assess the toxicity of lipophilic shellfish in Ecuador. There is limited local knowledge on HABs and their impacts on the health and livelihood of coastal communities, which contributes to the lack of monitoring, surveillance, and awareness strategies for HAB responses in this region (Torres, 2013, 2017).

This study utilizes the KAP behavioral framework to understand the underlying mechanisms that inform HAB health educational interventions, risk communication, and management. Global research has identified the importance of a broader social framework to manage HABs risk, and this research seeks to understand risk perception among coastal communities (fishermen, restaurant owners, and authorities in the health, environmental, and risk sectors), as a part of a larger study on the ocean climate drivers of HABs, on the coast of Ecuador. In addition, this study developed a stakeholder engagement process to discuss responses and communication strategies to raise awareness and inform future HAB events. Results from this study can provide insight into the current perceived risks of HABs by key stakeholder groups, and help to define strategies and policies to manage HABs in the coastal zone of Ecuador.

MATERIALS AND METHODS

Study Area

The study area comprises part the coastal zone of the Gulf of Guayaquil including four cities: Posorja and Playas in the Guayas province, and La Libertad and Santa Elena in the Santa Elena province (**Figure 1**). These cities range from about 18,000 and 24,000 in Posorja and Playas, respectively, to 96,000 in La Libertad and 144,000 inhabitants in Santa Elena (Independent National Electoral Commission [INEC], 2010a,b; Ricaurte-Quijano, 2013). The main economic activities of these cities include artisanal fisheries in Posorja, and tourism and commerce in Playas, Santa Elena, and La Libertad. These economic activities, which involve tourism, fisheries, and shrimp farming, and these peak in the rainy season from December to April (Hernández and Zambrano, 2007). Seafood cuisine such as clams, shellfish, crabs, and fish are an economic staple in these provinces, and are heavily consumed by tourists and locals.

Design Sampling and Interviews

To understand the underlying mechanisms of HAB risk perception on the coast of Ecuador, we employed a knowledge, attitudes, and practices (KAP) framework to this study (See **Supplementary Materials**). To do this, we created semi-structured surveys that explored the knowledge (K) of HABs, the attitudes (A) toward the occurrence of HABs, and the practices (P) or preventive behavior conducted in efforts to minimize potential health risks of HABs (World Health Organization [WHO], 2008; Kirkpatrick et al., 2014). The objective of the KAP survey was to identify knowledge levels and actions of different stakeholders during HAB events. The target population included fishermen, restaurant owners, and coastal authorities in the cities of Playas, Posorja, Santa Elena, and La Libertad in Ecuador.

Surveys were applied using a non-probabilistic sampling approach for two groups of stakeholders: (1) fishermen (n = 120) and restaurant owners (n = 61, total N_1 = 181), and (2) coastal authorities in the sectors of health (n = 8), environment and risk management (n = 12, total N_2 = 20) during the first 6 months of 2016. Most members of the authorities were heads of departments in health, environment, risk management, and marine protected areas. From this point forward, this group will be referred to as 'authorities.' The coastal community group was comprised of restaurant owners and fishermen, who were part of local associations in each respective study site.

The involvement process with the stakeholders included formal letters and introductory meetings to explain the objectives, methodology, and scope of the project by the researchers. Regional and local government offices, as well leaders of fishermen and restaurant associations were contacted in advance with that purpose in each locality. Surveys and interviews between researchers and the studied populations were conducted in-person for approximately 30 min each. Surveys included 23 questions that were divided into several sections: (1) demography and background information; (2) knowledge, attitudes, and practices related to red tides for each group; (3) climate perception related to red tides, and (4) health perception related to red tides. The term 'red tide' was used to signify an indicator of potential HABs. Most of the questions were closed answers of nominal, single scale, or selection by Likert scale. This scale is a five-point bipolar response range from a group of categories, asking people to indicate how much they agree or disagree, or believe to be true or false (Likert, 1932; Jamieson, 2004). The surveys were based on previous risk perception studies applied in Florida, and were tested with a small sample group of coastal public officers (Moore et al., 2008; Nierenberg et al., 2010; Kirkpatrick et al., 2014). A limitation of this study is the inclusion of coastal authorities, fishermen, and restaurant owners, and the exclusion of hotel managers, tourists, and other residents, in our surveys. A possible bias toward public interviewees vs. institutional agents need to be considered in the interpretation of the results.

Statistical Analyses

This study utilized contingency tables, Pearson's chi-square tests, and multiple correspondence analysis (MCA). The contingency tables were applied to explore if there was a significant relationship between the activity of the group (authorities, fishermen and restaurant owners) and subgroups (environmental and risk managers, health staff) and responses regarding their knowledge, attitudes, and practices to red tide events. The Cramer's V statistic was used to corroborate the independence or dependence of the two variables analyzed in each contingency table. This statistic is a measure of association, or correlation, between two categorical or nominal variables. The result is a score between 0 and 1, with 0 indicating no correlation and 1

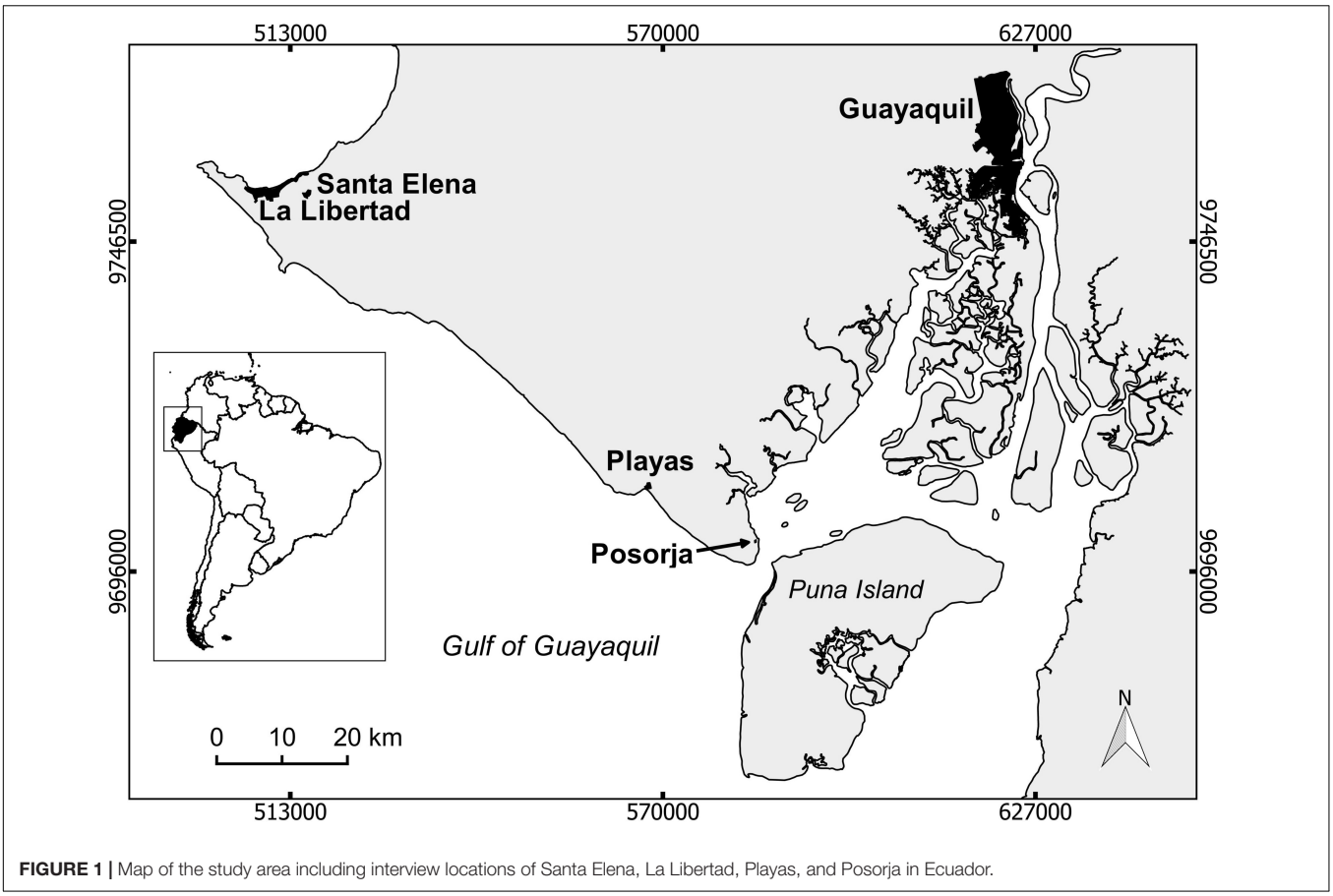


FIGURE 1 | Map of the study area including interview locations of Santa Elena, La Libertad, Playas, and Posorja in Ecuador.

indicating perfect correlation (Hope, 1968; Husson et al., 2011; Navarro, 2014). The null hypothesis of this study is that survey responses from both groups are independent of their respective principal activities or occupations. A MCA was used to determine the associations between the responses to the KAP surveys by the activity group, level of education, and if they considered themselves to be affected by red tides. Statistical software (R Program) was used for all statistical analyses.

Workshops for Coastal Stakeholders and Health Practitioners: Feedback, Communication, and Outreach

After the completion of interviews and surveys regarding red tides, a series of workshops were developed in the study sites to present the results and educate communities about drivers of potential HABs, related marine toxins, and specific climatic and oceanic events that occurred within local coastal areas. A total of four workshops were given: two workshops for fishermen and restaurant owners, and two for coastal managers regarding the environment, disaster risk reduction, and public health. In addition to presentations in the fields of biology, biomedicine, and oceanography, a discussion session was developed to learn about experiences and local knowledge of red tides within different stakeholder groups. Excerpts and experiences of several stakeholders are presented in this study.

RESULTS

Demography and Background

In the authorities, 12 (60%) were from health offices, and 8 (40%) were from environmental and risk management agencies. Within the coastal communities group, 120 (66%) respondents were fishermen, whereas 61 (34%) were restaurant owners. Fishermen and restaurant owners were older than authorities by an average of 10–15 years, and many members of this group had been living in the community for about 15 years longer than members of the authorities (**Table 1**). Most of the fishermen and restaurant owners had received some education, with a majority finishing elementary (52%) or high school (29%). All authorities had received post-secondary degree (100%). Men greatly outnumbered women in the fishermen and restaurant owner group at nearly 80%, while women comprised the majority of the authorities at 55%.

Knowledge, Attitudes, and Practices of Ecuadorian Red Tide

Knowledge varied greatly between the two groups (fishermen and restaurant owners, and authorities, $p < 0.001$) (**Table 2**). The authorities had greater knowledge of the causes of red tides (70% had an awareness of red tides on nearby beaches, and 100% had heard or seen of them), while approximately half

TABLE 1 | Demography and background information.

Demography and background	Fishermen and restaurant owners *n* = 181, *n* (%)	Authorities *n* = 20, *n* (%)
1. Age		
15–29	16 (8.84)	1 (5)
30–44	63 (34.81)	11 (55)
45–59	74 (40.88)	7 (35)
60–74	23 (12.71)	1 (5)
75–78	4 (2.1)	0 (0)
No response	1 (0.55)	0 (0)
2. Gender		
Female	37 (20.44)	11 (55)
Male	143 (79.01)	9 (45)
No response	1 (0.55)	0 (0)
3. Education level		
Primary school	94 (51.93)	0 (0)
High school	53 (29.28)	0 (0)
Post-secondary	9 (4.97)	20 (100)
No education	11 (6.08)	0 (0)
No response	14 (7.73)	0 (0)
4. Residence time		
0–14	18 (9.94)	7 (35)
15–29	40 (22.10)	5 (25)
30–44	48 (26.52)	4 (20)
45–59	39 (21.55)	3 (15)
60–74	15 (8.29)	1 (5)
75–78	2 (1.10)	0 (0)
No response	19 (10.50)	0 (0)

Question 4 demonstrates that the amount of time an interviewee had lived in the community was dependent on principal activity (p-value < 0.05). Cramer's V = 0.337 suggests moderate correlation.

(49%) of fishermen and restaurant owners had seen or heard of them, and roughly half (46%) had not. Finally, most authorities believed red tides do not occur frequently in coastal Ecuador (60%), while most fishermen and restaurant owners did not know (61%).

Attitudes pertaining to red tides within coastal Ecuador also varied between the two groups (**Table 2**). Regarding the option to avoid the beach if algae were present on the shore, the biggest response within the authorities was 'Strongly Agree' (35%), while the majority of fishermen and restaurant owners strongly disagreed with this response (55%). Additionally, when asked about avoiding the beach due to dead fish on the shore, the largest response from the authorities was 'Strongly Agree' (40%), while half of fishermen and restaurant owners strongly disagreed with this statement (50%).

There was a large difference in red tide practices between the two groups (**Table 2**). When asked if respondents would avoid eating seafood or fish in the event of a red tide, authorities were evenly divided (50% yes, 50% no), while the largest response within the fishermen and restaurant owner group was 'I don't know' (39%).

Subgroup Analyses: Fishermen vs. Restaurant Owners and Health vs. Environmental-Risk Management

Results show that the risk and environment subgroup was well informed about the causes of red tides (92% attributed these events to climate change and nutrients), while the majority of health authorities did not know the causes (63%) (**Table 3**). Attitudes also differed greatly, as the majority of risk and environment officials responded that they would avoid eating seafood or fish if there were red tides (67%), while the majority of health officials would not (75%). Both subgroups had similar responses in managing red tides, both had the largest responses in avoiding the beach when there are red tides. However, a greater percentage of risk and environment officials felt they had been affected by red tides (42% o) versus just 13% of health officials.

Figure 2 describe the responses of different groups (fisherman = F, restaurant owners = Ro, environment and risk authorities = E, and health officers = H) to the questions: what are the causes of HABs, how they perceived to be affected by HABs, and if they think they have been poisoned by seafood contaminated with toxins. Regarding the causes of red tides, environment and risk authorities were very knowledgeable about the drivers of HABs. They identified climate change as the first cause, followed by nutrients, while health officers (60%), restaurant owners (50%), and fishermen (90%) have a poor knowledge about the causes of HABs. When asked if they had been affected by the red tides, most health officials said no (85%), followed by the environment officers (65%), risk authorities (60%), and fishermen (50%). These results suggest low risk perception of HABs. Regarding if the groups think if they have been poisoned by seafood contaminated with toxins restaurant owners (75%) and fishermen (70%) believed they do not have been poisoned.

Women and men from both groups (fishermen and restaurant owners, and authorities) performed similarly in risk perception responses (**Table 3**). Women and men from the authorities had substantial knowledge of the possible causes of red tides (64% of women and 77% of men attributed it to climate change and nutrients), whereas the majority of women and men from the fishermen and restaurant owners group did not know potential causes (89% of women and 78% of men). Also, the majority of men and women in both groups felt they had not been affected by red tides (89% of women and 74% of men in the restaurant owners and fishermen group, and 73% of women and 67% of men from the authorities).

Climate and Human Health

When stakeholders were asked if climate had changed over the years, most of the respondents agreed that the climate is changing, including all of the authorities (100%) and most of the fishermen and restaurant owners (89%). When asked specifically about changes in temperature and precipitation, 59% of fishermen and restaurant owners and 60% of authorities perceived that these climate variables have changed (**Table 4**).

Health knowledge within the two groups was very similar; the majority of both groups believed that shellfish consumption

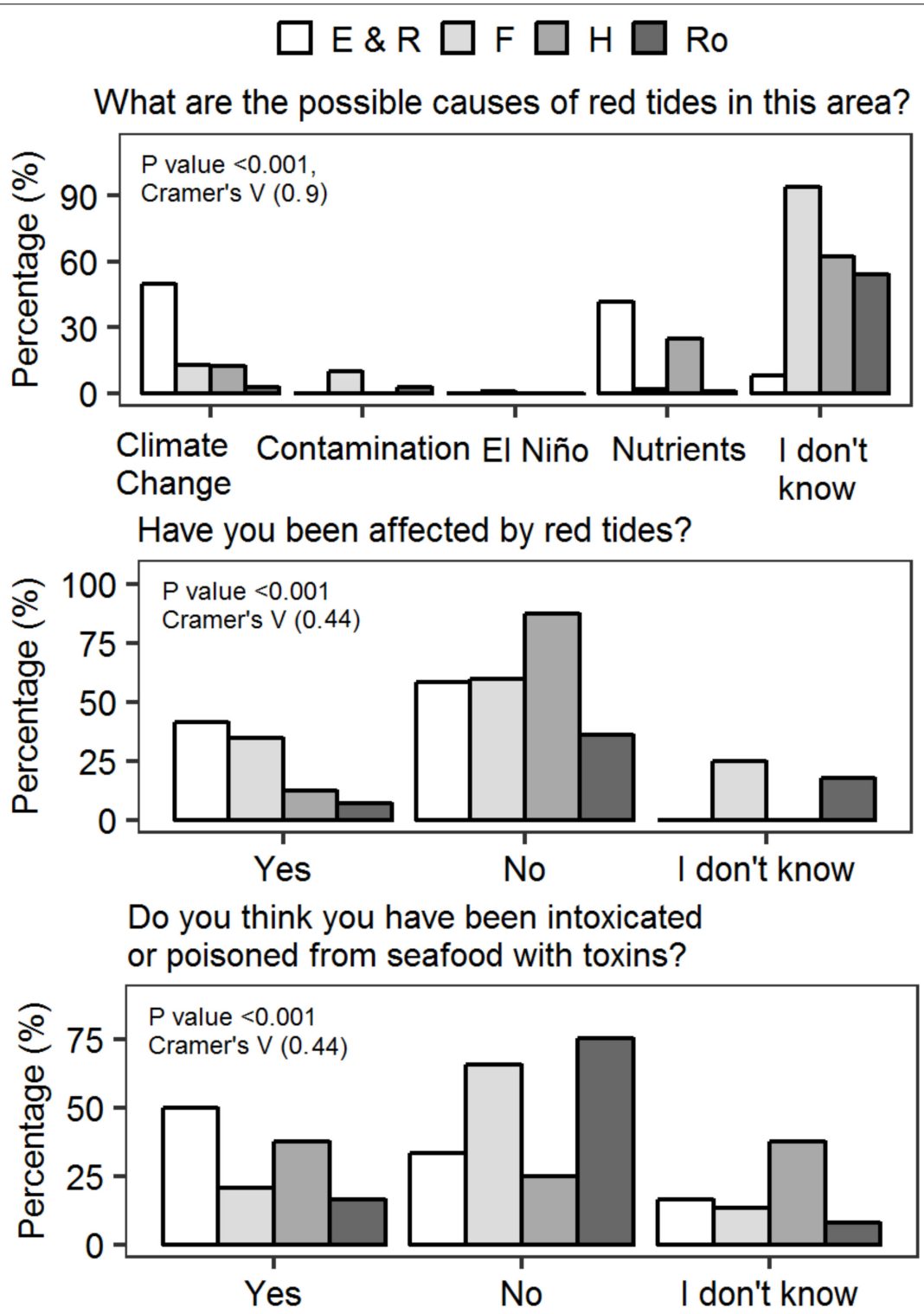


FIGURE 2 | Describes the response to tree questions as a function of group activity: fisherman (F), Ro (restaurant owners), environment and risk authorities (E), and health officers (H). Questions are related to causes of HABs, if they perceived to be affected by HABs, and if they think they have been poisoned by seafood contaminated with toxins.

TABLE 2 | Knowledge, attitudes and practices in fishermen and restaurant owners and authorities.

Knowledge	**Fishermen and restaurant owners *n* = 181, *n* (%)**	**Authorities *n* = 20, *n* (%)**	***p*-Value**	**Cramer's V correlation**
5. Have you seen or heard of red tides on this beach?				
Yes	88 (48.62)	20 (100)		
No	83 (45.86)	0 (0)	<0.001	0.581
I don't know	10 (5.52)	0 (0)		
6. What are the possible causes of red tides in this area?				
Climate change	16 (8.84)	7 (35)		
Contamination	13 (7.18)	0 (0)		
El Niño	1 (0.55)	0 (0)	<0.001	0.608
Nutrients	3 (1.66)	7 (35)		
I don't know	148 (81.77)	6 (30)		
7. Do red tides occur naturally?				
Yes	52 (28.73)	17 (85)		
No	29 (16.02)	1 (5)	<0.001	0.563
I don't know	100 (55.25)	2 (10)		
8. Do red tides occur frequently?				
Yes	14 (7.73)	8 (40)		
No	56 (30.94)	12 (60)	<0.001	0.674
I don't know	111 (61.33)	0 (0)		
Attitudes	**Fishermen and restaurant owners *n* = 181, *n* (%)**	**Authorities *n* = 20, *n* (%)**	***p*-Value**	**Cramer's V correlation**
9. I avoid the beach because of the presence of algae on the shore.				
Strongly disagree	99 (54.70)	6 (30)		
Disagree	4 (2.21)	3 (15)		
Neither agree nor disagree	8 (4.42)	2 (10)	<0.001	0.341
Agree	6 (3.31)	2 (10)		
Strongly agree	54 (29.83)	7 (35)		
I don't know	10 (5.52)	0 (0)		
10. I avoid the beach because of the dead fish on the shore.				
Strongly disagree	91 (50.28)	5 (25)		
Disagree	2 (1.10)	3 (15)		
Neither agree nor disagree	14 (7.73)	3 (15)	<0.001	0.359
Agree	4 (2.21)	1 (5)		
Strongly agree	59 (32.60)	8 (40)		
I don't know	11 (6.08)	0 (0)		
Practices	**Fishermen and restaurant owners *n* = 181, *n* (%)**	**Authorities *n* = 20, *n* (%)**	***p*-Value**	**Cramer's V correlation**
11. Would you avoid eating seafood or fish if there are red tides?				
Yes	62 (34.25)	10 (50)		
No	49 (27.07)	10 (50)	<0.001	0.483
I don't know	70 (38.67)	0 (0)		

An assessment of KAP within the study groups shows that all answers were dependent upon principal activity (p-value < 0.05). The null hypothesis (H0: X1 and X2 are independents) of this study is that survey responses from the stakeholders (X1), are independent of their respective principal activities (X2), this was rejected. Responses in the Knowledge and Practices sections had relatively strong correlations (Cramer's V = 0.483 – 0.674), whereas the Attitudes section had more moderate correlations (Cramer's V = 0.341–0.359).

can cause intoxication or illness (87% of restaurant owners and fishermen and 80% of authorities) (**Table 4**). However, the majority of restaurant owners and fishermen did not believe they had experienced seafood poisoning (69%), whereas the largest response group for authorities believed they had (45%). This question was oriented in general to respondents' experiences of seafood-related illnesses, and was not necessarily associated with consumption of seafood during red tides events.

Using a graphical representation of a MCA, we identified the correspondence between economic activity and level of education, for two main questions: "What are the causes of red tides?" and "Have you been affected by the red tides?" The **Figure 3** shows that fishermen are highly associated with the

TABLE 3 | Subgroup analysis of authorities in risk and environment and health.

Authorities	**Risk and environment *n* = 12, *n* (%)**	**Health *n* = 8, *n* (%)**	***p*-Value**	**Cramer's V correlation**
12. What are the possible causes of red tides in this area?				
Climate change	6 (50)	1 (12.5)		
Nutrients	5 (41.67)	2 (25)	<0.001	0.576
I don't know	1 (8.33)	5 (62.5)		
13. Would you avoid eating seafood or fish if there are red tides?				
Yes	8 (66.67)	2 (25)		
No	4 (33.33)	6 (75)	<0.001	0.413
14. Options for managing red tides: When there is a red tide, I do not. . .				
Go to the beach	6 (50)	3 (37.5)		
Fish	0 (0)	1 (12.5)		
Eat seafood	0 (0)	1 (12.5)		
All of the above	4 (33.33)	2 (25)	<0.001	0.512
Fish or eat seafood	2 (16.67)	0 (0)		
I don't know	0 (0)	1 (12.5)		
15. Have you been affected by red tides?				
Yes	5 (41.67)	1 (12.5)		
No	7 (58.33)	7 (87.5)	<0.001	0.321
Gender: Women	**Fishermen and restaurant owners *n* = 37, *n* (%)**	**Authorities *n* = 11, *n* (%)**	***p*-Value**	**Cramer's V correlation**
16. What are the possible causes of red tides in this area?				
Climate change	2 (5.41)	4 (36.36)		
Nutrients	2 (5.41)	3 (27.27)	<0.001	0.539
I don't know	33 (89.19)	4 (36.36)		
17. Have you been affected by red tides?				
Yes	4 (10.81)	3 (27.27)		
No	33 (89.19)	8 (72.73)	0.003	0.198
Gender: Men	**Fishermen and restaurant owners *n* = 144, *n* (%)**	**Authorities *n* = 9, *n* (%)**	***p*-Value**	**Cramer's V correlation**
18. What are the possible causes of red tides in this area?				
Climate change	15 (10.42)	3 (33.33)		
Nutrients	14 (9.72)	4 (44.44)	<0.001	0.571
I don't know	115 (79.86)	2 (22.22)		
19. Have you been affected by red tides?				
Yes	38 (26.39)	3 (33.33)		
No	106 (73.61)	6 (66.67)	0.284	0.027

The subgroup analyses on authorities (risk and environment, and health) and gender (fishermen and restaurant owners, and authorities) demonstrate that responses are dependent on principal activity (p-value < 0.05), except for the analysis on whether men from the two groups (fishermen and restaurant owners, and authorities) felt they have been affected by red tides (p-value = 0.284). There was a relatively strong correlation for most questions (Cramer's V > 0.413).

category 'I don't know' (in regards to the possible causes of red tides), 'Primary school' in terms of educational level, and the category 'No' in relation to being affected by red tides.

Authorities are somewhat associated with the 'nutrient' category of possible causes that generate red tides, and the 'Post-secondary' (high school or beyond) category of education level. In the case of health officers and environment and risk authorities, both well-educated groups, they had better knowledge of the complex interactions of HABs with nutrients. This result suggests that authorities may be willing to include nutrient management as part of a HABs management program. However, given that health and environment-risk authorities do not self-identify as affected by red tides suggests that they may have little interest in taking action to develop and implement a HABs management program.

DISCUSSION

The KAP surveys applied in this study contribute to our understanding of HABs risk perception, providing local socio-ecological information that can be used to inform policy development of ocean and human health on the coast of Ecuador and other similar tropical coastal countries. The study site has historically experienced red tides, and coastal communities such as fishermen, restaurant owners, and coastal managers are more

TABLE 4 | Perceptions on climate and health for groups of fishermen and restaurant owners and coastal authorities.

Climate and health	Fishermen and restaurant owners *n* = 181, *n* (%)	Authorities *n* = 20, *n* (%)	*p*-Value	Cramer's V correlation
20. During the time that you've lived here, have you noticed changes in the climate?				
Yes	161 (88.95)	20 (100)		
No	18 (9.94)	0 (0)	0.003	0.221
I don't know	2 (1.10)	0 (0)		
21. What changes have you noticed in the climate?				
Change in rainfall	26 (14.36)	3 (15)		
Change in air temperature	23 (12.71)	5 (25)		
Change in both	106 (58.56)	12 (60)	<0.001	0.278
No response	26 (14.36)	0 (0)		
22. Shellfish consumption causes intoxication.				
Yes	158 (87.29)	16 (80)		
No	22 (12.15)	4 (20)	0.249	0.062
I don't know	1 (0.55)	0 (0)		
23. Do you think you have been intoxicated or poisoned from seafood with toxins?				
Yes	35 (19.34)	9 (45)		
No	125 (69.06)	6 (30)	<0.001	0.553
I don't know	25(11.60)	5 (25)		

Climate and health analysis indicates that principal activity does affect interviewee responses (p-value < 0.05), except regarding whether or not shellfish consumption causes intoxication (p-value = 0.249). Correlations were relatively weak to moderate (Cramer's V = 0.062–0.278), except when interviewees were asked if they had been poisoned from seafood with toxins (Cramer's V = 0.553).

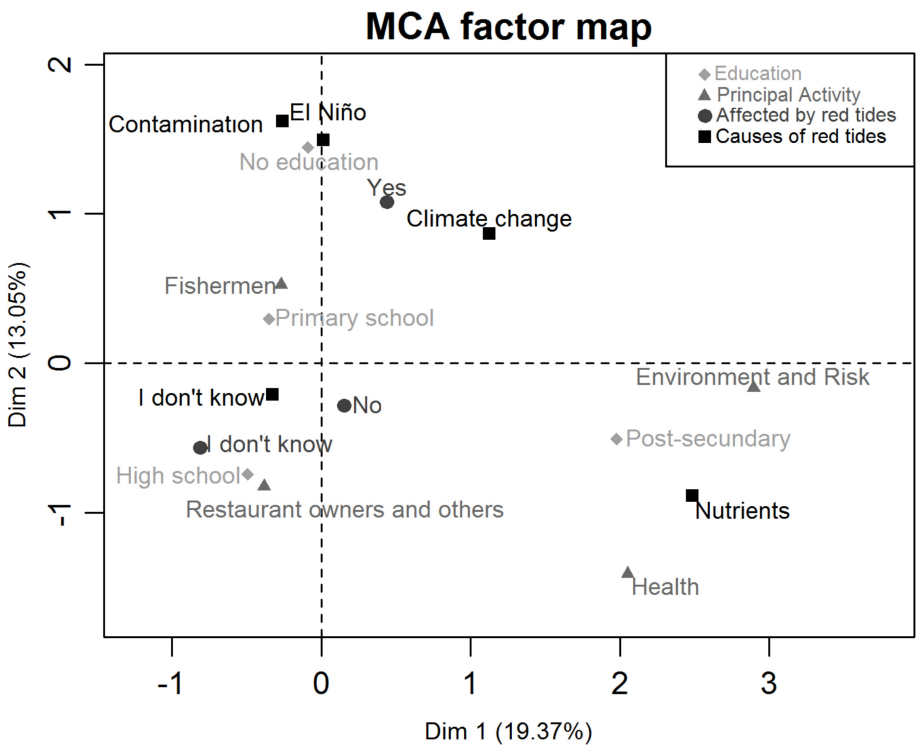


FIGURE 3 | Multiple correspondence analysis (MCA) using the 201 interviews considering four groups: fishermen, restaurant owners, health authorities, environment officers, and risk authorities, considering their activity and level of education in regards of two questions: What are the causes of red tides?" and "Have you been affected by the red tides?"

aware of their occurrences than their causes. Furthermore, there is a low risk perception of health-related consequences and, instead, greater concern with the aesthetic of the beaches and the impact on tourism. Interestingly, fishermen have developed adaptation strategies during the red tide events that reduce their HABs impact perception.

The coast of Ecuador has historically experienced red tides, and coastal communities such as fishermen, restaurant owners, and coastal managers are more aware of their occurrences than their causes. Furthermore, there is a low risk perception of health-related consequences and, instead, greater concern with the aesthetic of the beaches and the impact on tourism. Results from this study confirm that responses of individuals depend on their life experiences, livelihood or profession, and cultural values, thus coastal authority responses pertaining to HABs differed greatly from those of restaurant owners and fishermen. Additionally, responses from the authorities were tightly linked to members' previous experiences managing HABs and lessons learned regarding best practices (Taylor et al., 2014; Van Dolah et al., 2016). Furthermore, understanding the interaction between ecosystems and stakeholder activities is important in reducing the vulnerability of exposed populations to HABs, and monitoring coastal hazards and research of coastal ecosystems is an opportunity for stakeholder engagement.

Fishermen and Restaurant Owners: Experience and Livelihood

Risk perception is a social construction influenced by human factors, such as an individual's culture, gender, education level, socio-economic status, worldview, and previous experiences (Leiserowitz, 2006; Lin et al., 2011; Taylor et al., 2014; Van Dolah et al., 2016). In general, results demonstrate that risk perception of the respondents does indeed depend on the primary activity (livelihood or profession), education level, and life experience of each individual. MCA demonstrates that lack of education, lack of awareness of the origin of red tides, and low risk perception of these events coincide. However, this study identified that most of the fishermen who had lived 30 years or more along the coast were very aware of the existence of red tides, leading to the conclusion that greater observation and interaction with red tides promotes higher risk perception of the impact of HABs on livelihoods, but low risk perception of the impact of HABs on human health. Most knowledge in this group is derived by experiences from fishing efforts and observations of climate patterns and changes along the coastal zone. There is great uncertainty within coastal communities regarding the risk of eating shellfish during episodes of red tides; fishermen and restaurant owners do not consider it a risk (34%), and almost 40% of this group would not know what to do if there were a red tide.

Life experience is the main factor that influenced behavior and response to red tides in the fishermen and restaurant owners group in this study. Members of this group were influenced by their livelihoods, which were directly related to subsistence fishing and tourism. Most respondents are not willing to alter their practices during a HAB event for fear of loss of income. This group's low concern for the effects of red tides on human health was confirmed when 90% of this group felt they had not been affected by a HAB. During the workshop, respondents who felt that they had been affected by red tides mentioned several impacts: lack of fish, warmer ocean temperatures during the events, and the need to travel greater distances in search of a good catch. An aged fisherman expressed: "Red tides reduce fishing, and they come every time the weather is stronger, every 5 or 6 years." Another statement that is related to extreme events and climate change include: "The red tides appear from the currents of El Niño, and when they appear, the shellfish die," and "Climate change has affected marine currents because it brings very high temperatures and no fish."

About their practices, fishermen say: "When the red tides appear, I fish away from the coast to obtain my catch," or "Red tides are temporary and when they happen, many red crabs appear and I can catch them." About their practices, fisherman say: "When the red tides appear, I fish away from the coast to obtain my catch," or "Red tides are temporary and when they happens, many red crabs appear and I can catch them." Interestingly, one of the most important finding in this study is that fishermen have identified adaptation practices to respond to and reduce the impact of HABs on their livelihoods.

In relation to the health impacts, a fisherman said, "We have eaten fish during red tides and nothing has happened to us." This excerpt demonstrates that there was a misconception that both shellfish and fish are affected by red tides, thus potentially reducing risk perception among fishermen. On the other hand, several restaurant owners felt that HAB events had affected tourism: "Yes, we have been affected by red tides because customers are leaving." Or "Yes, algae affects us because the sea looks dirty, and tourists do not like it." Some respondents recommended that management options be established: "The Ministry of the Environment must create a measure to counteract the red tide; one of the possible causes of the red tide is the pollution of the rivers that flow into the sea." In relation to potential shellfish contaminated with toxins, restaurant owners said, "We eat all kinds of seafood at all times of the year, but seafood poisonings are produced by improper food storage and food in poor condition," suggesting that seafood-borne illnesses are dependent on seafood freshness and handling, but not related to HABs. The main concern of fishermen and restaurant owners is the protection of their livelihoods, and they expect local authorities to protect their activities from hazardous coastal events such as red tides and marine pollution.

Coastal Authorities: Awareness and Monitoring

Incorporating risk perception of coastal authorities into preventative measures and risk communication is critical during extreme events such as HABs. This study found that most of the authorities working in risk management and the environment believed that climate change and nutrients coming from wastewater from the cities and rivers were important drivers of red tides. Furthermore, there was a lower level

of uncertainty regarding the causes of red tides within this subgroup compared to the health subgroup in relation to human health impacts. Members of the environment and risk subgroup are first responders and monitors of coastal hazards, and are key components to early warning systems of coastal hazards within the communities and health sector. Their experiences managing and responding to red tides, along with their previous education, contributed to their greater knowledge and higher risk perception to these events. When the authorities was asked about options for managing red tides, they responded that they would avoid going to the beach, as well as eating seafood or fish during red tide events, and that it would be necessary to develop a communication strategy for the general public during HAB events. Currently, there are no regulations on food safety of wild shellfish, nor are there any programs on communication of coastal risks such as HABs, in Ecuador.

Health sector authorities and staff were found to be less informed than their counterparts in the environmental and risk sectors about red tides and their links to diarrheic, amnesic, neurologic, and paralytic syndrome produced by HABs. No cases of any of the syndromes have been reported or identified in emergency rooms in Ecuador. Additionally, there was a common misconception within the health group that cooking the shellfish would eliminate toxins. Moreover, the public health subgroup did not consider themselves to have been affected by HABs events (88%) and most would not stop eating seafood or fish during red tide events (75%). A very low risk perception of HABs on human health is related to limited awareness and understanding of red tides causes, processes, and impacts on the coastal area. However, at one workshop, health stakeholders expressed their concern and interest in knowing more about unusual cases of shellfish poisoning, and some local physicians and clinical staff expressed: "There is not a specific protocol of interventions during red tides," and "It would be very difficult to identify some of the diseases brought by the red tides."

Research in Latin America and in other locations across the world indicates the need to generate better scientific evidence for the potential presence of algal bloom toxins. Therefore, it is important to expand the existing monitoring of phytoplankton along the coast of Ecuador, and to implement testing that allows scientists to assess the presence, frequency, and levels of toxins in wild shellfish through an integrated risk assessment of human and coastal ecosystem health (Fleming et al., 2002; Heisler et al., 2008; Berdalet et al., 2015; Van Dolah et al., 2016; Vila et al., 2016; Torres, 2017). For the first time in Ecuador, we detected the presence of toxins from domoic acid in samples of *Anadara tuberculosa* in Santa Elena and Puerto Bolivar area (unpublished data). These toxins can produce DSP and are produced by a community of dinoflagellates (Grattan et al., 2016). Further monitoring and toxins analysis in seafood is recommended as part of a coastal monitoring program.

In regards to practices implemented by stakeholders to address the dangers of red tide events, our results suggest that while education within both subgroups contributed to a greater understanding of the ecological processes of red tides, personal experience of red tides and their impacts produced greater motivation to enact preventative measures. Therefore, a tailored training for emergency room staff, health promoters, and coastal managers should be part of a future communication and awareness program of HABs in Ecuador.

In the analysis on gender's role in HAB risk perception, results show that most women and men, independent of their livelihoods, did not believe that they had been affected by red tides (over 70%). Furthermore, it appeared that education, more so than gender, was a relevant factor in affecting survey responses within the group of authorities, as 60% were women with post-secondary (high school or higher) levels of education. Educated respondents identified climate change and nutrients as drivers of red tides during the surveys.

A main limitation of this study was the exclusion of services sectors like tour operators, hotel owners, permanent and seasonal residents. Those stakeholders are also affected by HABs events; interventions and preventive actions need to be considered for these and other populations living on the coast. In addition, may be a bias toward public interviewees versus institutional agents; however, we consider the assessment of the perception of public agents as a key step toward HABs policy development and implementation. Future research on the perceptions of coastal communities could include a more diverse stakeholders.

CONCLUSION

This study documents stakeholder's perceptions regarding HABs in a broad socio-cultural context in the Gulf of Guayaquil area. Our findings revealed relevant social issues related to general public awareness, risk perception, health and governance matters. These findings will help to strengthen strategic planning, which will contribute to the enhancement of current measures of monitoring and limiting the potential negative impacts of HABs on the population. The KAP surveys analyses demonstrate that limited awareness on the health and economic implications of HABs on coastal communities prevent authorities from developing integrated strategies that establish protocols of action for each sector. Communities are more concerned with the aesthetic quality of the beach than health impacts, as the display of red tides can detrimentally impact their tourism and fishing activities. And fishermen who have identified adaptation strategies to respond to HABs, do not think they are a problem to their livelihoods.

In addition, this study highlights the need for an integrated socio-ecological approach to face coastal hazards, which include HABs, coastal pollution, and others extreme events. Recommended future actions include: (a) a stakeholder engagement processes to delineate measures of monitoring coastal ecosystems and developing communication strategies between sectors, (b) develop scientific evidence of the presence of toxins by monitoring phytoplankton as well other indicators of marine ecosystem health, and (c) a HABs education and outreach program for public health practitioners, coastal managers, and community groups that helps to raise awareness and reduce potential public health and economic impacts during red tide events. This study contributes to the understanding of perceptions, cultural values, and perceived risks of HABs by key

stakeholders, which can help define strategies and policies to better manage HABs in the coastal zone of Ecuador.

AUTHOR CONTRIBUTIONS

MB-C was responsible for the study design, fieldwork implementation, research development, data analysis, and wrote the report. MP-C designed surveys, fieldwork implementation with communities, developed focus group, collated the data, and contributed to the study design. AC-M designed surveys and implemented the surveys with authorities, and data analysis. CK data analysis, literature review, and helped write the report. GMS design surveys, statistical analysis, and data interpretation. ME-C collated the data, literature review, and helped to write the report. RL field work implementation with health officers and authorities, developed focus group, and data analysis. OR-B statistical design and data interpretation. GT contributed to the study design, fieldwork implementation, developed focus groups, data interpretation, socialized results, and helped to write the report. All authors contributed to the study design, discussed the results, and reviewed and approved the final report.

FUNDING

Financial Support comes from the Transdisciplinary Project "Climate Variability and recurrence of harmful algae blooms and their impact on human health along with an estuarine-coastal gradient" (T2-DI-2014) funded by the Escuela Superior Politécnica del Litoral (ESPOL), Guayaquil, Ecuador. Fulbright Fellowship supported the participation of CK in the research.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2018.00365/full#supplementary-material

REFERENCES

Backer, L. C., and McGillicuddy, D. J. (2006). Harmful algal blooms: at the interface between coastal oceanography and human health. *Oceanography* 19, 94–106. doi: 10.5670/oceanog.2006.72

Bauer, M., Hoagland, P., Leschine, T. M., Blount, B. G., Pomeroy, C. M., Lampl, L. L., et al. (2010). The importance of human dimensions research in managing harmful algal blooms. *Front. Ecol. Environ.* 8, 75–83. doi: 10.1890/070181

Berdalet, E., Fleming, L. E., Gowen, R., Davidson, K., Hess, P., Backer, L. C., et al. (2015). Marine harmful algal blooms, human health and wellbeing: challenges and opportunities in the 21st century. *J. Mar. Biol. Assoc. U.K.* 2015, 61–91. doi: 10.1017/S0025315415001733

Boholm, A. (1998). Comparative studies of risk perception: a review of twenty years of research. *J. Risk Res.* 1, 135–163. doi: 10.1080/136698798377231

Brisson, G., Dubé, K., Doyon, S., and Lévesque, B. (2017). *Social Construction of Cyanobacteria Blooms in Quebec: A Matter of Perceptions and Risk Management*. Thousand Oaks, CA: SAGE Publications, doi: 10.1177/2158244017697361

Carnicer, O., García-Altares, M., Andree, K., Diogene, J., and Fernández-Tejedor, M. (2016). First evidence of *Ostreopsis* cf. ovata in the eastern tropical Pacific Ocean. Ecuadorian coast. *Bot. Mar.* 59, 267–274. doi: 10.1515/bot-2016-0022

Clément, A., Lincoqueo, M., Saldivia, M., Brito, C. G., Muñoz, F., Fernandez, C., et al. (2017). "Climatic anomalies and harmful flagellate blooms in Southern Chile," in *Marine and Fresh-Water Harmful Algae. Proceedings of the 17th International Conference on Harmful Algae*, eds L. A. O. Proença and G. M. Hallegraeff, Wellington.

Finkel, A. M. (2008). Perceiving others' perceptions of risk. *Ann. N. Y. Acad. Sci.* 1128, 121–137. doi: 10.1196/annals.1399.013

Fleming, L. E., Backer, L., and Rowan, A. (2002). "The epidemiology of human illnesses associated with harmful algal blooms," in *Handbook of Neurotoxicology*, ed. E. J. Massaro (Totowa, NJ: Humana Press)

Fleming, L. E., McDonough, N., Austen, M., Mee, L., Moore, M., Hess, P., et al. (2014). Oceans and human health: a rising tide of challenges and opportunities for Europe. *Mar. Environ. Res.* 99, 16–19. doi: 10.1016/j.marenvres.2014.05.010

García, C., Lagos, M., Truan, D., Lattes, K., Véjar, O., Chamorro, B., et al. (2005). Human intoxication with paralytic shellfish toxins: clinical parameters and toxin analysis in plasma and urine. *Biol. Res.* 38, 197–205. doi: 10.4067/S0716-97602005000200009

Glibert, P. M., Icarus, J., Artioli, Y., Beusen, A., Bouwman, L., Harle, J., et al. (2014). Vulnerability of coastal ecosystems to changes in harmful algal bloom distribution in response to climate change: projections based on model analysis. *Glob. Chang. Biol.* 20, 3845–3858. doi: 10.1111/gcb.12662

Grattan, L. M., Holobaugh, S., and Morris, J. G. (2016). Harmful algal blooms and public health. *Harmful Algae* 57, 2–8. doi: 10.1016/j.hal.2016.05.003

Guzman, L., Espinoza-Gonzalez, O., Pinilla, E., Martínez, R., Carbonell, P., Calderón, M. J., et al. (2017). "The *Alexandrium catenella* and PSP Outbreak in the Chilean Coast, the first in the Open Coast of the South East Pacific Ocean," in *Proceedings of The 7th International Conference on Harmful Algae*, (Florianopolis: ICHA Brazil).

Hallegraeff, G. M. (2010). Ocean climate change, phytoplankton community responses, and harmful algal blooms: a formidable predictive challenge. *J. Phycol.* 46, 220–235. doi: 10.1111/j.1529-8817.2010.00815.x

Heisler, J., Gilbert, P., Burkholder, J., Anderson, D., Cochlan, W., Dennison, W., et al. (2008). Eutrophication and harmful algal blooms: a scientific consensus. *Harmful Algae* 8, 3–13. doi: 10.1016/j.hal.2008.08.006

Hernández, F., and Zambrano, E. (2007). Inicio, duración y término de la estación lluviosa en cinco localidades de la costa ecuatoriana. *Acta Oceanogr. Pac.* 14, 7–11.

Hope, A. C. A. (1968). A simplified Monte Carlo significance test procedure. *J. R. Stat. Soc. Ser. B* 30, 582–598.

Husson, F., Le, S., and Pages, J. (2011). Exploratory multivariate analysis by example using R. *J. Stat. Softw.* 40, 1381–1385.

Independent National Electoral Commission [INEC] (2010a). *Fascículo Provincial Santa Elena*. Abuja: INEC.

Independent National Electoral Commission [INEC] (2010b). *Resultados Censo Poblacional Guayas*. Abuja: INEC.

Jamieson, S. (2004). Likert scales: how to (Ab)use them. *Med. Educ.* 38, 1217–1218. doi: 10.1111/j.1365-2929.2004.02012.x

Jiménez, R. (1989). "Red tide and shrimp activity in ecuador," in *Establishing a Sustainable Shrimp Mariculture Industry in Ecuador*, eds S. Olsen and L. Arriaga (Narragansett, RI: Coastal Resources Center, University of Rhode Island), 185–194.

Jiménez, R. (1993). "Ecological factors related to *Gyrodinium instriatum* "bloom" in the inner estuary of Gulf of Guayaquil," in *Toxic Phytoplankton "bloom"s in the Sea*, eds T. J. Smayda and Y. Shimizu (New York, NY: Elsevier).

Jiménez, R. (1996). "Annual cycle of *Prorocentrum* maximum red tides in the inner Estuary of the Gulf of Guayaquil Ecuador," in *Harmful and Toxic Algal "bloom"s*, eds T. Yasumoto, Y. Oshima, and Y. Fukuyo (Paris: UNESCO).

Kirkpatrick, B., Kohler, K., Byrne, M. M., and Studts, J. (2014). Florida red tide knowledge and risk perception: is there a need for tailored messaging? *Harmful Algae* 32, 27–32. doi: 10.1016/j.hal.2013.09.008

Kuhar, S. E., Nierenberg, K., Kirkpatrick, B., and Tobin, G. A. (2009). Public Perceptions of Florida red tide risks. *Risk Anal.* 29, 963–969. doi: 10.1111/j.1539-6924.2009.01228.x

Leiserowitz, A. (2006). Climate change risk perception and policy preferences: the role of affect. *Imagery Values Clim. Chang.* 77, 45–72. doi: 10.1007/s10584-006-9059-9

Likert, R. (1932). A technique for the measurement of attitudes. *Arch. Psychol.* 140:55.

Lin, Y., Huang, L., Nie, S., Liu, Z., Yu, H., Yan, W., et al. (2011). Knowledge, Attitudes and Practices (KAP) related to the Pandemic (H1N1) 2009 among Chinese general population: a telephone survey. *BMC Infect. Dis.* 11:128. doi: 10.1186/1471-2334-11-128

McCabe, R. M., Hickey, B. M., Kudela, R. M., Lefebvre, K. A., Adams, N. G., Bill, B. D., et al. (2016). An unprecedented coastwide toxic algal bloom linked to anomalous ocean conditions. *Geophys. Res. Lett.* 43, 366–310. doi: 10.1002/2016GL070023

Moore, S. K., Trainer, V. L., Mantua, N. J., Parker, M. S., Laws, E. A., Backer, L. C., et al. (2008). Impacts of climate variability and future climate change on harmful algal blooms and human health. *Environ. Health* 7(Suppl. 2):S4. doi: 10.1186/1476-069X-7-S2-S4

National Oceanic and Atmospheric Administration [NOAA] (2016). *West Coast Harmful Algal Bloom: NOAA Responds to Unprecedented Bloom that Stretches from Central California to the Alaska Peninsula*. Available at: https://oceanservice.noaa.gov/news/sep15/westcoast-habs.html.

Navarro, D. J. (2014). *Learning Statistics with R: A Tutorial for Psychology Students and Other Beginners, Version 0.5*. Adelaide: University of Adelaide.

Nierenberg, K., Byrne, M., Fleming, L. E., Stephan, W., Reich, A., Backer, L. C., et al. (2010). Florida red tide perception: residents versus tourists. *Harmful Algae* 9, 600–606. doi: 10.1016/j.hal.2010.04.010

Rav-Marathe, K., Wan, T. H., and Marathe, S. (2016). A systematic review on the KAP-O Framework for diabetes education and research. *Med. Res. Arch.* 4, 1–22.

Ricaurte-Quijano, C. (2013). *Self Organisation in Tourism Planning: Complex Dynamics of Planning, Decision-making, and Tourism Governance in Santa Elena, Ecuador*. Ph.D. thesis, University of Brighton, Brighton.

Rose, J. B., Epstein, P. R., Lipp, E. K., Sherman, B. H., Bernard, S. M., and Patz, J. A. (2001). Climate variability and change in the United States: potential impacts on water- and foodborne diseases caused by microbiologic agents. *Environ. Health Perspect.* 109(Suppl. 2), 211–221.

Slovic, P. (1987). Perception of risk. *Science* 236, 280–285. doi: 10.1126/science.3563507

Slovic, P. (2000). *Risk, society, and policy series. The perception of risk*. London, England: Earthscan Publications.

Taylor, A. L., Dessai, S., and Bruine de Bruin, W. (2014). Public perception of climate risk and adaptation in the UK: a review of the literature. *Clim. Risk Manag.* 4, 1–16. doi: 10.1016/j.crm.2014.09.001

Torres, G. (2013). Eventos de mareas rojas: estrategias de manejo preventivas en Ecuador. *Rev. Univ. Guayaquil.* 2001, 1–14.

Torres, G. (2015). Evaluación de mareas rojas durante 1968-2009 en Ecuador. *Acta Oceanogr. Pac.* 20, 89–98.

Torres, G. (2017). *Evaluación del Fitoplancton como un mecanismo preventivo a la ocurrencia de floraciones algales frente a las costas de Esmeraldas, Manta, La Libertad y Puerto Bolívar en Ecuador 2013- 2015*. Ph.D. thesis, Universidad Nacional Mayor de San Marcos, Lima.

Torres, G., and Palacios, C. (2007). . "Bloom" de Noctiluca scintilans y Ceratium dens en el Golfo de Guayaquil (2004). Informe científico. *Acta Oceanogr. Pac.* 14, 125–130.

Van Dolah, E. R., Paolisso, M., Sellner, K., and Place, A. (2016). Employing a socio-ecological systems approach to engage harmful algal bloom stakeholders. *Aquat. Ecol.* 50, 577–594. doi: 10.1007/s10452-015-9562-z

Vila, M., Abós-Herràndiz, R., Isern-Fontanet, J., Àlvarez, J., and Berdalet, E. (2016). Establishing the link between *Ostreopsis* cf. ovata blooms and human health impacts using ecology and epidemiology. *Sci. Mar.* 80(Suppl. 1), 107–115.

Wan, T. T. (2014). A transdisciplinary approach to health policy research and evaluation. *Int. J. Public Pol.* 10, 161–177. doi: 10.1504/IJPP.2014.063094

World Health Organization [WHO] (2008). *Advocacy, Communication and Social Mobilization for TB control: A Guide to Developing Knowledge, Attitude and Practice Surveys*. Geneva: World Health Organization.

WoRMS Editorial Board (2018). *World Register of Marine Species*. Available from http://www.marinespecies.org at VLIZ [accessed September 04, 2018]

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Borbor-Córdova, Pozo-Cajas, Cedeno-Montesdeoca, Mantilla Saltos, Kislik, Espinoza-Celi, Lira, Ruiz-Barzola and Torres. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Marine Science

ORIGINAL RESEARCH
published: 12 October 2018
doi: 10.3389/fmars.2018.00366

Abundance and Distribution of the Potentially Toxic Thecate Dinoflagellate *Alexandrium tamiyavanichii* (Dinophyceae) in the Central Mexican Pacific, Using the Quantitative PCR Method

David U. Hernández-Becerril[1], Winnie L. S. Lau[2], Kieng S. Hii[2], Chui P. Leaw[2], F. Varona-Cordero[1] and Po T. Lim[2]*

[1] *Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Ciudad de México, Mexico,*
[2] *Institute of Ocean and Earth Sciences, University of Malaya, Kuala Lumpur, Malaysia*

OPEN ACCESS

Edited by:
Juan José Dorantes-Aranda, Institute for Marine and Antarctic Studies (IMAS), Australia

Reviewed by:
Matthew John Harke, Columbia University, United States
Sai Elangovan S., National Institute of Oceanography (CSIR), India

***Correspondence:**
David U. Hernández-Becerril
dhernand@cmarl.unam.mx

Specialty section:
This article was submitted to Marine Ecosystem Ecology, a section of the journal Frontiers in Marine Science

Received: *03 July 2018*
Accepted: *24 September 2018*
Published: *12 October 2018*

Citation:
Hernández-Becerril DU, Lau WLS, Hii KS, Leaw CP, Varona-Cordero F and Lim PT (2018) Abundance and Distribution of the Potentially Toxic Thecate Dinoflagellate Alexandrium tamiyavanichii (Dinophyceae) in the Central Mexican Pacific, Using the Quantitative PCR Method. Front. Mar. Sci. 5:366. doi: 10.3389/fmars.2018.00366

Most of the toxic algal blooms in coasts of the Mexican Pacific are attributed to planktonic dinoflagellates. Recently, some new records of dinoflagellates producers of emergent toxins have been documented. The genus *Alexandrium* encompasses several toxic species which produce saxitoxin. In this work, the abundance and distribution of the potentially toxic species *Alexandrium tamiyavanichii* from coasts of the central Mexican Pacific were studied, following the method of quantitative real-time PCR. During the oceanographic cruise "MareaR IX" (19–30 April, 2017), carried out along coasts of the Mexican Pacific, hydrographic, and environmental variables were measured, and net and bottle samples were collected and preserved in modified saline ethanol buffer for analysis in the laboratory. In order to perform the qPCR method, the molecular target used was the ITS2 of the rDNA of *A. tamiyavanichii*. From 45 samples analyzed, 14 yielded positive results, showing the presence and abundance of the species in fixed stations of Cabo Corrientes, Manzanillo and Acapulco, with low densities (less than 40 cells/m^3), which is an evidence of the sensitiveness of the method. On the other hand, chains of cells of the species were found in net samples, in stations where its presence was detected by qPCR, confirming results by the method. General distribution showed presence of the species in two zones where upwellings were detected, but not at coastal stations, except in Acapulco where a more stratified water column was found. Vertical distribution indicated that highest densities were found at subsurface layers, in association with the chlorophyll *a* maxima (between 11 and 30 m depth). The results show the importance of assessing the abundance and distribution of a species which may be systematically monitored, and that the method of the qPCR may be very useful.

Keywords: *Alexandrium tamiyavanichii*, central Mexican Pacific, dinoflagellates, molecular tools, quantitative real time PCR

INTRODUCTION

The Mexican Pacific is a very large region which comprises various different areas with particular climatic and oceanographic conditions, including more temperate zones (e.g., western coasts of Baja California) and subtropical and tropical zones (e.g., the Gulf of California, the central Mexican Pacific and the Gulf of Tehuantepec) (Fiedler and Lavín, 2006). The phytoplankton composition may therefore be different within certain areas and zones, and also during different seasons. The central Mexican Pacific is considered a tropical region, which may also be divided according to its hydrographic and oceanographic characteristics as well as its seasonality (Kessler, 2006). In the zone of Cabo Corrientes, upwellings have been detected to occur by spring, whereas more to the south, other zones (e.g., close to Acapulco and surroundings) show more stable conditions, with a more stratified water column (Willett et al., 2006; Zamudio et al., 2007).

Harmful algae blooms (HABs) have been historically recorded along the coasts of the Mexican Pacific, most of these events being caused by planktonic dinoflagellates, for which responsible species have been usually well-recognized, the most commonly recorded are *Gymnodinium catenatum* and *Pyrodinium bahamense* (Hernández-Becerril et al., 2007), whereas other dinoflagellate species considered harmful or producing "emergent toxins" (for example Azaspiracid toxins or Pinnatoxins) have been recently identified: *Azadinium spinosum* and *Vulcanodinium rugosum*, although they have not been reported to produce blooms or toxins as yet (Hernández-Becerril et al., 2012, 2013).

Within the phytoplankton, one of the most important genera with species known to form blooms and produce toxins [mainly saxitoxins, which cause the Paralytic Shellfish Poisoning (PSP)] is the thecate dinoflagellate genus *Alexandrium* Halim. A number of works have reported blooms, toxin production, human intoxications, economic losses in many places around the world (e.g., Hashimoto et al., 2002; Beppu et al., 2008; Kon et al., 2015; Mohammad-Noor et al., 2018). In the Mexican Pacific the genus is represented by several species (Okolodkov and Gárate-Lizárraga, 2006), some of them responsible for producing blooms or toxins elsewhere. However, the species identification has been a problem and little is known about the distribution of toxic *Alexandrium* species in the central Mexican Pacific, apart from coastal areas. *Alexandrium tamiyavanichii* was described forming long chains (chains of 62 cells have been found), with a particularly shaped first apical (1′) and anterior sulcal plates (Sa), and a conspicuous and large pore at the posterior sulcal plate (Sp), besides the apical pore complex (Po) (Balech, 1995), and it is considered a toxic species producing saxitoxin and other toxins. This species has been previously found and described in the central Mexican Pacific (Esqueda-Lara and Hernández-Becerril, 2010).

However, observation of these cells under microscope is tedious, time-consuming and requires trained personnel in phytoplankton identification (Cembella and Taylor, 1986; Steidinger and Moestrup, 1990). Therefore, systematic and rapid manners are needed to provide more accurate information on the potentially toxic phytoplankton species by using the advent of molecular tools. Molecular-based species-specific assays, such as the quantitative real-time PCR (qPCR), have proven to be a reliable and rapid method for assessing and monitoring the abundance and distribution of toxic phytoplankton species, especially those of problematic identification, as those belonging to the genus *Alexandrium* (Lim et al., 2007). For instance, qPCR has been developed and implemented to study the distribution of *A. tamiyavanichii* in the South China Sea-Sulu Sea (Kon et al., 2015). Through this assay, it was found that *A. tamiyavanichii* is highly abundant offshore of Kuching, southern Borneo with 150 cells L^{-1} (Kon et al., 2015).

In qPCR assays, a pair of good primers is important to amplify the targeted DNA region. It enables amplification of the targeted DNA region with no other unintended target regions (Ye et al., 2012). The qPCR primer is usually designed with an amplicon size of less than 150 bp (Arya et al., 2005; Arvidsson et al., 2008; Ye et al., 2012). Other criteria in primer region are: 18–30 nucleotide length, GC content of 40–60%, more than one mismatches to non-target DNA or organism, melting temperature of forward and reverse primer should be similar or within 2°C and improbable with formation of primer dimer or heterodimer (Arya et al., 2005; Ye et al., 2012). On the other hand, slope of the calibration curve used for absolute quantification qPCR is in the range of −3.1 to −3.6, with the amplification efficiency (AE) of 90–110% and the R^2 greater than 0.99.

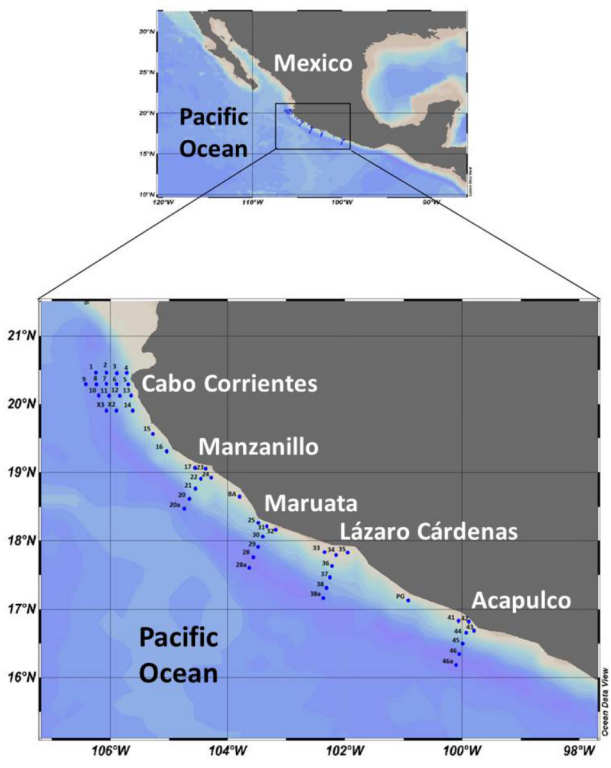


FIGURE 1 | Map of the study area, showing the fixed stations along transects in five zones of the central Mexican Pacific.

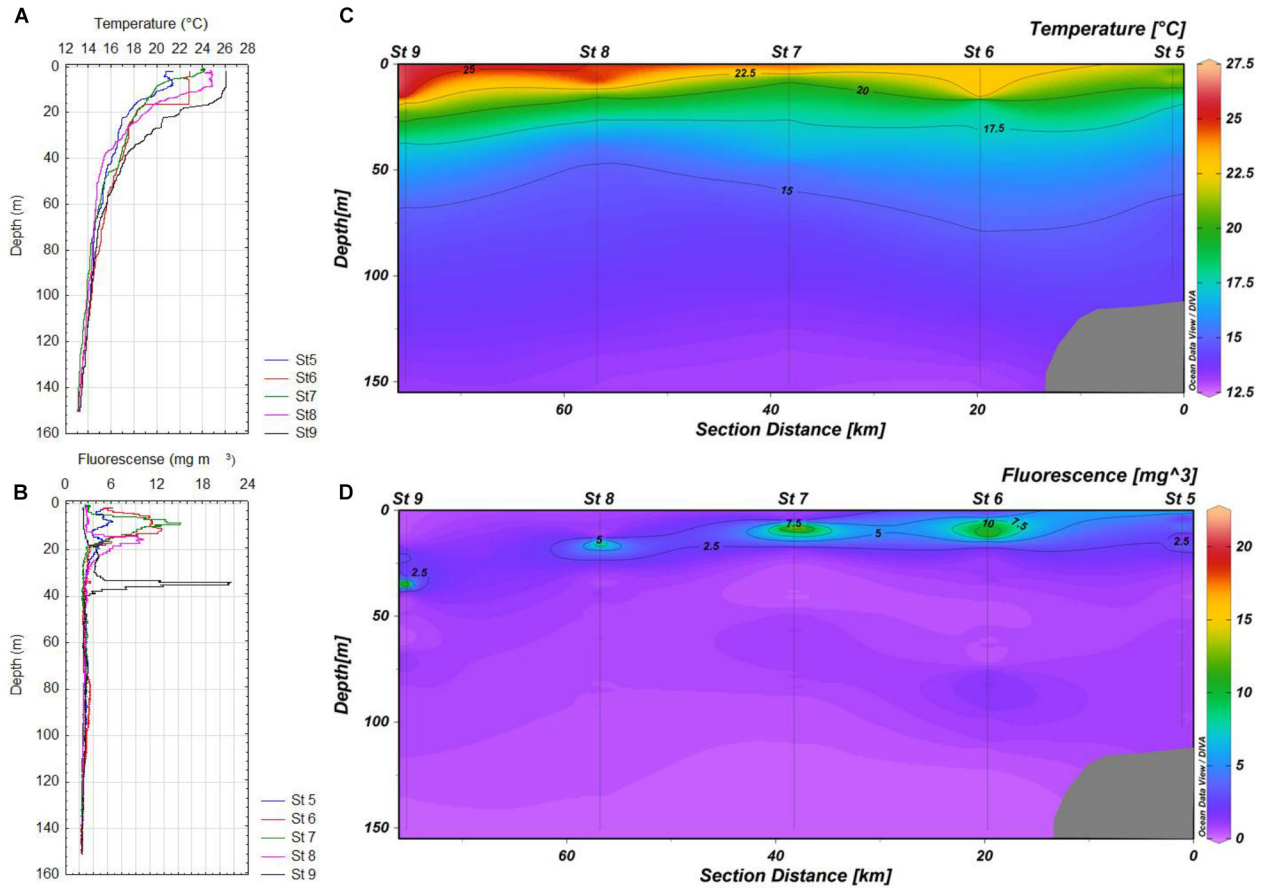


FIGURE 2 | (A,B) Vertical profiles of temperature and *in situ* fluorescence for stations 5-9 of the transect in Cabo Corrientes, respectively. **(C,D)** Maps of the distribution of temperature and *in situ* fluorescence for stations 5-9 in Cabo Corrientes, respectively.

In this paper, we give an account of the abundance and distribution of the toxic thecate dinoflagellate *A. tamiyavanichii* from the central Mexican Pacific using a real-time quantitative PCR (qPCR) assay.

MATERIALS AND METHODS

Study Area

This study was conducted in an area of the central tropical Mexican Pacific, between 16° 20.421 and 20° 27.500 N, and 100° 02.922 and 106° 15.052 W (**Figure 1**). This area sustains a great diversity of organisms and important pelagic fisheries, with different mechanisms of natural fertilization such as mesoscale phenomena (plumes and eddies) and upwellings, and shallow thermoclines reducing stratification and keeping relatively high phytoplankton biomass values (López-Sandoval et al., 2009). The area is located within an oxygen minimum zone (Paulmier and Ruiz-Pino, 2009; Ulloa et al., 2012), and some hydrographic features might affect the vertical distribution of biological properties such as chlorophyll *a*.

Data and samples were measured and collected during the oceanographic cruise "MareaR IX," carried out 19–30 April, 2017, on board the Research Vessel "El Puma," considering five zones: Cabo Corrientes (stations 1-14, X2, X3), Manzanillo (stations 17, 20a-24), Maruata (stations 25, 28a-32), Lázaro Cárdenas (stations 33-38a), and Acapulco (stations 41-46a) (**Figure 1**). There are additional intermediate stations (15, 16, BA, and PG), but only stations in perpendicular transects were sampled (5-9, 20a-23, 28a-31, 34-38a, 42-46a) (**Figure 1**).

Hydrographic Data and Water Sampling

Forty-one fixed stations were visited during the cruise (**Figure 1**) to obtain hydrographic data (temperature, salinity, dissolved oxygen) in vertical profiles, using a CTD (Seabird SBE 911 PLUS), fitted with an additional sensor for fluorescence (WET Labs ECOAFL/FL), and water samples. Transects perpendicular to the coastline of five stations were set for each of the five zones (**Figure 1**). For this study, water samples were collected with Niskin bottles of 10 L, from nine stations, at five depths, according to the *in situ* fluorescence (chlorophyll a) maximum layers or peaks: 1 L per sample was filtered through nylon membrane filters (47 mm diameter, 0.2 μm) filters with a vacuum pump.

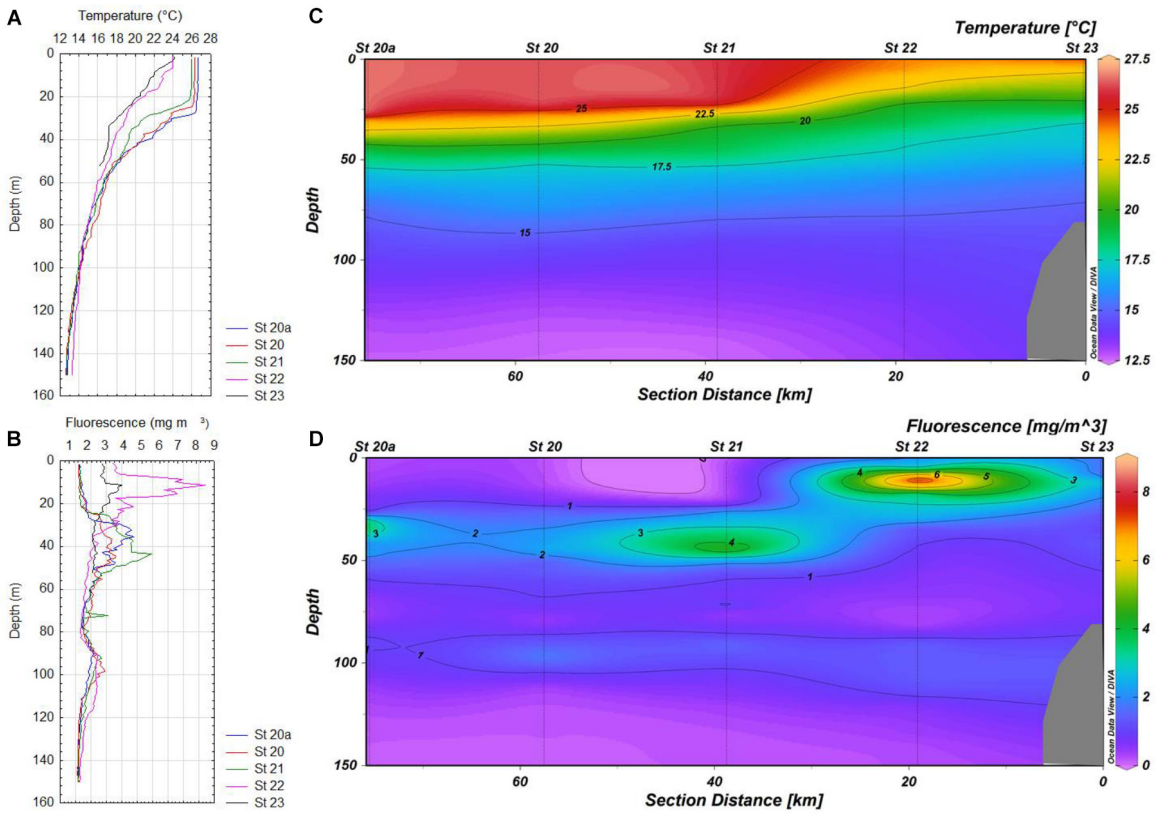


FIGURE 3 | (A,B) Vertical profiles of temperature and *in situ* fluorescence 20a-23 of the transect in Manzanillo, respectively. **(C,D)** Maps of the distribution of temperature and *in situ* fluorescence 20a-23 in Manzanillo, respectively.

Each filter was kept in 10 mL of modified saline ethanol solution into a 15 mL sterile centrifuge tubes and stored at −20°C until analysis (Miller and Scholin, 1998). Additionally, net (54 μm mesh) samples were obtained at all 41 stations, following vertical hauls from 120 m to surface or depending on the depth of the stations. The net samples were observed under the microscope.

Laboratory Analysis

Environment DNA Extraction

One milliliter of saline ethanol-preserved samples was transferred into 1.5 mL centrifuge tube and harvested the cells pellet by centrifugation (200 × *g*, 10 min), followed the removal of the saline ethanol supernatant from the cell pellet. The cell pellet was rinsed with 1 mL of TE buffer (Tris 1 M, EDTA 0.5 M, pH 8) and cells were spun again (200 × *g*, 10 min). Environmental DNA (eDNA) of the samples was extracted by using DNeasy® Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction as described in Kon et al. (2015). The eDNAs were kept at −20°C until further analysis.

qPCR Assay for *Alexandrium tamiyavanichii* Cell Quantification

Real-time quantitative PCR (qPCR) assay of *A. tamiyavanichii* quantification of Kon et al. (2015) was employed in this study for the detection and quantification of *A. tamiyavanichii*. Prior to qPCR, specificity of the species-specific primers and probe (TamiaiiF, TamiaiiR, and Tamia-probe; Kon et al., 2015) were confirmed by DNA sequencing of the amplicons.

The qPCR assay was performed using an Applied Biosystems® 7500 Fast Real-time PCR system (Applied Biosystems, Life Technologies, Austin, TX, United States). The 20 μL-reactions contained 1× Taqman® Fast Advanced Master Mix (Applied Biosystems), 200 nM of Taqman® hydrolysis probe, 300 nM of each forward and reverse primer and 2 μL of eDNA template. The reaction consisted of a holding stage at 50°C for 2 min and 95°C for 20 s, followed by 40 cycles of 95°C for 3 s and 60°C for 30 s. All samples were run in triplicate. Each qPCR run included a no-template control and a positive control. The threshold cycle (C_q) was determined from the exponential phase of all amplification plots using the default settings. qPCR in this study was conformed to the MIQE guidelines (Minimum Information for Publication of qPCR Experiments; Bustin et al., 2009).

The calibration curve of DNA in this study was constructed using the gBlock® synthetic gene fragment (Integrated DNA Technologies, Coralville, IA, United States) of the ITS2 rDNA of *A. tamiyavanichii*. The synthetic gene fragment was diluted into a 10-fold serial dilution (1×10^2–10^8 copies) as templates in qPCR run. Each reaction was performed in triplicate. The calibration curve was constructed based on the triplicate Cq values against the log-transformed copy numbers; linear

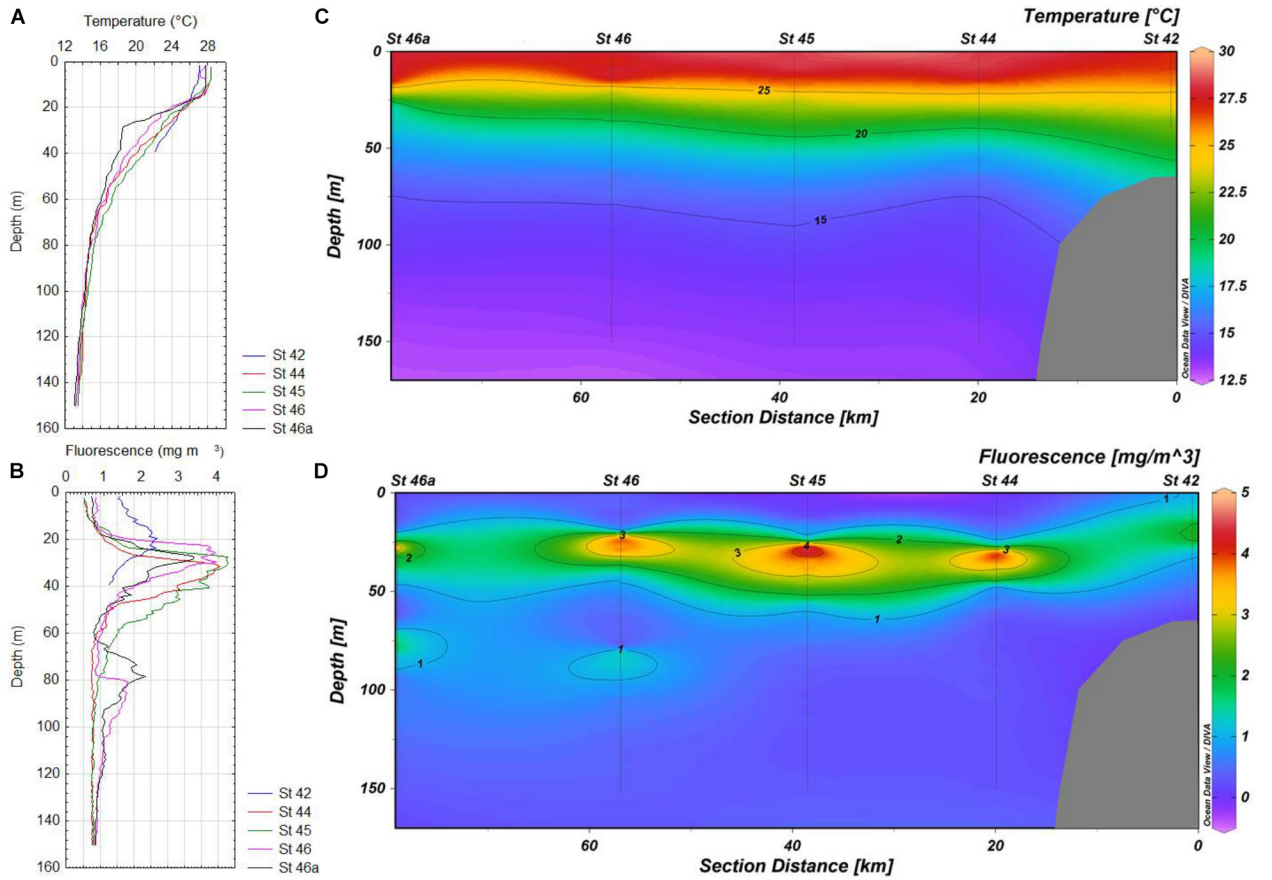


FIGURE 4 | (A,B) Vertical profiles of temperature and *in situ* fluorescence for stations 42-46a of the transect in Acapulco, respectively. **(C,D)** Maps of the distribution of temperature and *in situ* fluorescence for stations 42-46a in Acapulco, respectively.

regression was performed onto the calibration curve to determine the R^2 value and slope. AE was calculated as AE = $[10^{(-1/\text{slope})} - 1] \times 100\%$.

Gene copies number of the *A. tamiyavanichii* ITS2 rDNA in each eDNA sample was defined based on the slope of simple linear regression of the calibration curve, followed by quantification of the number of cells based on the ITS2 copy numbers per *A. tamiyavanichii* cell of 527,835 ± 7617 ITS2 copies $cell^{-1}$ as described in Kon et al. (2015).

Microscopic Identification

Microscopic species identification was made by calcoflour white staining on the thecal plates of dinoflagellates and observed under an inverted microscope (Olympus IX51, Tokyo, Japan), equipped with a mercury lamp and a UV filter set, at 400–1000X magnification. Species identification was confirmed once the distinctive morphological features of *A. tamiyavanichii* were observed, and microphotographs were taken of the cells and chains found in the samples. These features include an oblique posterior margin of the first apical plate (1′), a triangular to trapezoid-shaped of precingular part of the anterior sulcal plate (Sa), a characteristic apical pore complex (Po), and a longer than wide posterior sulcal plate (Sp) with a large pore.

Data Analysis

Sampling map and graphic interpolations were generated using Ocean Data View (version 4.7.10) with the DIVA algorithm for variable resolution in a rectangular grid (Schlitzer, 2016). Vertical profiles of environmental variables were made with the STATISTICA 10 software (StatSoft, Inc.) and principal component analysis (PCA), using abiotic and biotic data (abundances and fluorescence) as quantitative variables and transect name as qualitative supplementary variables, was made with XLSTAT 2018.5 (Base version, Addinsoft).

RESULTS

Hydrography and Oceanographic Conditions

In April, 2017, general hydrographical conditions along the Pacific coast showed a conspicuous thermal gradient from the north zone (Cabo Corrientes) toward south zones (Acapulco). Surface water was colder (about 21.3°C) in the coastal station (St 5) in Cabo Corrientes, than at the coastal station (St 42) in Manzanillo, where temperature reached 27.5°C (**Figures 2A,C**,

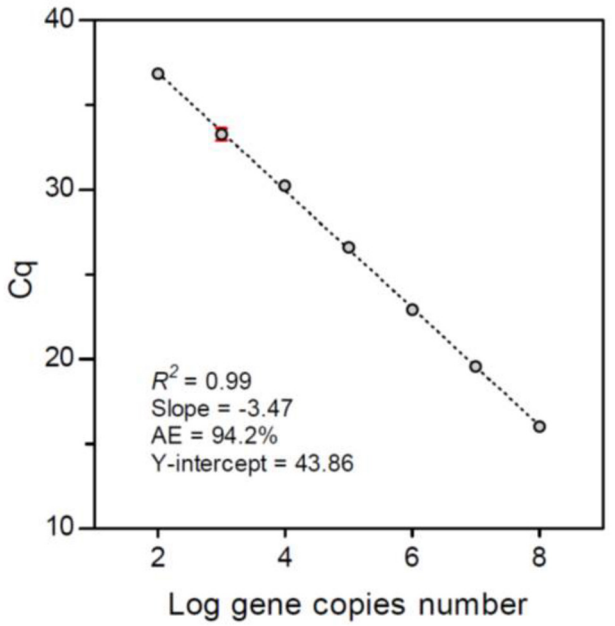


FIGURE 5 | Calibration curve of the cycle threshold (Cq) constructed using the synthetic gene fragment of *Alexandrium tamiyavanichii* ITS2 rDNA. Error bars show the standard deviations from the triplicate amplifications.

3A,C). We also noted shallower thermoclines in Cabo Corrientes, where they were located below 20 m depth, whereas thermoclines were deeper (up to 30 m) in Manzanillo and Acapulco (**Figures 3A,C**, **4A,C**). Values and distribution of salinity did not change considerably and they are not shown.

Most subsurface fluorescence (chlorophyll *a*) maxima (SCM) were located above the main thermoclines in the zone of Cabo Corrientes, except at the most oceanic station (St 9), where this layer was detected lower (37 m depth), with very high values (more than 20 mg m^{-3}), probably as result of a coastal upwelling (**Figures 2B,D**), whereas in the other zones (Manzanillo and Acapulco) only two stations (St 22 and 23) showed SCM above the main thermoclines, as they were located below the thermoclines (**Figures 3B,D**, **4B,D**). Values of the chlorophyll *a* in the coastal areas were less than 4 mg m^{-3}.

Deeper (80–90 m depth) chlorophyll *a* maxima (DCM) were also found at stations 6 and 9, but due to the scales used they are not very conspicuous (**Figures 2B,D**). Vertical distribution of chlorophyll *a* was more variable in Manzanillo, where SCM were located above the thermoclines at the most inshore stations (Sts 22 and 23), whereas the rest of stations (21, 20, and 20a) these maxima were found lower (30–48 m depth), with the highest concentration at station 21 (more than 6 mg m^{-3}) (**Figures 3B,D**). At station 42 (Acapulco) only a SCM was found, located at 20 m depth (**Figures 4B,D**). DCM were also detected in almost all stations, except coastal stations, from 90 to 120 m depth, and concentrations just lower than 2 mg m^{-3} (**Figures 2D**, **3D**, **4B,D**).

Oceanographic conditions of the zones where *A. tamiyavanichii* was detected, indicated a conspicuous ascent of colder water toward inshore locations (St 5 in Cabo Corrientes and St 23 in Manzanillo), which was more evident in Cabo Corrientes than in Manzanillo (**Figures 2C**, **3C**), whereas in the Acapulco zone, the coastal station (St 42) showed a more stratified condition (with the main thermocline located at 26 m depth) (**Figures 4A,C**). The isotherms of 22.5°C raised close to coast in the zones of Cabo Corrientes and Manzanillo, strongly suggesting the occurrence of upwellings (**Figures 2C**, **3C**).

Alexandrium tamiyavanichii Abundance and Distribution

The linear dynamic range of seven-order magnitudes (10^2–10^8 copies) showed AE of 94.2% and slope of −3.47 (R^2 = 0.99), Y-intercept was 43.86 (**Figure 5**). We have obtained 45 samples for this study, from which only 14 yielded positive results, at two stations in Cabo Corrientes, two in Manzanillo and one in Acapulco, with cell densities ranging from 35 to 25,180 cells m^{-3} (**Figures 6A–C** and **Table 1**). *Alexandrium tamiyavanichii* cell densities were low in general (less than 30 cells L^{-1}) (**Table 1**). Overall cells abundance of *A. tamiyavanichii* increased in the order of Cabo Corrientes, St 7 > Manzanillo, St 22 > Acapulco, St 42 > Manzanillo, St 23 > Cabo Corrientes St 8 (**Table 1**). The higher abundances (25,180 cells m^{-3}) of *A. tamiyavanichii* were located at an intermediate, more oceanic station (which has more than 2000 m depth), not in a coastal area, at St 7 in the Cabo Corrientes zone.

Regarding the vertical distribution of the species, we detected a maximum abundance layer associated with the SCM (about 15 m) in Cabo Corrientes, with a cell density of 25,180 cells m^{-3} (**Table 1**), whereas in Manzanillo the maximum cell density was found close to the surface (5 m depth), and the lowest cell densities were detected at Acapulco, St 42 with the depth of 18 m (35 cells m^{-3}) (**Figures 6A–C** and **Table 1**). Cells of *A. tamiyavanichii* were detected as deep as 150 m depth (40.61 cells m^{-3}) only at St 7, Cabo Corrientes (**Table 1**).

Additionally, cells of *A. tamiyavanichii* were also identified in one of the positive result sample. The cells have also been confirmed with light microscopy (LM) and fluorescence microscopy, with the addition of calcofluor (**Figures 7A–C**). This fact has confirmed the reliability of the results obtained from the qPCR assay in this study.

Statistical Analysis

In accordance with the PCA, temperature, depth, salinity and dissolved oxygen explained 94.14% of the variability (**Figure 8**). Temperature and oxygen correlated positively with the first component and negatively with salinity and upwelling index which reflects the influence of cold water, while the second axis correlated positively with the upwelling index. Fluorescence and *A. tamiyavanichi* abundances correlated positively with the first components which suggest its preference for warmer waters and more light. Due to the influence of temperature and dissolved oxygen, samples from Manzanillo and Acapulco were closer than Cabo Corrientes.

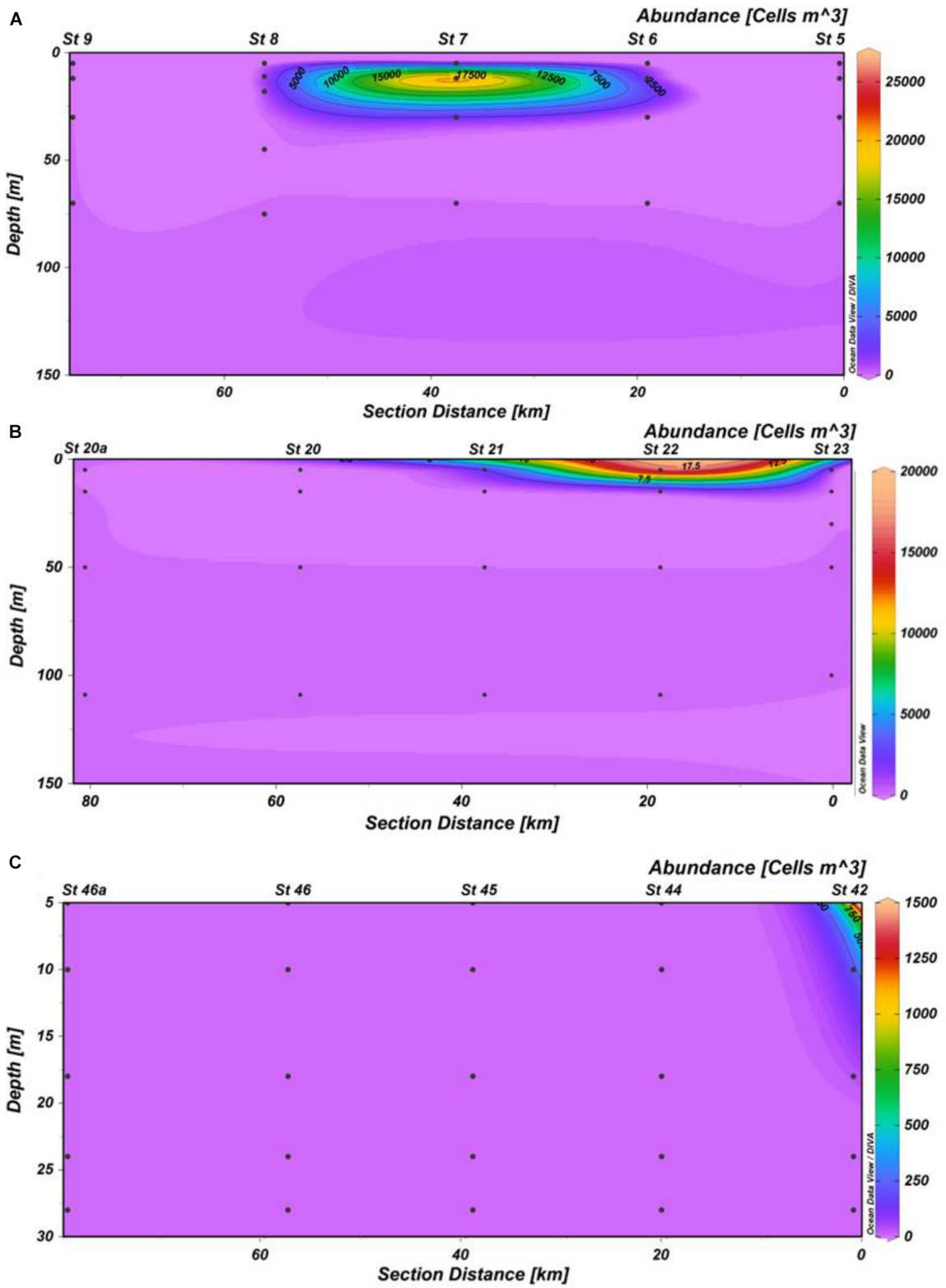


FIGURE 6 | Vertical distribution of *Alexandrium tamiyavanichii* cell densities (cells m^{-3}) in three transects. **(A)** Cabo Corrientes; **(B)** Manzanillo; **(C)** Acapulco.

DISCUSSION

Due to the importance of species of the genus *Alexandrium* producing toxins in the marine phytoplankton, the diversity and taxonomy of the genus has been dealt with by various authors (Ogata et al., 1990; Balech, 1995; Usup et al., 2002a,b; Mackenzie et al., 2004; Nguyen and Larsen, 2004; Lim et al., 2007), and several works have followed the use of molecular tools to assess the *Alexandrium* species presence and distribution around the world oceans (Scholin et al., 1994, 1995; Galluzzi et al., 2004; Menezes et al., 2010; Nagai, 2011; Kon et al., 2015).

This is the first report of the abundance and distribution of *A. tamiyavanichii* in the Mexican Pacific, as well as the use of qPCR to asses these characteristics. We were able to detect cell densities of the species as low as less than 30 cells L^{-1}. The highest abundance (25,180 cells m^{-3}) of *A. tamiyavanichii* was located at an intermediate, fairly oceanic station (St 7, which is more than 2000 m deep), coinciding with the SCM (12 m depth), in the Cabo Corrientes zone, not in more coastal stations, such as it occurred in the other two zones, Manzanillo and Acapulco, where the species was present in detectable cell densities at more coastal areas, close to the surface. Only in that sample (St 7, at 12 m depth) was the limit of *A. tamiyavanichii* cell density (20–40 cells L^{-1}) surpassed, where toxin (saxitoxin) concentration, causing PSP, represents a threat for human health for shellfish consumption (García et al., 2004).

There is a considerable variation in abundances of *A. tamiyavanichii* among the three transects (e.g., Cabo Corrientes, Manzanillo, and Acapulco), showing a heterogeneous distribution in the gradient coast to ocean, and also in the vertical distribution (**Figures 6A–C**). No cells were detected in the remote stations from the coast, mostly oceanic, in all the three transects (e.g., offshore, such as St 9, 20a, and 46a) (**Figures 6A–C**). The highest density of the species coincided with the subsurface chlorophyll maximum layer in St 7, in Cabo Corrientes zone (**Figures 2B,D**, **6A** and **Table 1**), and also a considerable number of cells (19,218 cells m^{-3}) of *A. tamiyavanichii* was found close to the surface (5 m depth) at St 22, in Manzanillo, almost coinciding with the subsurface chlorophyll maximum layer (between 5 and 10 m depth) (**Figures 3B,D**, **6B** and **Table 1**). In St 42 (Acapulco) there was no coincidence of the highest density of the species and the SCM (**Figures 4B,D**, **6C**).

We may speculate that the distribution of the species corresponds to two possible explanations: (1) the cells of *A. tamiyavanichii* were transported off the coast, far from the coastal area, by physical forcing, for in this zone (Cabo Corrientes) an upwelling was detected, most possibly induced by winds, or (2) the species was able to maintain high cell densities in environmental conditions (e.g., a more stratified water column) which were more favorable for its ecological requirements, because in the other stations where the species was detected, the oceanographic conditions were more stable along the water column.

To explain the first hypothesis, we found that prior to the start of sampling in Cabo Corrientes an intense period of upwelling occurred (April 5–17, Bakun index CUI = 109 m^3 s^{-1} 100 m^{-1}), which gradually decreased toward the end of the cruising (April 25, CUI = 40 m^3 s^{-1} 100 m^{-1}), when satellite and *in situ* chlorophyll measurements were the lowest in Acapulco (<1.6 mg m^{-3}, **Supplementary Figure S1**). This strong upwelling event, prior to the cruise, may explain the isotherms elevation in the Cabo Corrientes area and the displacement of the warmer water mass off the coast (>25°C, stations St8 and St9), and the local shoaling of the Subtropical Subsurface Water (StSsW) (Fiedler and Talley, 2006), opposite to the oceanographic conditions occurring in Acapulco, where upwelling was weak at the time of sampling; coastal upwelling events have been previously documented for the coasts of Colima and Michoacán (Davies et al., 2015) and at the entrance of the Gulf of California and Cabo Corrientes (Pelayo-Martínez et al., 2017).

The second hypothesis assumes a combination of physiological characteristics, such as mobility, photosynthesis and cyst production, and environmental features (e.g., small-scale physical turbulence) which are found in the St 42 in Acapulco (**Figures 4A,C**). It has been reported that the distribution of toxic and non-toxic *Alexandrium* species, in other areas of the world, does not overlap (Anderson et al., 2012), which partially explains why only in some sites of the current study area, *A. tamiyavanichii* was detected.

TABLE 1 | Cell abundances of *Alexandrium tamiyavanichii* found in samples of the central Mexican Pacific by using qPCR assay.

Station	Depth (m)	Cell density (cells L^{-1})	(cells m^{-3})
7	5	0.47	475.83
	12	25.18	25,179.8
	30	1.74	1,739.18
	70	0.062	61.89
	150	0.041	40.61
8	5	—	—
	11	0.11	110.36
	18	0.046	45.78
	45	0.04	39.54
	75	—	—
22	5	19.22	19,218.1
	15	—	—
	50	—	—
	109	—	—
	150	—	—
23	5	0.4	397.24
	15	0.15	145.02
	30	—	—
	50	—	—
42	5	1.38	1380
	10	0.232	232.16
	18	0.035	35.35
	24	—	—
	28	—	—

Stations, depths, and abundances (both in cells L^{-1} and cells m^{-3}) are indicated.

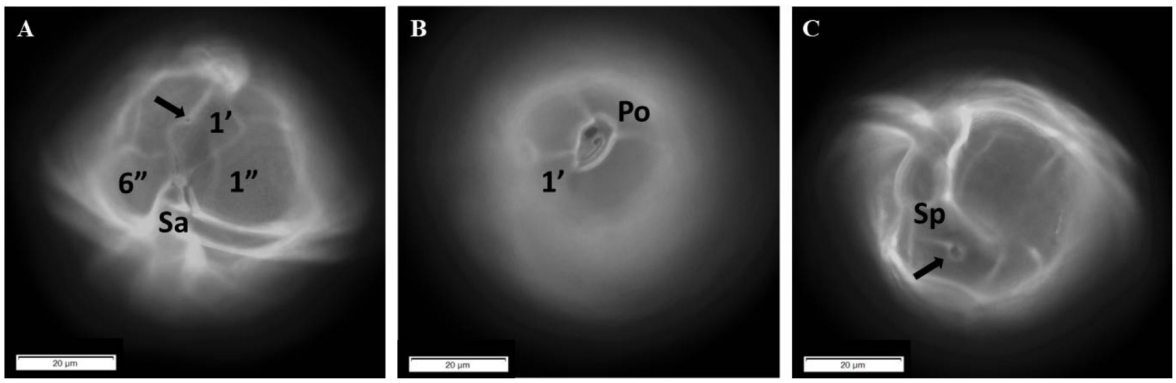


FIGURE 7 | Micrographs of *Alexandrium tamiyavanichii* in fluorescent microscopy showing some distinctive morphological characters. **(A)** Ventral view of a cell with the first apical plate (1′), the first and sixth precingular plate (1′′ and 6′′, respectively), and the anterior sulcal plate (Sa); the arrow points to the ventral pore. **(B)** Apical view showing the apical pore complex (Po) and the first apical plate (1′). **(C)** Antapical view with the posterior sulcal plate (Sp) and its large pore (arrowed).

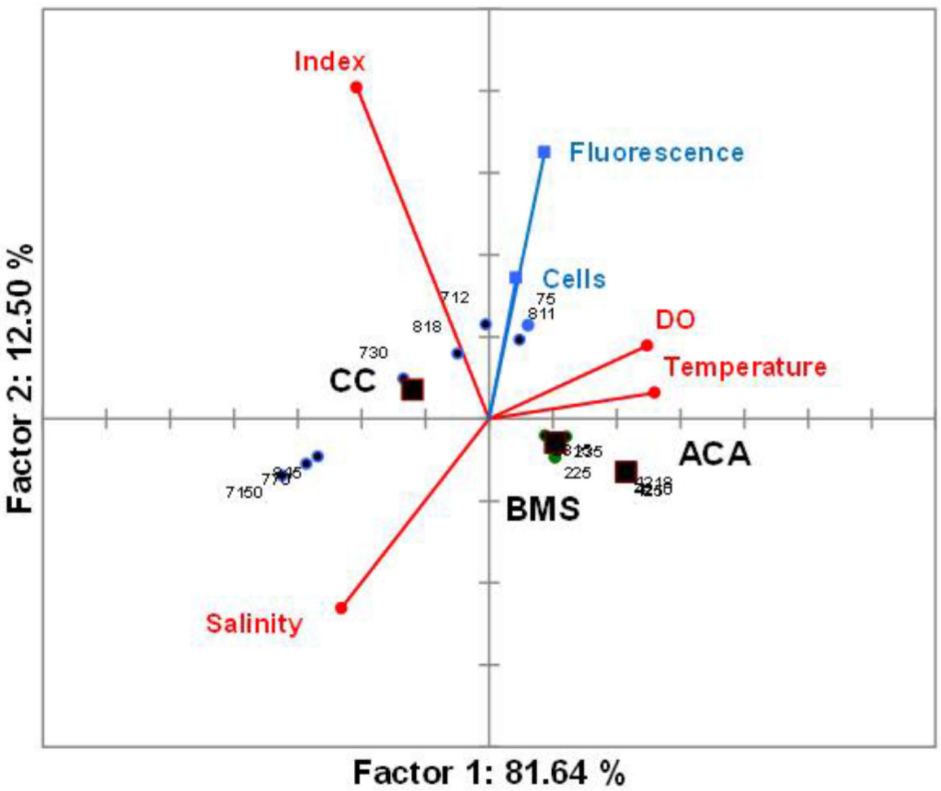


FIGURE 8 | Principal component analysis (PCA) biplot (variables and samples) of the two first components from data of environmental and biotic variables of the central Mexican Pacific. Circles represent the samples and squares are the centroids for qualitative variables. CC, Cabo Corrientes; BMS, Manzanillo; and ACA, Acapulco transects.

Statistical analyses support the visual relationships (**Figure 8**) and coincidence between the fluorescence (chlorophyll *a*) highest values and the highest *A. tamiyavanichii* abundances in Cabo Corrientes (**Figures 2B,D**, **6A**), and also the relationships with other environmental features such as temperature and dissolved oxygen, and induce to consider some preferences in environmental conditions the species may have: more stratified water column. At St 7, low abundances (40.61 cells m^{-3}) of *A. tamiyavanichii* were yet detected, deeper into the water column, up to 150 m depth (**Table 1**).

The cell densities of *A. tamiyavanichii* found in this study are comparable (e.g., the same magnitude order) to numbers reported in a different area, with a similar irregular distribution (regarding horizontal and vertical distribution), following the

same study method (Kon et al., 2015). Additionally, similar genetic structures are to be interpreted in populations found in various localities in waters of Malaysia and the populations studied in this work, for we pursued the same protocol.

It is plausible to consider that *Alexandrium* species may become an important component of the phytoplankton community in the Mexican Pacific, with the possibility of increasing their populations or expanding their geographic distribution, although until now there are not many reports of blooms or toxic episodes of the species of the genus (Hernández-Becerril et al., 2007). The use of molecular tools may improve our knowledge of the biodiversity and taxonomy of the phytoplankton in the Mexican Pacific, and aims the rapid and systematic reports of species involved in harmful events. The sensitivity of the method followed in this paper proved to be adequate for assessing the presence and distribution of *A. tamiyavanichii*.

AUTHOR CONTRIBUTIONS

DH-B designed the oceanographic cruise plan, obtained environmental data and samples, took samples for analysis, wrote and submitted the manuscript. WL made samples and data analysis. KSH made samples and data analysis and wrote part of the manuscript. FV-C performed the hydrographic and oceanographic information and statistical analysis. CPL supported samples analysis and edited part of the manuscript. PTL supported research stay and samples analysis.

FUNDING

Partial support for this study was provided by PAPIIT, DGAPA, UNAM (Project No. IN206516). Instituto de Ciencias del Mar y Limnología, Coordinación de la Investigación Científica (CIC, UNAM) approved and supported the use of the R/V "El Puma" (oceanographic cruise "MareaR IX," 19–30 April, 2017), and a short scientific stay of the senior author (DUH-B) to the Institute of Ocean and Earth Sciences, University of Malaya, Malaysia (June, 2017). Molecular work was supported by HiCOE IOES-2014C to PTL.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2018.00366/full#supplementary-material

FIGURE S1 | Mean surface chlorophyll in three zones of the central Mexican Pacific (Cabo Corrientes, Manzanillo and Acapulco), during April 19 **(left)** and April 27 **(right)**. Data from: https://coastwatch.pfeg.noaa.gov/erddap/griddap/erdMH1chla8day.graph?chlorophyll[(2017-04-19T00:00:00Z)][(25.85417):(15.14583)][(-110.4375):(-99.43749)]&.draw=surface&.vars=longitude%7Clatitude%7Cchlorophyll&.colorBar=%7C%7C%7C%7C%7C&.bgColor=0xffc cccff.

REFERENCES

Anderson, D. M., Alpermann, J. T., Cembella, A. D., Collos, Y., Masseret, E., and Montresor, M. (2012). The globally distributed genus *Alexandrium*: multifaceted roles in marine ecosystems and impacts on human health. *Harmful Algae* 14, 10–35. doi: 10.1016/j.hal.2011.10.012

Arvidsson, S., Kwasniewski, M., Riaño-Pachón, D. M., and Mueller-Roeber, B. (2008). QuantPrime – a flexible tool for reliable high-throughput primer design for quantitative PCR. *BMC Bioinformatics* 9:465. doi: 10.1186/1471-2105-9-465

Arya, M., Shergill, I. S., Williamson, M., Gommersall, L., Arya, N., and Patel, H. R. (2005). Basic principles of real-time quantitative PCR. *Expert Rev. Mol. Diagn.* 5, 209–219. doi: 10.1586/14737159.5.2.209

Balech, E. (1995). *The Genus Alexandrium Halim (Dinoflagellata)*. Cork: Sherkin Island Marine Station.

Beppu, R., Nojima, K., Tsuruda, S., Gomez-Delan, G., Barte-Quilantang, M., Taniyama, S., et al. (2008). Occurrence of PSP-producing dinoflagellate *Alexandrium tamiyavanichi* in Bingo-Nada, the central coastal water of the Seto Inland Sea, Hiroshima Prefecture, Japan. *Mar. Pollut. Bull.* 56, 758–763. doi: 10.1016/j.marpolbul.2007.12.005

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., et al. (2009). The MIQE Guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin. Chem.* 55, 611–622. doi: 10.1373/clinchem.2008.112797

Cembella, A. D., and Taylor, F. J. R. (1986). Electrophoretic variability within the *Protogonyaulax tamarensis/catenella* species complex; pyridine linked dehydrogenases. *Biochem. Syst. Ecol.* 14, 311–321. doi: 10.1016/0305-1978(86)90107-9

Davies, S. M., Sánchez-Velasco, L., Beier, E., Godínez, V. M., Barton, E. D., and Tamayo, A. (2015). Three-dimensional distribution of larval fish habitats in the shallow oxygen minimum zone in the eastern tropical Pacific Ocean off Mexico. *Deep Sea Res. Part I* 101, 118–129. doi: 10.1016/j.dsr.2015.04.003

Esqueda-Lara, K., and Hernández-Becerril, D. U. (2010). *Dinoflagelados microplanctónicos marinos del Pacífico central de México (Isla Isabel, Nayarit y costas de Jalisco y Colima)*. Mexico City: Universidad Nacional Autónoma de México, 206.

Fiedler, P. C., and Lavín, M. F. (2006). Introduction: a review of eastern tropical Pacific oceanography. *Prog. Oceanogr.* 69, 94–100. doi: 10.1016/j.pocean.2006.03.006

Fiedler, P. C., and Talley, L. D. (2006). Hydrography of the eastern tropical Pacific: a review. *Prog. Oceanogr.* 69, 143–180. doi: 10.1016/j.pocean.2006.03.008

Galluzzi, L., Penna, A., Bertozzini, E., Vila, M., Garcés, E., and Magnani, M. (2004). Development of a real-time PCR assay for rapid detection and quantification of *Alexandrium minutum* (a dinoflagellate). *Appl. Environ. Microbiol.* 70, 1199–1206. doi: 10.1128/AEM.70.2.1199-1206.2004

García, C., Bravo, M. C., Lagos, M., and Lagos, N. (2004). Paralytic shellfish poisoning: post-mortem analysis of tissue and body fluid samples from human victims in the Patagonia fjords. *Toxicon* 2, 149–158. doi: 10.1016/j.toxicon.2003.11.018

Hashimoto, T., Matsuoka, S., Yoshimastsu, S., Miki, K., Nishibori, N., Nishio, S., et al. (2002). First paralytic shellfish poison (PSP) infestation of bivalves due to toxic dinoflagellate *Alexandrium tamiyavanichii*, in the southeast coasts of the Seto, Inland Sea, Japan. *J. Food Hyg. Soc. Jpn.* 43, 1–5. doi: 10.3358/shokueishi.43.1

Hernández-Becerril, D. U., Alonso-Rodríguez, R., Álvarez-Góngora, C., Barón-Campis, S. A., Ceballos-Corona, G., Herrera-Silveira, J., et al. (2007). Toxic and harmful marine phytoplankton and microalgae (HABs) in Mexican coasts. *J. Env. Sci. Health Part A* 42, 1349–1363. doi: 10.1080/10934520701480219

Hernández-Becerril, D. U., Barón-Campis, S. A., and Escobar-Morales, S. (2012). A new record of *Azadinium spinosum* (Dinoflagellata) from the tropical Mexican Pacific. *Rev. Biol. Mar. Oceanogr.* 47, 553–557. doi: 10.4067/S0718-19572012000300016

Hernández-Becerril, D. U., Rodríguez-Palacio, M. C., and Lozano-Ramírez, C. (2013). Morphology and life stages of the potentially pinnatoxin producing thecate dinoflagellate *Vulcanodinium rugosum* from the tropical Mexican Pacific. *Bot. Mar.* 56, 535–540. doi: 10.1515/bot-2013-0079

Kessler, W. S. (2006). The circulation of the eastern tropical Pacific: a review. *Prog Oceanogr.* 69, 181–217. doi: 10.1016/j.pocean.2006.03.009

Kon, N. F., Teng, S. T., Hii, K. S., Yek, L. H., Aazani, M., Lim, H. C., et al. (2015). Spatial distribution of toxic *Alexandrium tamiyavanichii* (Dinophyceae) in the southeastern South China Sea-Sulu Sea: a molecular-based assessment using real-time quantitative PCR (qPCR) assay. *Harmful Algae* 50, 8–20. doi: 10.1016/j.hal.2015.10.002

Lim, P.-T., Leaw, C.-P., and Ogata, T. (2007). Morphological variation of two *Alexandrium* species responsible for paralytic shellfish poisoning in Southeast Asia. *Bot. Mar.* 50, 14–21. doi: 10.1515/BOT.2007.003

López-Sandoval, D. C., Lara-Lara, J. R., Lavín, M. F., Álvarez-Borrego, S., and Gaxiola-Castro, G. (2009). Productividad primaria en el Pacífico oriental tropical adyacente a Cabo Corrientes, México. *Ciencias Mar.* 35, 169–182. doi: 10.7773/cm.v35i2.1530

Mackenzie, L., de Salas, M., Adamson, J., and Beuzenberg, V. (2004). The dinoflagellate genus *Alexandrium* (Halim) in New Zealand coastal waters: comparative morphology, toxicity and molecular genetics. *Harmful Algae* 3, 71–92. doi: 10.1016/j.hal.2003.09.001

Menezes, M., Varela, D., de Oliveira Proença, L. A., da Silva, T. M., and Paredes, J. (2010). Identification of the toxic alga *Alexandrium tamiyavanichii* (Dinophyceae) from northeastern Brazil: a combined morphological and rDNA sequence (partial LSU and ITS) approach. *J. Phycol.* 46, 1239–1251. doi: 10.1111/j.1529-8817.2010.00918.x

Miller, P. E., and Scholin, C. A. (1998). Identification and enumeration of cultured and wild *Pseudo-nitzschia* (Bacillariophyta) using species-specific LSU rDNA-targeted fluorescent probes and filter-based whole cell hybridization. *J. Phycol.* 34, 371–382. doi: 10.1046/j.1529-8817.1998.340371.x

Mohammad-Noor, N., Adam, A., Lim, P. T., Leaw, C. P., Lau, W. L. S., Liow, G. R., et al. (2018). First report of paralytic shellfish poisoning (PSP) caused by *Alexandrium tamiyavanichii* in Kuantan Port, Pahang, East Coast of Malaysia. *Phycol. Res.* 66, 37–44. doi: 10.1111/pre.12205

Nagai, S. (2011). Development of a multiplex PCR assay for simultaneous detection of six *Alexandrium* species (Dinophyceae). *J. Phycol.* 47, 703–708. doi: 10.1111/j.1529-8817.2011.00976.x

Nguyen, N.L., and Larsen, J. (2004). "Gonyaulacales," in *Potentially Toxic Microalgae of Vietnamese Waters*, Eds J. Larsen and N. L. Nguyen (Lund: Opera Botanica a Societate Botanica Lundensi), 140, 73–117.

Ogata, T., Pholpunthin, P., Fukuyo, Y., and Kodama, M. (1990). Occurrence of *Alexandrium cohorticula* in Japanese coastal waters. *J. Appl. Phycol.* 2, 351–356. doi: 10.1007/BF02180925

Okolodkov, Y. B., and Gárate-Lizárraga, I. (2006). An annotated checklist of dinoflagellates (Dinophyceae) from the Mexican Pacific. *Acta Bot. Mex.* 74, 1–154. doi: 10.21829/abm74.2006.1008

Paulmier, A., and Ruiz-Pino, D. (2009). Oxygen minimum zones (OMZs) in the modern ocean. *Prog. Oceanogr.* 80, 113–128. doi: 10.1016/j.pocean.2008.08.001

Pelayo-Martínez, G., Olivos-Ortiz, A., Franco-Gordo, C., Quijano-Scheggia, S., Gaviño-Rodríguez, J., Kono-Martínez, T., et al. (2017). Physical, chemical and zooplankton biomass variability (inshore-offshore) of Mexican Central Pacific during El Niño-La Niña 2010. *Lat. Am. J. Aquat. Res.* 45, 67–78. doi: 10.3856/vol45-issue1-fulltext-7

Schlitzer, R. (2016). *Ocean Data View*. Available at: http://odv.awi.de/ [accessed May 29, 2018].

Scholin, C. A., Hallegraeff, G. M., and Anderson, D. M. (1995). Molecular evolution of the *Alexandrium tamarense* 'species complex' (Dinophyceae): dispersal in the North American and West Pacific regions. *Phycologia* 34, 472–485. doi: 10.2216/i0031-8884-34-6-472.1

Scholin, C. A., Herzog, M., Sogin, M., and Anderson, D. M. (1994). Identification of group- and strain-specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). II. Sequence analysis of a fragment of the LSU rRNA gene. *J. Phycol.* 30, 999–1011. doi: 10.1111/j.0022-3646.1994.00999.x

Steidinger, K. A., and Moestrup, T. (1990). "The taxonomy of Gonyaulax, Pyrodium, *Alexandrium, Gessnerium*, *Protogonyaulax* and *Goniodoma*," in *Toxic Marine Phytoplanton*, Eds F. Graneli, B. Sundstrom, L. Edler, and D. M. Anderson (New York, NY: Elsevier), 522–523.

Ulloa, O., Canfield, D. E., Delong, E. F., Letelier, R. M., and Stewart, F. J. (2012). Microbial oceanography of anoxic oxygen minimum zones. *Proc. Nat. Acad. Sci. U.S.A.* 109, 15996–16003. doi: 10.1073/pnas.1205009109

Usup, G., Leaw, C.-P., Asmat, A., and Lim, P.-T. (2002a). *Alexandrium* (Dinophyceae) species in Malaysian waters. *Harmful Algae* 1, 265–275.

Usup, G., Pin, L. C., Ahmad, A., and Teen, L. P. (2002b). Phylogenetic relationship of *Alexandrium tamayavanichii* (Dinophyceae) to other *Alexandrium* species based on ribosomal RNA gene sequences. *Harmful Algae* 1, 59–68. doi: 10.1016/S1568-9883(02)00003-3

Willett, C. S., Leben, R., and Lavín, M. F. (2006). Eddies and tropical instability waves in the eastern tropical Pacific: a review. *Prog. Oceanogr.* 69, 218–238. doi: 10.1016/j.pocean.2006.03.010

Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., and Madden, T. L. (2012). Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics* 13:134. doi: 10.1186/1471-2105-13-134

Zamudio, L., Hurlburt, H. E., Metzger, E. J., and Tilburg, C. E. (2007). Tropical wave-induced oceanic eddies at Cabo Corrientes and the Maria Islands, Mexico. *J. Geophys. Res.* 112, 1–17. doi: 10.1029/2006JC004018

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Hernández-Becerril, Lau, Hii, Leaw, Varona-Cordero and Lim. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Marine Science

ORIGINAL RESEARCH
published: 06 November 2018
doi: 10.3389/fmars.2018.00411

Large-Scale Sea Turtle Mortality Events in El Salvador Attributed to Paralytic Shellfish Toxin-Producing Algae Blooms

Oscar Amaya[1], Rebeca Quintanilla[1], Brian A. Stacy[2], Marie-Yasmine Dechraoui Bottein[3], Leanne Flewelling[4], Robert Hardy[4], Celina Dueñas[5] and Gerardo Ruiz[1]*

[1] Laboratorio de Toxinas Marinas, Facultad de Ciencias Naturales y Matemática, Universidad de El Salvador, San Salvador, El Salvador, [2] National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Office of Protected Resources at University of Florida, Gainesville, FL, United States, [3] Environment Laboratories, Department of Nuclear Science and Application, International Atomic Energy Agency, Monaco, Monaco, [4] Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute, St. Petersburg, FL, United States, [5] Gerencia de Vida Silvestre, Ministerio de Medio Ambiente y Recursos Naturales, San Salvador, El Salvador

OPEN ACCESS

Edited by:
Juan José Dorantes-Aranda, Institute for Marine and Antarctic Studies (IMAS), Australia

Reviewed by:
Gustaaf Marinus Hallegraeff, University of Tasmania, Australia
Pedro R. Costa, Instituto Português do Mar e da Atmosfera (IPMA), Portugal

***Correspondence:**
Rebeca Quintanilla
rebekquintanilla@gmail.com

Specialty section:
This article was submitted to Marine Ecosystem Ecology, a section of the journal Frontiers in Marine Science

Received: *01 August 2018*
Accepted: *16 October 2018*
Published: *06 November 2018*

Citation:
Amaya O, Quintanilla R, Stacy BA, Dechraoui Bottein M-Y, Flewelling L, Hardy R, Dueñas C and Ruiz G (2018) Large-Scale Sea Turtle Mortality Events in El Salvador Attributed to Paralytic Shellfish Toxin-Producing Algae Blooms. Front. Mar. Sci. 5:411. doi: 10.3389/fmars.2018.00411

In late October and early November 2013 and 2017, hundreds of sea turtles were found dead along the Pacific coastline of El Salvador. The dead turtles were in good body condition and did not have any injuries or other major anomalies. In order to determine the role of paralytic shellfish toxins (PST) in this mass mortality, tissue samples, including blood, flipper, liver, kidney, stomach and intestinal contents, of dead green turtles (*Chelonia mydas*) and olive ridley turtles (*Lepidochelys olivacea*) were analyzed for PST using a radioactive receptor binding assay, enzyme-linked immunosorbent assay, and high performance liquid chromatography. Highest values of PST were detected in enteric contents in the 2013 event (7,304.1 μg STX eq kg^{-1}) and in gastric contents during the 2017 event (16,165.0 μg STX eq kg^{-1}). During these events, remotely-sensed chlorophyll-*a* and fluorescence line height imagery revealed anomalies suggestive of algal blooms off the coast of El Salvador. In the 2017 event, *Pyrodinium bahamense* was observed in samples of gastrointestinal contents from affected sea turtles. Seawater from the region where dead sea turtles were found was also analyzed, but saxitoxin-producing species were found in low abundance (5400 cell/L in 2013 and 672 cell/L in 2017), which may reflect limited sampling. Although threshold levels of toxicity in sea turtle species are not well-characterized, our evidence suggests that these large events were the result of PST-producing algal blooms and that these blooms are a major cause of sea turtle mortality in this region.

Keywords: sea turtle, paralytic shellfish poisoning, saxitoxin, receptor binding assay, HABs

INTRODUCTION

Harmful algal blooms (HABs) negatively affect not only human health, but also that of marine ecosystems. These effects include hypoxia/anoxia events, decreased water clarity, and altered feeding behavior and toxicosis of marine fauna (Zingone and Enevoldsen, 2000). Toxin-producing algal blooms are recurrent off the coast of El Salvador and are predominately paralytic shellfish

poisoning events, which are produced by several paralytic shellfish toxin (PST)-producing dinoflagellates, such as *Pyrodinium bahamense* var. *compressum*, *Gymnodinium catenatum,* and *Alexandrium* spp. These events have caused human intoxication and death, as well as mortality of marine fauna (Espinoza et al., 2013). Globally, exposure to PST toxins has been associated with mortality of marine mammals (Geraci et al., 1989; Van Dolah et al., 2003; Lefebvre et al., 2016), sea birds (Shumway et al., 2003; Shearn-Bochsler et al., 2014), estuarine turtles (Hattenrath-Lehmann et al., 2017), and sea turtles (Maclean, 1975; Licea et al., 2008).

Four of the seven sea turtle species found in the world nest or forage in El Salvador and its waters. All species are considered vulnerable, endangered, or critically endangered by the International Union for the Conservation of Nature (IUCN, 2018) Red List. In 2005, over 200 sea turtles, including green turtles (*Chelonia mydas*) and olive ridley turtles (*Lepidochelys olivacea*), were found dead along the coast of El Salvador. The mortality event was attributed to PST based on concentrations found in brain samples which were as high as of 6278.0 μg STX eq kg^{-1} (Licea et al., 2008). Additionally, in 2010 two hawksbill turtles (*Eretmochelys imbricata*) were found dying after a *Pyrodinium bahamense* bloom. One of the turtles was found with 1212.5 μg STX eq kg^{-1} in brain tissue, and both individuals had diarrhea and exhibited erratic swimming, disorientation, and reduced activity (Licea et al., 2013). However, the limited toxin analytical data prevented confident attribution of clinical signs to PSP (Licea et al., 2013).

Two additional large sea turtle mortality events occurred in El Salvador waters in recent years. In early October 2013, the Ministry of Environment and Natural Resources (MARN) received a report from fishermen of dozens of dead sea turtles floating far from the coast, drifting northwest. In a subsequent report, 40 dead sea turtles were found on beaches of La Paz and La Libertad during September and October, and an undetermined number on beaches in Usulután. On October 28th, 2017, an estimated 400 dead sea turtles were found floating approximately 12 miles off the coast of Bahía de Jiquilisco. In the following days, 160 turtles were observed around 7 miles off the coast of Puerto Parada, of which 60% were *L. olivacea* and 40% were *C. mydas*. Between November 3rd and 6th, another 47 sea turtles were found floating off the coast of Usulután and La Paz and 25 *L. olivacea* individuals were detected 10 miles off El Cordoncillo in La Paz. No other dead marine organisms were observed during either event. The turtles were in good nutritional condition based on muscle mass and body fat and did not have any apparent injuries or other abnormalities to explain the cause of death.

PST-producing HABs were again suspected and samples of tissues, gastrointestinal contents, and seawater were collected for biotoxin analysis and phytoplankton identification. In addition, data collected by satellite (MODIS-Aqua) were reviewed for evidence of possible algal blooms. Herein, we report the results of these analyses as evidence that both mortality events were caused by PST exposure, demonstrating that PST-producing algal blooms are a substantial cause of sea turtle mortality in the region.

METHODS

Paralytic Shellfish Toxins Analyses

Tissue samples from *C. mydas* and *L. olivacea* were collected by the MARN from different sites along the coast during stranding events in 2013 and 2017 (**Figure 1**) and analyzed for PST at three different laboratories. In 2013, stomach, intestines, liver and kidneys were taken from 13 *C. mydas* that stranded in El Amatal-Toluca, San Diego, and El Pimental were analyzed by Laboratorio de Toxinas Marinas at the University of El Salvador (LABTOX-UES); 24 samples consisting of brain, enteric content, kidney, liver, and salt gland were analyzed by the International Atomic Energy Agency (IAEA) Environment Laboratories in Monaco; and 7 samples consisting of enteric contents, liver, kidney, and lung from 5 *C. mydas* that stranded in El Amatal and El Pimental and one *L. olivacea* that stranded in Barra de Santiago, were analyzed by the Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute (FWRI). CITES export and import permits were obtained for all samples exported to the U.S.

In the 2017 event, 25 samples consisting of whole blood, serum, soft tissue from flippers from 23 *C. mydas,* and liver and intestinal contents from one *L. olivacea* were analyzed by LABTOX-UES. Additionally, 28 samples consisting of sea turtle liver, stomach contents, and enteric contents from 14 *C. mydas* and one *L. olivacea* were analyzed by FWRI.

Samples were analyzed for PST at LABTOX-UES and IAEA Environment Laboratories using a receptor binding assay (RBA) that is the AOAC official method of analysis (OMA-2011-27) (Amaya et al., 2012; Van Dolah et al., 2012; Dechraoui Bottein and Clausing, 2017). Briefly, the RBA measures competition between a radiolabeled ^{3}H-STX and the PSTs present in samples for binding to voltage gated sodium channels in brain membrane preparations. The quantification is obtained against a standard curve generated using increasing concentrations of unlabeled STX reference material (S. Hall, United States Food and Drug Administration/Center for Food Safety and Applied Nutrition, Washington, DC). Toxin concentration is reported as micrograms of STX equivalents per kilogram of sample (μg STX eq. kg^{-1}). The assay format described in the present study provides quantitative determination of the compound toxicity of PST in turtle extracts using a MicroBeta Trilux 1450 LSC PerkinElmer with 96-well microplate. For analyses conducted by LABTOX-UES, detection limits were 150 μg STX eq. kg^{-1} in 2013 and 70 μg STX eq. kg^{-1} in 2017.

FWRI analyzed samples for PST using two methods, enzyme-linked immunosorbent assay (ELISA) and high performance liquid chromatography (HPLC). Some changes in laboratory methodology occurred between 2013 and 2017. Frozen samples were completely thawed and homogenized before sub-sampling. To extract PST, 0.1M HCl was added at a ratio of 1:1 (w:v) in 2013 or 1:10 (w:v) in 2017. The mixture was adjusted to pH 2.5-4, boiled for 5 min in a water bath, and then centrifuged at 3,000 × g for 20 min. The supernatant was retained.

Samples from the 2013 event were analyzed for PST using HPLC with pre-column oxidation and fluorescence detection (Lawrence and Niedzwiadek, 2001) only. The HPLC

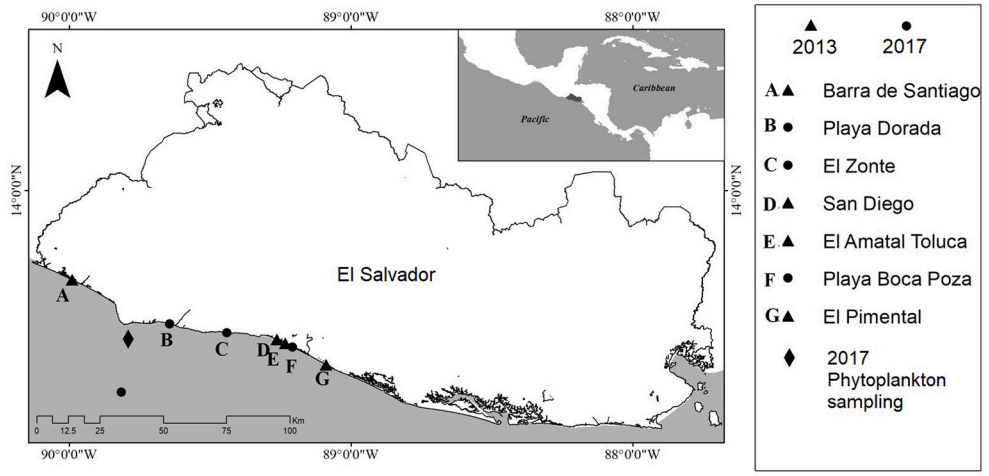


FIGURE 1 | Location of sea turtle strandings in 2013 and 2017 and phytoplankton sampling locations in 2017.

system consisted of a Shimadzu (Tokyo, Japan) chromatograph equipped with an SCL-10A VP controller, LC-10 AD pump, SIL-10AF autosampler, CTO-10AS VP column oven, and RF-10A XL fluorescence detector. Sample clean-up and PST separations were performed as described in Lawrence and Niedzwiadek (2001). Certified reference standards of STX, NEO, dcSTX, B1, GTX 1/4, GTX 2/3, dcGTX-2/3, and C1/2 (National Research Council, Canada) were used for instrument calibration. Detection limits varied for each congener, ranging between 6 and 18 ng mL^{-1} extract and 12–36 $\mu g\ kg^{-1}$ tissue. Total HPLC-FL results are expressed as STX equivalents calculated using experimentally derived toxicity factors for each congener (Oshima, 1995).

In 2017, sample extracts were first screened using the Abraxis Saxitoxin (PSP) ELISA. All extracts were diluted with provided sample diluent to 0.01 g tissue equivalent per ml or less prior to loading on the plate. The Abraxis Saxitoxin (PSP) ELISA is a direct competitive ELISA specific to STX, with recognition of other PST to varying degrees. All samples and standards were run in duplicate wells, and the plate was analyzed at a wavelength of 450 nm on a BioTek μQuant^{TM} microplate spectrophotometer. The kit is calibrated using STX, and results are expressed as STX equivalents. The limit of detection for the ELISA as performed was 2 μg STX eq. kg^{-1}. Toxin confirmation and characterization were obtained for a subset of sample extracts using HPLC-FL as described above. Liver samples selected for HPLC confirmation were re-extracted at a ratio of 1:1 (w:v) to yield a more concentrated sample extract.

Phytoplankton Analyses

Sampling campaigns were carried out to monitor toxic microalgae during both mortality events. In 2013, samples were taken in La Libertad and Acajutla in October 15th and 16th. In 2017, samples were taken on November 7th 2017, at Los Cóbanos (**Figure 1**). This site was selected because the Ministry of the Environment and Natural Resources received reports of sea turtle stranding in the central and west coast of El Salvador, after the dead sea turtles were located floating in Bahía de Jiquilisco. Water samples were collected at five points along a 15 nautical mile transect perpendicular to Los Cóbanos coastline. Samples for phytoplankton quantification were collected using a Niskin bottle and using a phytoplankton net.

Phytoplankton abundance was estimated using an inverted microscope, following the Utermöhl method (Utermöhl, 1958). Additionally, in the 2017 event, intestinal contents of one *L. olivacea* and one *C. mydas* were screened for the presence of toxic microalgae using an inverted microscope by LABTOX-UES; and two gastric and two enteric contents were examined by FWRI using light microscopy.

Satellite Imagery Analyses

We examined corresponding chlorophyll-*a* (Chl) and normalized fluorescence line height (NFLH) imagery to visualize the approximate location and spatiotemporal extent of a potential algae bloom. The NFLH imagery provide an alternative source of imagery that is capable of detecting blooms but is less sensitive to interference by non-algal substances common in coastal waters (e.g., dissolved organic matter; Hu et al., 2005).

RESULTS

Paralytic Shellfish Toxins Analyses

During both events, PST were detected in most sample types from all sea turtles. Out of the 75 tissue samples analyzed, 63 were positive for PST and 12 were below the detection limits (**Figure 2**). Those samples that were below detection limits included heart and fat, and well as blood, serum, and flipper soft tissue sampled during 2017.

Highest PST concentrations were detected in enteric contents in the 2013 event (7304.1 μg STX eq. kg^{-1} using RBA method) and in gastric contents during the 2017 event (16165.0 μg STX eq. kg^{-1} using ELISA) (**Table 1**). Both values were found in *C. mydas* individuals. In general, PST concentrations found in 2017 were

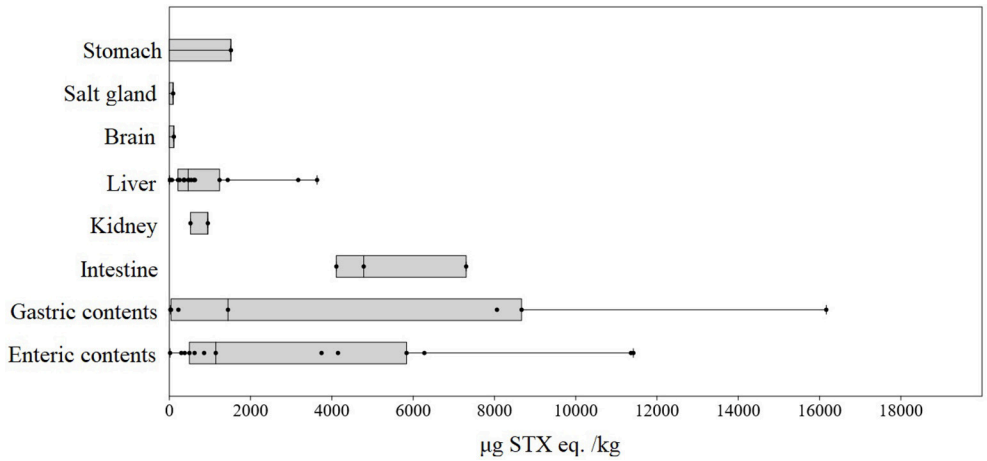


FIGURE 2 | PST concentration (µg STX eq. kg^{-1}) in sea turtles (*C. mydas* and *L. olivacea*) per sample type from both 2013 and 2017 mortality events.

higher than those found in 2013. PST concentrations in tissues from brain, lung and salt glands were generally lower, ranging from 94.1 (RBA) to 136.0 µg (HPLC-FL) STX eq. kg^{-1} ($n = 6$) (**Figure 1**). Particularly in the brain, the concentration was 111.1 µg STX eq. kg^{-1}.

During 2013 event, minimum PST concentrations were found in *C. mydas* intestinal contents with 18.8 µg STX eq. kg^{-1}; while in 2017, minimum PST concentration were found in *C. mydas* liver with 4.0 µg STX eq. kg^{-1}.

Ten of the samples analyzed at FWRI in 2017 were selected for HPLC-FL analysis, and the presence of PST was confirmed in all 10 samples. Of the PST monitored for (STX, NEO, dcSTX, B1, GTX 1/4, GTX 2/3, dcGTX-2/3, and C1/2) only STX, B1, and dcSTX were detected (**Table 2**). The toxin profile was dominated by STX, which represented 63–82% of the toxin present. B1 accounted for 14–33%, and dcSTX was present at low levels (0–7% of the total).

Phytoplankton Analyses

The most abundant potential producer of PST found in the phytoplankton samples from 2013 was *Gymnodinium catenatum* (5,400 cells L^{-1}) in Acajutla. Other PST-producing species were detected in low abundance, such as *Pyrodinium bahamense* and *Alexandrium monilatum* (**Table 3**). During 2017, *Alexandrium sp.* was the only potential PST-producing microalgae detected in water samples from Los Cóbanos, with a low abundance of 672 cell per liter. The most abundant phytoplankton species found in the area were the diatoms *Dactyliosolen fragilissimus* and *Pseudo-nitzschia* spp., with 110,235 cell L^{-1} and 4,033 cell L^{-1}, respectively (**Table 3**).

In contrast to the water samples, microscopy of gastrointestinal contents of dead turtles from 2017 found *Pyrodinium bahamense* cells and thecae in gastric and enteric contents from two *C. mydas*. No PST-producing dinoflagellates were found in the enteric contents of an additional *C. mydas* and one *L. olivacea*, but whole cells of *Planktoniella sol, Scrippsiella trochoidea,* and *Prorocentrum compressum* were found in low abundance.

Satellite Imagery Analyses

During early September 2013, a potential dinoflagellate bloom was present off the western coast of El Salvador (**Figures 3A,B**). Another suspect bloom appeared during mid-September 2013 along the central coast (**Figures 3C,D**) and persisted through the end of the month (**Figures 3E,F**).

Throughout October 2017, a potential dinoflagellate bloom was present off El Salvador's central coast (**Figure 4**). In addition, a potential offshore bloom appeared to the south of the central coast during late October (**Figures 4C,D**).

DISCUSSION

Previous sea turtle mass mortality events suspected to be caused by PST-producing dinoflagellate blooms have been reported on the Pacific coast of Mexico and Central America, and in Papua New Guinea; however, very limited information about these events is available (Maclean, 1975; Licea et al., 2008, 2013; Meave del Castillo et al., 2008).

There are significant challenges associated with investigating sea turtle mortality events in many regions of the world, including the remote nature of many coastal areas, limited logistical resources, and inevitable delays between initial observations and response to reports. Although our sample size and some aspects of our data reflect these challenges, we were able to characterize two relatively large sea turtle mortality events in El Salvador and provide evidence that exposure to PST was the most likely cause. This evidence includes postmortem findings consistent with acute toxicosis, detection of relatively high concentrations of PST in gastrointestinal contents and tissues, observation of PST-producing dinoflagellates within gastrointestinal contents, and remote-sensing data suggestive of dinoflagellate blooms concurrent with sea turtle mortality.

TABLE 1 | Maximum PST concentrations for each sample type using Receptor Binding Assay, HPLC-FL and ELISA in sea turtle tissues from 2013 and 2017 mortality events.

Year	Location	Species	Sample type	μg STX eq. kg^{-1}	References
2005-2006	El Salvador	*Lepidochelys olivacea*	Not specified	6278.0***	Licea et al., 2008
2010	Jiquilisco Bay, El Salvador	*Eretmochelys imbricate*	Not specified	1,212.5***	Licea et al., 2013
2015	Flanders Bay, USA	*Malaclemys terrapin*	Heart	125.0***	Hattenrath-Lehmann et al., 2017
2013	El Pimental, El Salvador	*C. mydas*	Enteric content	7,304.1*	This study
	El Pimental, El Salvador	*C. mydas*	Liver	3,635.4*	
	San Diego, El Salvador	*C. mydas*	Stomach	1,515.3*	
	El Pimental	*C. mydas*	Kidney	977.4*	
	Barra de Santiago, El Salvador	*L. olivacea*	Brain	111.1*	
	El Pimental	*C. mydas*	Salt gland	94.1*	
	El Amatal-Toluca, El Salvador	*C. mydas*	Lung	136.0***	
2017	Sihuapilapa, El Salvador	*C. mydas*	Gastric content	16,165.5**	This study
	El Zonte, El Salvador	*C. mydas*	Enteric content	11,418.6**	
	Sihuapilapa, El Salvador	*C. mydas*	Liver	706.1***	

**RBA, **ELISA, ***HPLC. DL, detection limit.*

TABLE 2 | Results of analysis for saxitoxin (STX) and gonyautoxins (GTX) using high performance liquid chromatography with fluorescence detection (HPLC-FL) for 2017 mortality event.

Case number	Species	Location	Sample type	HPLC-FL (μg/kg)		
				STX	B1 (GTX-5)	dcSTX
15	*C. mydas*	Playa Dorada	EC	2,290.5	652.3	<DL
15	*C. mydas*	Playa Dorada	L	244.6	129	15.6
14	*C. mydas*	Playa El Zonte	L	378.6	186.6	19
14	*C. mydas*	Playa El Zonte	EC	4,185.1	2,010	257.1
14	*C. mydas*	Playa El Zonte	GC	1,936.6	714.8	<DL
12	*C. mydas*	Playa El Majahual	EC	5,998.7	2,390.1	653.2
11	*C. mydas*	Playa El Maguey	GC	6,683.3	1,615.9	365.7
10	*C. mydas*	Playa El Maguey	EC	11,614.2	2,002.5	566.5
4	*C. mydas*	Playa Sihuapilapa	GC	7,026.6	3,092.9	283.8
3	*C. mydas*	Playa Sihuapilapa	L	679.5	238.7	24.1

Same case numbers represent samples from the same individual. EC, enteric contents; L, liver; GC, gastric content.

In *Chelonia mydas*, higher concentrations of STX has been found in the gastro-intestinal tract contents rather than tissues (Capper et al., 2013). Toxicity thresholds have not been defined for PST in chelonians or other reptiles. Given their conservation status, exposure studies in sea turtles are not feasible and laboratory studies using non-imperiled chelonians have not yet been conducted. Attribution of clinical effect and mortality currently relies on exclusion of other possible causes and comparison of toxin exposures associated with mortality during known blooms, as well as exposure in turtles that are clinically stable or those that died from apparently unrelated causes. The dead turtles found during both events described herein were consistent with the general features of sea turtle mortality attributed to brevetoxicosis, another biotoxin that acts on neuronal voltage-gated sodium channels (Fauquier et al., 2013; Walker et al., 2018). These features include strandings of turtles in good nutritional condition without injuries, other abnormalities, or known association with other causes of mortality. Similar to a mortality event involving diamondback terrapins (*Malaclemys terrapin)* associated with

TABLE 3 | Concentration of the most abundant phytoplankton species found in La Libertad and Acajutla during the 2013 event, and in Los Cóbanos during 2017.

Species	Cell abundance (cells/L)		
	2013		2017
	La Libertad	Acajutla	Los Cóbanos
Alexandrium monilatum	ND	4080	ND
Alexandrium sp.	ND	ND	672
Chaetoceros affinis	ND	ND	2,689
Cochlodinium polykrikoides	880	520	ND
Dactyliosolen fragilissimus	ND	ND	110,235
Gonyaulax sp.	ND	ND	672
Guinardia striata	ND	ND	3,361
Gymnodinium catenatum	480	5400	ND
Gyrodinium instriatum	120	480	ND
Oxytoxum sp.	ND	ND	1,344
Pseudo-nitzschia spp.	ND	ND	4,033
Pyrodinium bahamense	20	ND	ND

ND, not detected.

a PST-producing *Alexandrium* bloom (Hattenrath-Lehmann et al., 2017), all turtles were found dead thus we were unable to ascertain whether affected turtles exhibited signs of neurotoxicosis. However, as previously reviewed, abnormal neurological signs including, erratic swimming, disorientation, and reduced activity were observed in stranded turtles found in 2010, one of which had detectable STX in brain tissue (Licea et al., 2013).

The PST concentrations detected in sea turtles that were found during both events were similar to those reported in 2006 (Licea et al., 2008) and 2010 (Licea et al., 2013), the only other published accounts of sea turtle mortality associated with PSP that includes toxin analyses. Our values were much higher than detected in the aforementioned *M. terrapin* mortality event (Hattenrath-Lehmann et al., 2017). In the case of PST found in one sample from brain tissue in 2013, the value is lower than found in brain tissue from the 2005 to 2006 mortality event (Licea et al., 2008). Two of the authors (Stacy and Flewelling, unpublished data) have screened sea turtles found stranded in Florida for PST as part of health studies and investigation of mortality events. In those results, concentrations typically are below detectable limits (10 ng/g) or well below those documented in this report, including within areas that experience periodic PST-producing blooms. Thus, although some degree of asymptomatic PST exposure almost certainly occurs in sea turtles, we have not found evidence of widespread background exposure similar to that measured during these events as a complication related to interpretation of analytical results. Nonetheless, broader sampling is needed of sea turtles during mortality events and PST-producing blooms and from unrelated circumstances with which to better understand PST exposure in Central America.

The opportunistic nature of sampling during these events did not allow conclusions to be drawn regarding differences in toxin concentrations among turtles and sample types other than variation is attributable to potential differences in dose, timing of exposure, toxin absorption and metabolism, and analytical methods. There are notable differences in the assays performed, which were determined by logistical considerations during each event. For example, RBA measures overall PST toxicity, whereas HPLC measures concentrations of specific congeners (Costa et al., 2009). ELISA is very sensitive and useful for screening for exposure, and analytical confirmation was achieved for the 2017 animals screened by ELISA. However, we did not have the ability to compare results of the same samples across all three methods. Future efforts should endeavor to standardize both sampling and analytical methods to the degree possible.

We observed anomalies in remotely-sensed imagery suggesting possible dinoflagellate blooms during both events. Unfortunately, available water samples were inadequate to confirm that the anomalies observed in satellite imagery were blooms of PST-producing species; corresponding *in situ* water samples are essential to confirm the presence of a bloom (Hu et al., 2005). The spatiotemporal extent of water sampling was extremely limited and was not considered representative of the anomalies detected by satellite. Nevertheless, the possibility that the observed anomalies were caused by blooms of *Pyrodinium bahamanse* and the likelihood that such blooms contributed to these mortality events are strengthened by concurrent detection of PST exposure in turtles, detection of *Pyrodinium bahamense* in the gastrointestinal contents of some turtles, and the observed toxin profiles. PST toxin profiles reported for *P. bahamense* vary somewhat from location to location, but generally contain fewer toxins than those described for other PST-producing dinoflagellates (Wiese et al., 2010). Profiles consisting of STX, NEO, dcSTX, GTX5, and GTX6 were reported in *P. bahamense* clones both from Palau (Harada et al., 1982, 1983) and Malaysia (Usup et al., 1994). *Pyrodinium bahamense* isolates from the Philippines and the southeastern US contained only STX, GTX5, and dcSTX (Landsberg et al., 2006; Gedaria et al., 2007), similar to the PST profile observed in the turtles analyzed in the present study. Although we were unable to confirm and identify blooms of specific species, the remotely-sensed data provided insight into the possible locations and extent of the blooms that may have resulted in toxin exposure and caused sea turtle mortality. Hopefully, this technology will be used to inform real-time sampling during future events.

Another consideration relevant to collection and interpretation of environmental data is the potential for persistent toxin exposure following dissipation of blooms. In marine animal mortality events attributed to brevetoxicosis, animals are not only exposed to high toxin levels during bloom periods, but also to toxins circulating through food webs for weeks or months after a bloom has dissipated (Landsberg et al., 2009). The same potential may exist for PST.

The number of sea turtles found likely reflects a minority of affected animals. Previous studies of beach strandings have estimated that around 5–20% of sea turtles that die at sea are subsequently found on shore (Hart et al., 2006; Mancini et al., 2011; Koch et al., 2013). Therefore, actual mortality

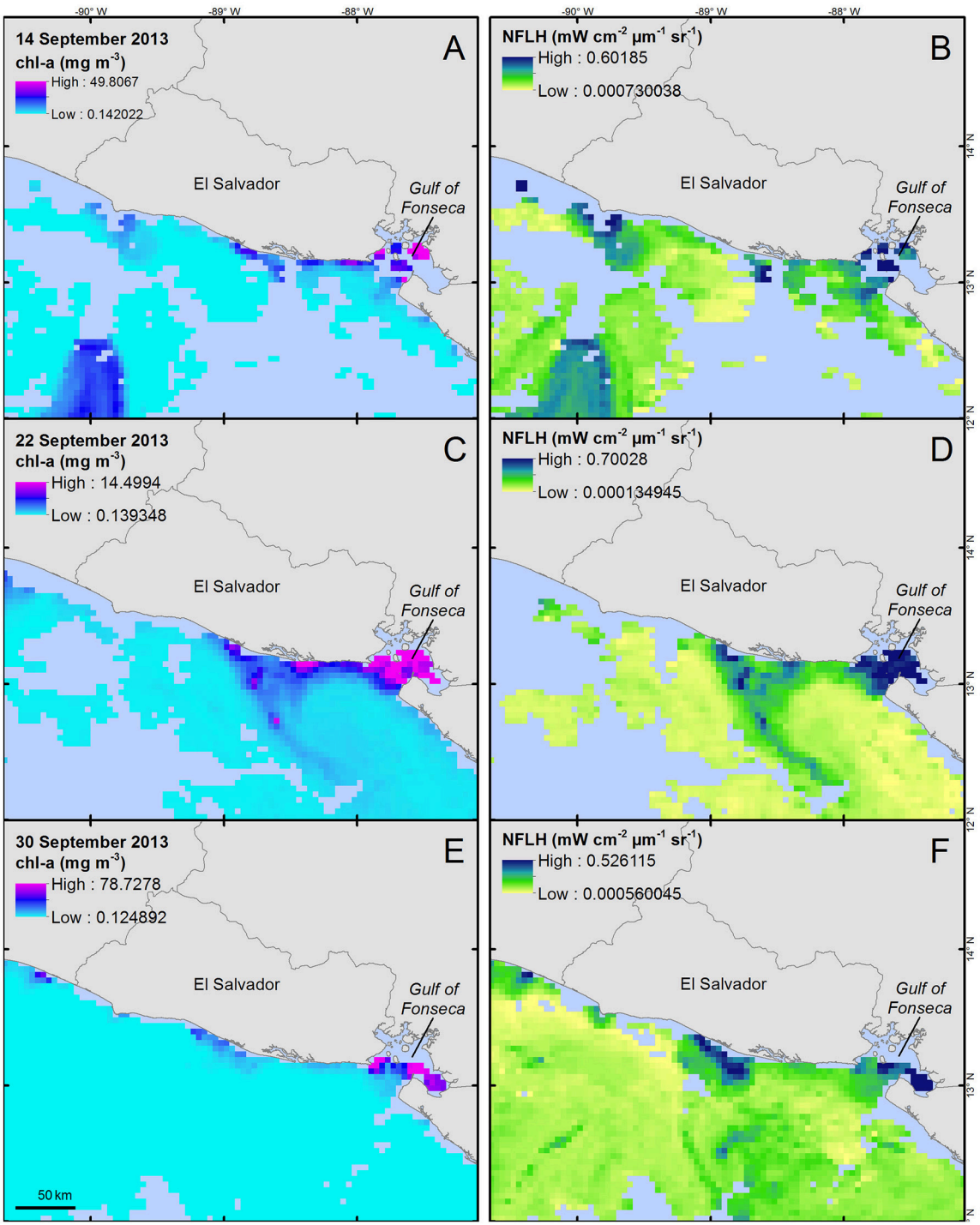


FIGURE 3 | Chlorophyll-a concentration (**A,C,E**, units = mg m^{-3}) and normalized fluorescence line height (**B,D,F**, units = mW cm^{-2} μm^{-1} sr^{-1}) for three 8-day time periods during September 2013. Data were collected from the MODIS-Aqua sensor and provided by NASA's Giovanni system (Acker and Leptoukh, 2007). Corresponding images represent 8-day means for each parameter ending on the dates displayed in the images; **(A,B)** represent 6–14 September data, **(C,D)** represent 14–22 September, and **(E,F)** represent 22–30 September. All maps are shown at an equal spatial scale.

from both events could have numbered in the hundreds or even thousands of sea turtles. Moreover, the 2013 event may have been widespread based on the extent of the potential bloom observed in remotely-sensed imagery and reported concurrent sea turtle strandings in Guatemala (Brittain et al., 2014).

Given the status of sea turtles and efforts that are required to manage significant anthropogenic threats, an

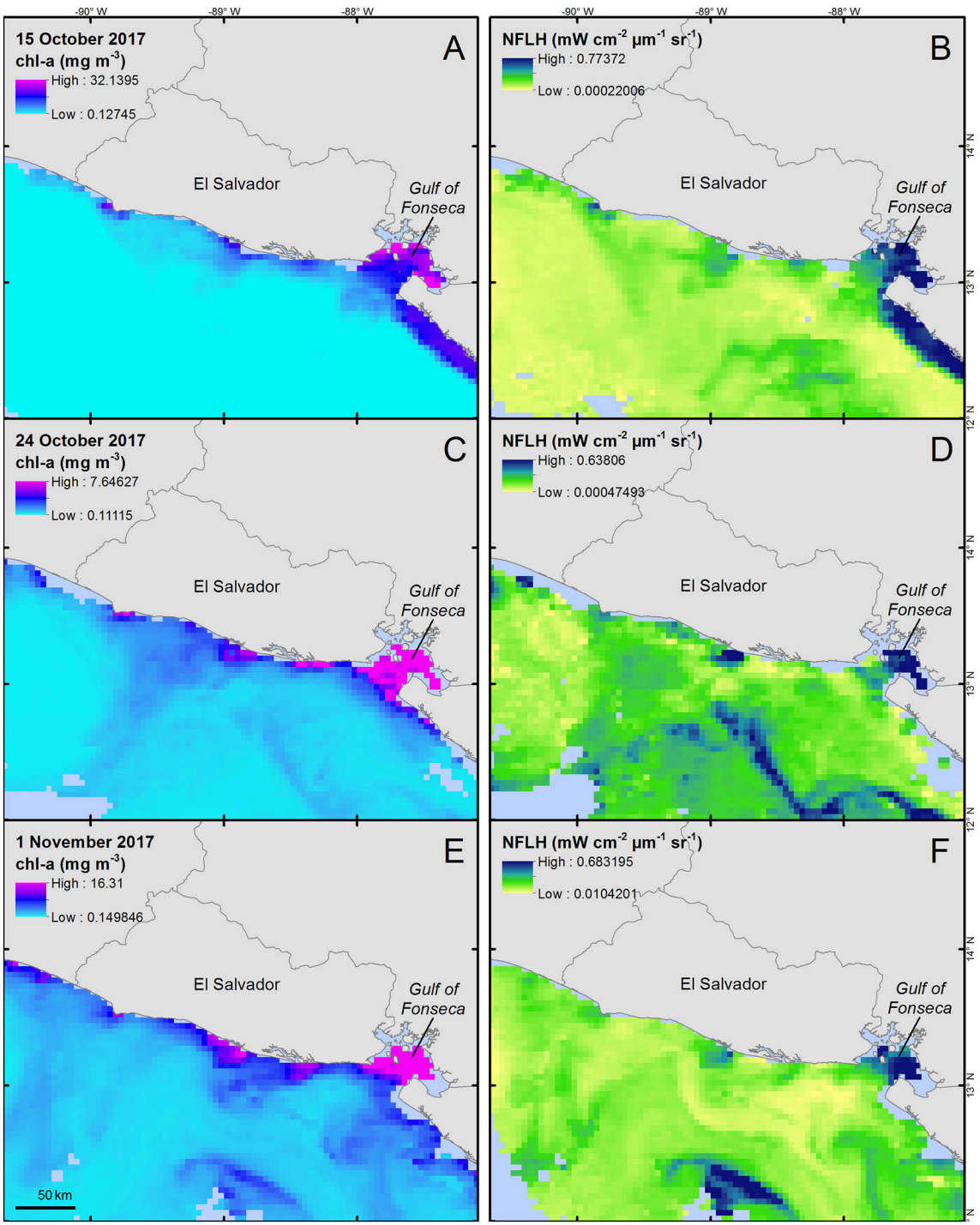


FIGURE 4 | Chlorophyll-a concentration (**A,C,E**, units = mg m^{-3}) and normalized fluorescence line height (**B,D,F**, units = mW cm^{-2} μm^{-1} sr^{-1}) for three 8-day time periods during October 2017. Data were collected from the MODIS-Aqua sensor and provided by NASA's Giovanni system (Acker and Leptoukh, 2007). Corresponding images represent 8-day means for each parameter ending on the dates displayed in the images; **(A,B)** represent 8–15 October data, **(C,D)** represent 16–24 October, and **(E,F)** represent 24 October−1 November. All maps are shown at an equal spatial scale.

understanding of various causes of mortality is important for conservation and management. The number of sea turtle deaths attributed to HABs on the Pacific coast of Central America is notable and warrants further effort to understand the factors that contribute to these events.

AUTHOR CONTRIBUTIONS

OA, RQ, and M-YD contributed conception and design of the study. RQ, LF, and BS organized the database. RQ performed the statistical analysis. OA wrote the first draft of the manuscript. RQ, M-YD, BS, RH, LF, CD, and GR wrote sections of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

ACKNOWLEDGMENTS

We thank the International Agency of Atomic Energy through projects RLA7020 and RL7022, the staff from the Ecosystems and Wildlife Department of the Ministry of the Environment and Natural Resources, the guards of Los Cóbanos Marine Protected Area and Puerto Parada, staff from Governance and Territory Department of the Ministry of the Environment and Natural Resources, the Network of Local Observers of MARN, Civil National Police, NGOs PROCOSTA-ICAPO, FUNZEL and ACOTOMBSAB, and the people who supported in the collection of tissues and stranding information. The International Atomic Energy Agency is grateful to the Government of the Principality of Monaco for the support provided to its Environment Laboratories. We also thank the U.S. Department of State for facilitating diagnostic analyses and April Granholm (FWRI) and Sugandha Shankar (FWRI) for performing toxin analyses. Satellite imagery analyses and visualizations used in this study were produced with the Giovanni online data system, developed and maintained by the NASA GES DISC.

REFERENCES

Acker, J. G., and Leptoukh, G. (2007). Online Analysis Enhances Use of NASA Earth Science Data. *Eos. Trans. AGU* 88, 14–17. doi: 10.1029/2007EO020003

Amaya, O., Ruiz, G., Rivera, W., and Espinoza, J. (2012). Application of a Receptor Binding Assay to the Analyses of PSP Toxins in Four Species of Shellfish in El Salvador. *Harmful Algae News* 45, 14–15.

Brittain, R., Lucas, S., Handy, S., and Nunny, R. (2014). Sea Turtle Strandings and Mortalities on the Southeast Coast of Guatemala. *Marine Turtle Newsl.* 143, 10–13.

Capper, A., Flewelling, L. J., and Arthur, K. (2013). Dietary Exposure to Harmful Algal Bloom (HAB) Toxins in the Endangered Manatee (Trichechus Manatus Latirostris) and Green Sea Turtle (*Chelonia mydas*) in Florida, USA. *Harmful Algae* 28, 1–9. doi: 10.1016/j.hal.2013.04.009.

Costa, P. R., Baugh, K. A., Wright, B., RaLonde, R., Nance, S. L., Tatarenkova, N., et al. (2009). Comparative determination of paralyic shellfish toxins (PSTs) using five different toxin detection methods in shellfish species collected in the Aleutian Islands, Alaska. *Toxicon* 54, 313–320. doi: 10.1016/j.toxicon.2009.04.023

Dechraoui Bottein, M.-Y., and Clausing, R. J. (2017). "Receptor-Binding Assay for the Analysis of Marine Toxins: Detection and Mode of Action." in *Recent Advances in the Analysis of Marine Toxins*, Vol. 78, eds J. Diogene and M. Campas (Amsterdam: Elsevier B. V.), 277–301. doi: 10.1016/bs.coac.2017.08.004

Espinoza, J., Amaya, O., and Quintanilla, R. (2013). *Atlas de Fitoplancton Marino*. 1st Edn. San Salvador: Universidad de El Salvador.

Fauquier, D. A., Flewelling, L. J., Maucher, J., Manire, C. A., and others (2013). Brevetoxin in blood, biological fluids, and tissues of sea turtles naturally exposed to Karenia brevis blooms in central west Florida. *J. Zoo Wildl. Med.* 44, 364–375. doi: 10.1638/2012-0164R.1

Gedaria, A. I., Luckas, B., Reinhardt, K., and Azanza, R. V. (2007). Growth response and toxin concentration of cultured *Pyrodinium bahamense* var. compressum to varying salinity and temperature conditions. *Toxicon* 50, 518–529. doi: 10.1016/j.toxicon.2007.04.021

Geraci, J. R., Anderson, D. M., Timperi, R. J., St. Aubin, D. J., Early, G. A., Prescott, J. H., et al. (1989). Humpback whales (*Megaptera novaeangliae*) fatally poisoned by dinoflagellate toxin. *Can. J. Fish. Aquatic Sci.* 46, 1895–1898 doi: 10.1139/f89-238

Harada, T., Oshima, Y., Kamiya, H., and Yasumoto, T. (1982). Confirmation of paralytic shellfish toxins in the dinoflagellate *Pyrodinium bahamense* var. compressa and bivalves in Palau. *Bull. Jpn. Soc. Sci. Fish* 48, 821–825. doi: 10.2331/suisan.48.821

Harada, T., Oshima, Y., and Yasumoto, T. (1983). Natural occurrence of decarbamoylsaxitoxin in tropical dinoflagellate and bivalves. *Agric. Biol. Chem.* 47, 191–193.

Hart, K., Mooreside, P., and Crowder, L. (2006). Interpreting the spatio-temporal patterns of sea turtle strandings: going with the flow. *Biol. Conserv.* 129, 283–290. doi: 10.1016/j.biocon.2005.10.047

Hattenrath-Lehmann, T. K., Ossiboff, R. J., Burnell, C. A., Rauschenberg, C. D., Hynes, K., Burke, R. L., et al. (2017). The role of a PSP-producing Alexandrium bloom in an unprecedented diamondback terrapin (*Malaclemys terrapin*) mortality event in Flanders Bay, New York, USA. *Toxicon* 129, 36–43. doi: 10.1016/j.toxicon.2017.02.006

Hu, C., Muller-Karger, F. E., Taylor, C., Carder, K. L., Kelble, C., Johns, E., et al. (2005). Red tide detection and tracing using MODIS fluorescence data: a regional example in SW Florida coastal waters. *Remote Sens. Environ.* 97, 311–321. doi: 10.1016/j.rse.2005.05.013

IUCN (2018). *The IUCN Red List of Threatened Species*. Version 2018-1. Available online at: http://www.iucnredlist.org

Koch, V., Peckham, H., Mancini, A., and Eguchi, T. (2013). Estimating at-sea mortality of marine turtles from stranding frequencies and drifter experiments. *PLoS ONE* 8:e56776. doi: 10.1371/journal.pone.0056776

Landsberg, J. H., Flewelling, L. J., and Naar, J. (2009). Karenia brevis Red Tides, Brevetoxins in the Food Web, and Impacts on Natural Resources: Decadal Advancements. *Harmful Algae* 8, 598–607. doi: 10.1016/j.hal.2008.11.010

Landsberg, J. H., Hall, S., Johannessen, J. N., White, K. D., Conrad, S. M., Abbott, J. P., et al. Steidinger (2006). Saxitoxin puffer fish poisoning in the United States, with the first report of *Pyrodinium bahamense* as the putative toxin source. *Environ. Health Perspect* 114, 1502–1507. doi: 10.1289/ehp.8998

Lawrence, J. F., and Niedzwiadek, B. (2001). Quantitative determination of paralytic shellfish poisoning toxins in shellfish by using prechromatographic oxidation and liquid chromatography with fluorescence detection. *J. AOAC* Inter. 84, 1099–1108.

Lefebvre, K. A., Quakenbush, L., Frame, E., Huntington, K. B., Sheffield, G., Stimmelmayr, R., et al. (2016). Prevalence of algal toxins in Alaskan marine mammals foraging in a changing arctic and subarctic environment. *Harmful Algae* 55, 13–24. doi: 10.1016/j.hal.2016.01.007

Licea, S., Navarrete, A., Casta-eda, V., and Guzmán, J. B. (2013). "Monitoring Program for Harmful Algal Blooms in Salvadoran Waters: Report of *Pyrodinium bahamense* from November 2009 to June 2010," in *Proceedings of the 14th International Conference on Harmful Algae*, eds K. A. Pagou and G. M. Hallegraeff (International Society for the Study of Harmful Algae and Intergovernmental Oceanographic Commission of UNESCO), 120–22.

Licea, S., Navarrete, A., Rodriguez, R., Bustillos, J., Martínez, B., and and, C., Ramirez (2008). "Monitoring a Bloom of Pyrodinium Bahamense Var. Compressus Occurring in El Salvador, Guatemala and Mexico (November 2005-March 2006)," in *12th Proceedings of the International Conference on Harmful Algae*, eds G. Doucette, H. Enevoldsen, A. Godhe, G. Hallegraeff, B. Luckas, N. Lundholm, J. Lewis, K. Rengefors, K. Sellner, K. Steidinger, P. Tester, and A. Zingone (Copenhague: International Society for the Study of Harmful Algae and Intergovernmental Oceanographic Commission of UNESCO), 219–220.

Maclean, J. L. (1975). Red tide in the Morobe district of Papua New Guinea. *Pac. Sci.* 29, 7–13.

Mancini, A., Koch, V., Seminoff, J. A., and Madon, B. (2011). Small-scale gill-net fisheries cause massive green turtle Chelonia mydas mortality in Baja California Sur, Mexico. *Oryx* 46, 69–77. doi: 10.1017/S0030605310001833

Meave del Castillo, E., Rodríguez, R., and Vargas, M. (2008). "Blooms of *Pyrodinium bahamense* var. compressum along the Pacific Coast of Central America and southern Mexico," in *Proceedings of the 12th International Conference on Harmful Algae*, eds G. Doucette, H. Enevoldsen, A. Godhe, G. Hallegraeff, B. Luckas, N. Lundholm, J. Lewis, K. Rengefors, K. Sellner, K. Steidinger, P. Tester, and A. Zingone (Copenhague: International Society for the Study of Harmful Algae and Intergovernmental Oceanographic Commission of UNESCO), 212–215.

Oshima, Y. (1995). Postcolumn derivation liquid chromatographic method for paralytic shellfish toxins. *J. AOAC Int.* 78, 528–532.

Shearn-Bochsler, V., Lance, E. W., Corcoran, R., Piatt, J., Bodenstein, B., Frame, E., et al. (2014). Fatal paralytic shellfish poisoning in Kittlitz's murrelet (*Brachyramphus brevirostris*) nestlings, Alaska, USA. *J. Wildl. Dis*. 50, 933–937. doi: 10.7589/2013-11-296

Shumway, S. E., Allen, S. M., and Dee Boersma, P. (2003). Marine birds and harmful algal blooms: sporadic victims or under-reported events? *Harmful Algae* 2, 1–17. doi: 10.1016/S1568-9883(03)00002-7

Usup, G., Kulis, D. M., and Anderson, D. M. (1994). Growth and toxin production of the toxic dinoflagellate *Pyrodinium bahamense* var. compressum in laboratory cultures. *Nat. Toxins* 2:254–262. doi: 10.1002/nt.2620020503

Utermöhl, H. (1958). Zur Vervollkommnung Der Quantitativen Phytoplankton-Methodik: Mit 1 Tabelle Und 15 Abbildungen Im Text Und Auf 1 Tafel. *SIL Commun.* 9, 1–38. doi: 10.1080/05384680.1958.11904091

Van Dolah, F. M., Doucette, G. J., Gulland, F. M. D., Rowles, T. L., and Bossart, G. D. (2003). "Impacts of algal toxins on marine mammals," in Toxicology of Marine Mammals, eds J. G. Vos, G. D. Bossart, M. Fournier, T. J. O'Shea (London: Taylor & Francis), 247–269.

Van Dolah, F. M., Fire, S. E., Leighfield, T. A., Mikulski, C. M., and Doucette, G. J. (2012). Determination of Paralytic Shellfish Toxins in Shellfish by Receptor Binding Assay: Collaborative Study. *J. AOAC Int.* 95, 795–812. doi: 10.5740/jaoacint.CS2011.

Walker, J. S., Shaver, D. J., Stacy, B. A., Flewelling, L. J., Broadwater, M. H., and Wang, Z. (2018). Brevetoxin exposure in sea turtles in south Texas (USA) during Karenia brevis red tide. *Dis. Aquat. Organ.* 31, 145–150 doi: 10.3354/dao03194

Wiese, M., D'Agostino, P. M., Mihali, T. K., Moffitt, M. C., and Neilan, B. A. (2010). Neurotoxic alkaloids: saxitoxin and its analogs. *Mar. Drugs* 8, 2185–2211. doi: 10.3390/md8072185

Zingone, A., and Enevoldsen, H. O. (2000). The diversity of harmful algal blooms: a challenge for science and management. *Ocean Coast. Manag.* 43, 725–748. doi: 10.1016/S0964-5691(00)00056-9

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Amaya, Quintanilla, Stacy, Dechraoui Bottein, Flewelling, Hardy, Dueñas and Ruiz. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Marine Science

PERSPECTIVE
published: 08 November 2018
doi: 10.3389/fmars.2018.00409

Addressing the Problem of Harmful Algal Blooms in Latin America and the Caribbean- A Regional Network for Early Warning and Response

Tomasa Cuellar-Martinez[1], Ana Carolina Ruiz-Fernández[1], Carlos Alonso-Hernández[2], Oscar Amaya-Monterrosa[3], Rebeca Quintanilla[3], Hector Leonel Carrillo-Ovalle[4], Natalia Arbeláez M.[5], Lisbet Díaz-Asencio[2], Silvia M. Méndez[6], Maribelle Vargas[7], Ninoska Fabiola Chow-Wong[8], Lorelys Rosario Valerio-Gonzalez[9], Henrik Enevoldsen[10] and Marie-Yasmine Dechraoui Bottein[11]*

[1] Unidad Académica Mazatlán, Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Mazatlán, Sinaloa, Mexico, [2] Centro de Estudios Ambientales de Cienfuegos, Cienfuegos, Cuba, [3] Marine Toxins Laboratory LABTOX-UES, Faculty of Natural Sciences and Mathematics, University of El Salvador, San Salvador, El Salvador, [4] Universidad de San Carlos de Guatemala USAC, Centro de Estudios del Mar y Acuicultura-CEMA, Guatemala, Guatemala, [5] Instituto de Investigaciones Marinas y Costeras (INVEMAR), Santa Marta, Colombia, [6] Dirección Nacional de Recursos Acuáticos, Montevideo, Uruguay, [7] Centro de Investigación en Estructuras Microscópicas, Universidad de Costa Rica, San José, Costa Rica, [8] Centro para la Investigación en Recursos Acuáticos de Nicaragua (CIRA/UNAN-Managua), Universidad Nacional Autónoma de Nicaragua-Managua, Managua, Nicaragua, [9] Escuela de Ciencias Aplicadas del Mar, Universidad de Oriente, Cumaná, Venezuela, [10] IOC Science and Communication Centre on Harmful Algae, University of Copenhagen, Copenhagen, Denmark, [11] International Atomic Energy Agency, Environment Laboratories, Monaco, Monaco

OPEN ACCESS

Edited by:
Juan José Dorantes-Aranda, Institute for Marine and Antarctic Studies (IMAS), Australia

Reviewed by:
Gustaaf Marinus Hallegraeff, University of Tasmania, Australia
Marcos Mateus, Universidade de Lisboa, Portugal

***Correspondence:**
Ana Carolina Ruiz-Fernández
caro@ola.icmyl.unam.mx

Specialty section:
This article was submitted to Marine Ecosystem Ecology, a section of the journal Frontiers in Marine Science

Received: *24 July 2018*
Accepted: *15 October 2018*
Published: *08 November 2018*

Citation:
Cuellar-Martinez T, Ruiz-Fernández AC, Alonso-Hernández C, Amaya-Monterrosa O, Quintanilla R, Carrillo-Ovalle HL, Arbeláez MN, Díaz-Asencio L, Méndez SM, Vargas M, Chow-Wong NF, Valerio-Gonzalez LR, Enevoldsen H and Dechraoui Bottein M-Y (2018) Addressing the Problem of Harmful Algal Blooms in Latin America and the Caribbean- A Regional Network for Early Warning and Response. Front. Mar. Sci. 5:409. doi: 10.3389/fmars.2018.00409

Harmful algal blooms (HABs) constitute a worldwide problem, affecting aquatic ecosystems, public health and local economies. Supported by the International Atomic Energy Agency since 2009, Latin America and the Caribbean (LAC) countries, including Brazil, Chile, Colombia, Costa Rica, Cuba, Dominican Republic, El Salvador, Guatemala, Haiti, Mexico, Nicaragua, Panama, Uruguay and Venezuela, have integrated a regional network for early warning of HABs and biotoxins in seafood. Technical capacities have been developed at regional level to identify toxic species, evaluate biota toxicity, and to perform retrospective analysis of HAB occurrence. This network involves 58% of the coastal LAC countries, two regional reference centers (in El Salvador and Cuba), 14 well equipped institutions, and 177 professionals trained to contribute to the operation of HAB and biotoxin monitoring programs. All countries from the network have reported planktonic and benthic toxic species, and in selected cases, associated with toxin in biota. Dinocyst abundance analysis in ^{210}Pb-dated sediment cores have shown that some harmful species have been present in the region for at least 100 years ago, and that both coastal water pollution and climate change are important drivers for HAB occurrence. Efforts must be made to enrich the data base records on HAB events occurred in LAC, better understand key environmental variables that control HABs and expand coverage of HAB monitoring to all coastal countries in LAC to promote sustainable development of the region.

Keywords: HAB, biotoxin, nuclear techniques, laboratory network, IAEA-technical cooperation

INTRODUCTION

Harmful effects of phytoplankton blooms (abundance increment over background levels) may be associated with oxygen depletion, production of phycotoxins, mucilage, reactive oxygen species and polyunsaturated fatty acids, and physical damage to fish gill tissue; high mortalities of marine organisms including fish, marine mammals and sea turtles have been associated with algal blooms (Anderson, 2017). Worldwide, harmful algal blooms (HABs) appear to have increased in frequency, geographic extent and intensity, due to the increase in nutrient discharges to aquatic ecosystems, and climate variability (Heisler et al., 2008) as well as the introduction of exotic species (van den Bergh et al., 2002). Most of the HAB forming species are dinoflagellates, accounting for as much as 100 taxa in the marine environment (Moestrup et al., 2009).

In Latin America and the Caribbean region (LAC), between 1970 and 2007, ∼7800 human intoxications, including 119 human fatalities, were mainly associated with Paralytic Shellfish Poisoning (PSP) in the Pacific and Atlantic coasts, and Ciguatera Fish Poisoning (CFP) in the Caribbean zone (**Table 1** and **Supplementary Table S1**). There is no exact measure of the economic impacts of HABs in LAC; however, analysis of specific cases suggest that they are severe. In Mexico, 61 HAB events caused ∼2,500 days of sanitary closures between 2003 and 2014 (Comisión Federal de Protección contra Riesgos Sanitarios [COFEPRIS], 2018), affecting local economies due to bans of shellfish harvesting or extracting; and in 2002, in Bahía de Todos Santos (Pacific coast), a bloom of *Ceratium furca* caused a 15-million dollar loss in a mass mortality episode of farmed tuna (Orellana-Cepeda et al., 2004). In Chile, in 2016, a bloom of *Pseudochattonela* cf. *verruculosa* affected the salmon farming, with losses over 500 million dollars (Clément et al., 2016). Besides the multiple impacts of HABs in LAC, the scarcity of qualified personnel and properly equipped laboratories acted against the establishment of prevention and mitigation measures. Surveillance of toxic HAB occurrence and/or control of biotoxins in seafood has been unequally developed in the region, and only a few countries have regular monitoring programs for local or export trades.

Over the last decade, countries of LAC have developed capacities to better manage HABs, and strengthen cooperation within the region, through the creation of the "Regional Monitoring and Response Network for Marine Resources and Coastal Environments in Latin America and Greater Caribbean." This effort was supported by the International Atomic Energy Agency (IAEA) Technical Cooperation Program (projects RLA/7/012, RLA/7/014, RLA/7/020 and RLA/7/022). This work describes selected network achievements in terms of capacity building and establishment/improvement of monitoring programs; and presents examples of the information generated, including HAB events, toxic species and biotoxins identification, and historical reconstruction of HABs.

METHODOLOGY

Microalgae Species

Harmful algal bloom monitoring programs within the network followed the methods in Reguera et al. (2011, 2016). Phytoplankton was sampled with nets and Niskin bottles and quantified through the Utermöhl method using inverted microscopes; and benthic dinoflagellates were collected from seagrass and macroalgaes, and counted using Sedgewick-Rafter chambers using compound microscopes.

Toxins

Paralytic shellfish toxins (PSTs) and ciguatoxins were analyzed in marine biota using the receptor binding assay (RBA; IAEA, 2013), that quantifies the toxin potency, by determining, through a scintillation counter, the concentration of tritiated toxin standards, which compete with the toxin from sample extracts, for binding to voltage-gated sodium channels in a rat brain membrane preparation (Van Dolah et al., 2012).

Dinocysts

The analysis of dinocysts (cyst/g, dry weight) in ^{210}Pb dated sediment cores was performed following de Vernal et al. (2010). Briefly, sediment samples, added with *Lycopodium* spores as marker for quantitative analysis, are treated with a mixture of strong acids to clean the sample, which is used to prepare permanent slides to be counted in optic microscopes. The relation of environmental records (e.g., temperature or rainfall) and dinocysts abundances was examined using multivariate analysis (Cuellar-Martinez et al., 2018).

RESULTS AND DISCUSSION

The strategy followed by the network included: (i) capacity building (e.g., improvement of analytical infrastructure and formation of human resources through specialized courses and hands-on trainings); and (ii) standardization of sampling and analytical methods for microalgae and toxin quantification, and the retrospective analysis of HAB event occurrence. The most important activities and findings obtained are described below.

Capacity Building

As of 2018, the network involves laboratories in 14 countries (**Supplementary Table S2**), including Brazil, Chile, Colombia, Costa Rica, Cuba, Dominican Republic, El Salvador, Guatemala, Haiti, Mexico, Nicaragua, Panama, Uruguay and Venezuela, representing 58% of the Member States along the Atlantic and Pacific coasts of Latin America and 14% of the Caribbean coasts. As part of the network activities since 2009, the countries (except Chile and Brazil, who recently joined the network) developed pilot HAB monitoring programs in sampling sites off their coastline (**Figure 1**); and through 17 training courses, 177 persons were trained on diverse topics, including taxonomy and identification of harmful microalgae, dinocysts and foraminifera; analysis of phycotoxins (including RBA); data analysis (submission of data to the

TABLE 1 | HAB records from countries of the Latin America and Caribbean network in the Harmful Algae Event Database (Harmful Algae Event Database [HAEDAT], 2018).

Country	Years	HAB events	Species frequently reported[1] (number of HAB events)	Harmful event impact
Brazil*	1990–2016	7	*Dinophysis* sp.[2] (2)	Massive shellfish mortalities and human intoxication.
Chile	1972–2018	39	*Alexandrium catenella*[3] (17), *Chatonella verruculosa* (5), *Leptocylidrus minimus* (4)	113 human intoxication and 15 fatalities, harvest and shellfish farms closures, salmon mortalities.
Colombia	1994–2017	27	Species not identified (8), *Synechocystis* sp. (6), *Mesodinium* sp. (3)	Mass fish deaths.
Costa Rica*	1989–2003	3	*Pyrodinium bahamense*[3] (2)	20 human intoxications and 1 fatality, harvest sites closure.
Cuba	2003–2015	7	*Cochlodinium polykrikoides* (3)	Not reported.
Dominican Republic*	2015	1	Not identified	Not reported.
El Salvador	2001–2018	13	*P. bahamense*[3] (4), *C. polykrikoides* (3)	7 human intoxications and 1 fatality, sea turtle deaths.
Guatemala	1987–2004	7	*P. bahamense*[3] (4)	26 human fatalities.
Haiti	no data	–	–	–
Mexico*	1979–2014	49	*Gymnodinium catenatum*[3] (10), CFP (9), *Gambierdiscus* spp.[4] (9), *P. bahamense*[3] (5)	High toxin concentrations, 25 human intoxications by CFP.
Nicaragua*	2005–2018	4	*Phaeocystis* sp. (2)	Presence of mucilage, foam, bad smell, 50 human intoxications.
Panama*	2001	1	CFP	Not reported.
Uruguay	1980–2016	53	*G. catenatum*[3] (13), *D. acuminata*[2] (12), *Dinophysis* spp.[2] (5), *D. ovum*[2] (4), *A. tamarense*[3] (3), *Akashiwo sanguinea* (3), *M. rubrum* (3)	Shellfish closure and harvesting ban
Venezuela*	1956–2010	12	Diverse species (one per event)	Not reported.

[1] Species in ≥3 events, except countries marked with; Species associated with DSP[2], PSP[3], and CFP[4].*

international Harmful Algae Event Database [HAEDAT], 2018, and retrospective reconstruction of HABs occurrence through the study of dated sediment cores); ocean acidification; and communication skills to disseminate scientific findings to stakeholders (**Supplementary Figure S1**).

In order to perform the different monitoring activities, prioritized by the countries based on their needs and capacities, each laboratory of the network received specific equipment and consumables (e.g., Utermöhl and Sedgewick-Rafter counting chambers, Niskin bottles, phytoplankton nets, multiparameter probes for water quality and inverted microscopes), some of them inaccessible within the region. Liquid scintillation counters and minor equipment for toxin extraction and quantification using RBA, were provided to laboratories in El Salvador, Nicaragua, Colombia, Cuba and Costa Rica; and alpha and gamma spectrometry capacities were established in Colombia, Cuba, Mexico, Nicaragua and El Salvador, for retrospective studies on HAB forming species in ^{210}Pb dated sediment cores.

Pilot Monitoring Programs

Standardization of Methods Used in the Network

The collaborative work among the network laboratories led to production of manuals and guides, that led to significant findings and numerous scientific documents. The "Guide for designing and implementing a plan to monitor toxin-producing microalgae," prepared through a collaboration between scientists from the network (and other countries), the International Oceanographic Commission (IOC) and the IAEA, includes standardized sampling and counting procedures (Reguera et al., 2011, in Spanish); and has been expanded to include methods to study benthic HABs (Reguera et al., 2016, in English).

Microalgae Blooms Observations and Impacts in the Network Countries

In the Colombian Caribbean Coast, three blooms caused by *Cochlodinium* spp. and other three by *Mesodinium* cf. *rubrum* occurred in Santa Marta Bay and nearby areas, between 2010 (Malagón and Perdomo, 2013) and 2017; and in Ciénaga Grande de Santa Marta, about nine episodes of mass fish deaths were attributed to low oxygen concentrations associated with HABs (2014–2017; unpublished data). Additionally, in Chengue Bay, Tayrona National Park, 14 potentially harmful benthic dinoflagellates were identified in seagrass meadows, including *Prorocentrum* and *Ostreopsis* as the most frequent genus (Arbeláez-Merizalde et al., 2017). *Prorocentrum lima* was the most representative species on *Thalassia testudinum* and its maximum abundances were associated with salinities close to 35, high temperatures (> 29°C) and low nutrient (N-P)

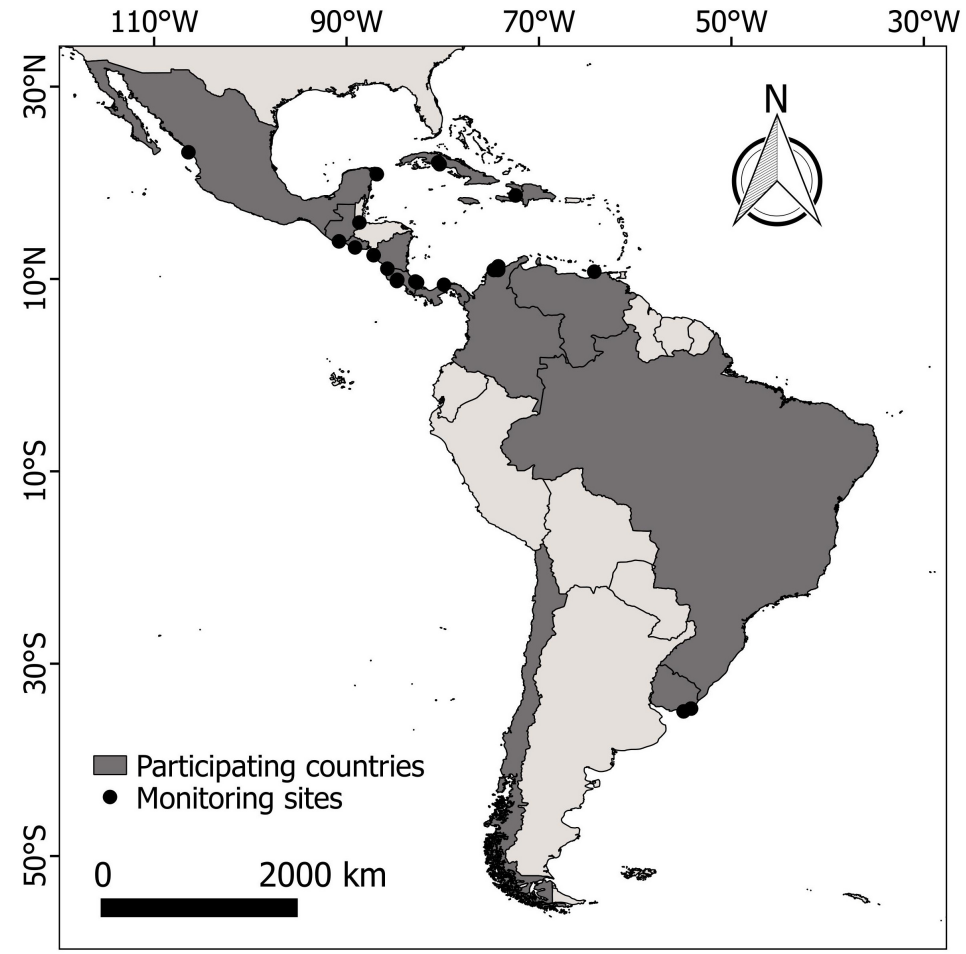


FIGURE 1 | Monitoring sites within the technical cooperation IAEA projects on HABs. Mexico: Mazatlán and Puerto Morelos, Cuba: Cienfuegos Bay and Ensenada de Guajimilco, Dominican Republic: Playa Caleta, Haiti: Port-au-Prince, Guatemala: Puerto Quetzal and Amatique Bay, El Salvador: all the coast, Nicaragua: Port of Corinto and El Ostional Bay, Costa Rica: Cahuita National Park, Gulf of Nicoya, Uvita Island and Puerto Vargas National Park, Panama: Punta Galeta, Colombia: Ciénaga Grande de Santa Marta and Chengue Bay, Venezuela: Cubagua Island, Uruguay: Punta del Este and La Paloma.

concentrations, during an ENSO period (Arbeláez-Merizalde and Mancera-Pineda, 2016).

In Cienfuegos Bay, Cuba and adjacent coasts, ∼20 potentially harmful species were identified between 2007 and 2009. *Gymnodinium catenatum*, *Pyrodinium bahamense* and *Dinophysis ovum* were recorded for the first time (Moreira-González et al., 2013). A bloom of *Heterocapsa circularisquama* occurred in 2009 (Moreira-González, 2010); and another of *Vulcanodinium rugosum* in 2015, left 60 cases of skin lesions (Moreira-González et al., 2016a). The HABs in Cienfuegos Bay were associated with the restricted water circulation in small enclosed areas and discharges of urban/industrial effluents (Moreira-González et al., 2014). A bloom of *Cochlodinium polykrikoides* in 2014 in Guanaroca Lagoon was related with extreme weather conditions associated with ENSO (Moreira-González et al., 2016b). Outbreaks by *Phaeocystis* sp. occurred in Cayo Largo del Sur in 2012 (Loza et al., 2013), and by *Chattonella* sp. in La Redonda Lagoon in 2013 (Moreira-González and Comas-González, 2014). *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* have been quantified in the southern Cuban coast, and a high diversity of *Gambierdiscus* species was revealed through qPCR assays (Díaz-Asencio et al., 2016).

In El Salvador, *P. bahamense* blooms and high levels of saxitoxins in shellfish occurred in 2011 and 2012 (Espinoza et al., 2013a). Also, in 2012, a bloom of *Alexandrium peruvianum/ostenfeldii* at Los Cóbanos was associated with sea turtle deaths (Espinoza et al., 2013b); and a bloom by *C. polykrikoides* caused abundant scum and mass fish mortalities in the coast of La Libertad (Espinoza et al., 2013b). A massive sea turtle death in 2013, was associated with *G. catenatum*, and *P. bahamense* (Amaya et al., 2014). Regarding epiphytic dinoflagellates, the most abundant taxon is *Prorocentrum lima* (80 cells/g), and *Gambierdiscus* sp. and *Ostreopsis* sp. are present in low densities (4 and 9 cell/g respectively; Quintanilla and Amaya, 2017).

On the Pacific coast of Nicaragua, in 2005, a *P. bahamense* bloom caused 50 human intoxications and one death

(Chow et al., 2010). In Punta de El Este and La Paloma, Uruguay, between 2010 and 2018, fourteen toxic events by *Dinophysis* cf. *ovum*, two by *G. catenatum* and two by both species, produced PST concentrations in bivalves above the regulatory level (0.8 mg STXeq/kg meat) and positive mouse bioassay results for Diarrhetic Shellfish Poisoning (DSP). In the Caribbean coast of Guatemala, monitoring reports since 2015, include *Prorocentrum* spp. (14–5,492 cell/g), *Ostreopsis* sp. (0–165 cell/g), *Gambierdiscus* spp. (0–16 cell/g, **Supplementary Figure S2**) and *Coolia* sp. (0–7 cell/g) (unpublished data). Whereas, in the southern Caribbean coast of Costa Rica, the reports since 2016, included *Coolia monotis*, *C. tropicalis*, *Ostreopsis* spp. *Prorocentrum* spp. and *Gambierdiscus* spp. (unpublished data).

Despite *Gambierdiscus* species being responsible for CFP, and having been reported in Colombia, Cuba, El Salvador, Guatemala and Costa Rica, none of these studies informed intoxication cases owing to this taxon.

Nuclear Techniques for the Study of HABs

Toxin analysis

In El Salvador, between 2010 and 2017, more than 300 RBA analyses of PST have been performed in marine biota (e.g., puffer fish, snails, crabs, mussels, clams, oysters, shells, phytoplankton and sea turtles). PST concentrations at risk for consumption have been regularly found in *Crassostrea iridiscens* oysters, with concentrations reaching levels as high as 28.09 mg STXeq/kg in 2011 (Amaya et al., 2012, 2014). In 2013, ∼200 sea turtles died, and RBA analysis indicated the presence of saxitoxins in diverse tissues of some turtle (Amaya et al., 2014). Owing to the permanent HAB monitoring program, and the toxins quantification by RBA, the intoxication cases associated with PSP have diminished in El Salvador, since both actions allow providing scientific information and early warnings to decision makers, which facilitates rapid responses to enforce shellfish harvest and trade bans, and protect the population from the consumption of contaminated seafood.

Ciguatoxins were detected for the first time in fish tissues from Cuba (Díaz-Asencio et al., 2016) using a recently optimized RBA method. Guidance on RBA use and quality control checks for toxin screening of fish samples is provided in Díaz-Asencio et al. (2018). This optimized protocol supports a full validation of the assay, a necessary step to develop and implement a regulatory monitoring program for ciguatoxins in seafood products.

Retrospective studies of the occurrence of potential harmful species

Historical reconstruction of HAB events have been conducted in Mexico through the analysis of dinocyst abundances in ^{210}Pb dated sediment cores. A sediment core collected in April 2016, in Punta Caracol (Mexican Caribbean coast; **Figure 1**), recorded very scarce dinocysts (total abundance: 178 ± 34 cyst/g) with all species belonging to *Spiniferites* taxa (*Spiniferites* spp., *S. ramosus*, *S. hyperacanthus, S. belerius*, *S. mirabilis*, **Supplementary Figure S2**, unpublished data).

In the Gulf of Tehuantepec (Pacific coast) and San Jose Lagoon (Gulf of California), *Polysphaeridium zoharyi* (cyst of *P. bahamense*) was the most representative species and was found in the sediments since more than 100 years ago. Both studies indicated that climate variability might be an important driver in the dynamics of this species. The highest *P. zoharyi* fluxes in the Gulf of Tehuantepec were associated with low sea surface temperatures (La Niña episodes) and increasing rainfall (El Niño events) (Sanchez-Cabeza et al., 2012); and in San Jose Lagoon, with increments in rainfall and in minimum atmospheric temperatures (Cuellar-Martinez et al., 2018).

HAB Records in an International Database

The network recognized the lack of HAB records in LAC and found synergy with HAEDAT to integrate information about HABs that have occurred in the region. The network laboratories have provided 184 new data within the past 6 years. The database HAEDAT was consulted in June 2018 and until then, 223 HAB events occurred in countries from the network (between 1 and 53 records per country; **Table 1**), the oldest being from 1956.

The common taxa in the region are *Alexandrium*, *Pyrodinium*, *Cochlodinium* and *Gymnodinium.* The most reported HAB forming species are *Alexandrium catenella* (Chile), *G. catenatum* (Mexico and Uruguay) and *P. bahamense* (Mexico, El Salvador, Costa Rica and Guatemala). In recent years the occurrence of *C. polykrikoides* blooms has become evident in Cuba and El Salvador.

Harmful Algae Event Database is a good platform to highlight the problem of HAB occurrence in LAC countries and facilitate the incorporation of results emanating from the monitoring programs. However, based on the current records available, the appraisal of the present situation regarding HABs in LAC would be largely underestimated, since not all HAB events are registered yet. For instance, despite CFP being a threat to human health in the Caribbean (∼2400 intoxication cases, **Supplementary Table S1**), the records in HAEDAT are still scarce (19 events). It is important to maintain constant efforts to update the information to maximize the benefits of the database for coastal management and to improve the global perspective about HABs.

CHALLENGES AND PERSPECTIVES ON HAB RESEARCH IN LAC THROUGH THE NETWORK

The main obstacles encountered by the network to establish and sustain continued HAB monitoring programs, are (a) lack of knowledge and awareness of the magnitude of the HAB problem in the region; (b) dependency on importation for many reagents and materials needed for the monitoring programs; (c) high turn-over of trained personnel; and (d) lack of financial resources.

Currently, under the project RLA/7/022 "Strengthening Regional Monitoring and Response for Sustainable Marine and Coastal Environments" the immediate objectives include: dissemination of the information on the installed capacity

of nuclear technologies and their applications for monitoring of stressors in the marine-coastal environments in LAC; the integration of strategic partners in the network activities to stablish long lasting flows of validated information; and the consolidation of processes to transfer network-generated information to stakeholders, for evaluation of socio-economic impacts derived from environmental damage in the region, which would allow mitigation and remediation decisions.

Recent network efforts aim at communicating the activities and major accomplishments to decision makers, international institutions and general public. The dissemination of these achievements is expected to help improving the understanding and recognition of the threat that HABs represent to public health and sustainable coastal management. Improving the network visibility can also facilitate access to international funding and promote cooperation with other HAB-related networks. Locally, this could lead to stronger governmental engagement to maintain and expand HAB and biotoxin monitoring programs, with the ultimate goal of safeguarding seafood safety and the generation of scientific knowledge to better prevent, control and mitigate HAB occurrence and impacts.

The establishment of early warning systems in the rest of the network countries, the adoption of molecular techniques to improve the identification of HAB forming species, and the study of HABs in freshwater environments, certainly should be included in further efforts. Also, the network intends to improve the study of other marine stressors such as sea level rise and contaminants; and capacity is currently being built for ocean acidification monitoring and microplastic quantification.

CONCLUSION

Despite recurrent toxic blooms and drastic consequences for human health and the economy of LAC countries, response strategies have been quite unequal in the region. The creation of a network enabled countries to work together, with the support of IAEA technical cooperation projects, to build on their existing skills and capacity. Regional efforts have led to successful establishment of mature, standardized and up-to-date methodologies for identification and quantification of potentially harmful species, for toxin analysis and for temporal reconstruction of HAB occurrences. Scientific information and HAB occurrence data have been generated, and pilot surveillance of HABs biotoxins allowed the establishment of an early warning system in El Salvador.

Most countries in the region are challenged by the lack of resources to maintain continuous monitoring programs or expand monitoring efforts beyond HABs. It is therefore essential to strengthen south-south collaborations within the network laboratories and among other LAC countries, to promote alliances with local and international institutions, and take steps forward the sensitization and awareness of local authorities and general public about the threat of HABs in the region. The effective transfer of the scientific results obtained through the network are a key step to support the coastal management and to facilitate decision making toward the prevention and mitigation of HAB impacts in LAC.

AUTHOR CONTRIBUTIONS

MYDB and ACRF contributed to the conception and design of the study. TCM, RQ, and LRVG organized the database. TCM performed the statistical analysis. TCM and ACRF wrote the first draft of the manuscript. MYDB, CAH, OAM, RQ, HLCO, NA, LDA, SMM, MV, and NFCW wrote sections of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

FUNDING

IAEA supported projects RLA/7/020 and RLA/7/022 RLA/7/020 and RLA/7/022, ELS/7/002, ELS/7/003, ELS/7/005, and ELS/7/007.

ACKNOWLEDGMENTS

The IAEA is grateful to the Government of the Principality of Monaco for the support provided to its Environment Laboratories, and to NOAA for its support to the implementation of RBA. The authors are thankful to Florence Descroix Comanducci for her scientific and Technical support in the implementation of RLA7014 and ELS7002 projects, to Anne de Vernal, Beatriz Reguera, Patricia Tester, and Santiago Fraga for the expertise and advice they have been providing to the network, and to L.H. Pérez-Bernal, E. Cruz-Acevedo, H. Bojórquez-Leyva, G. Ramírez-Reséndiz, and C. Suárez-Gutiérrez, S. Rendón-Rodríguez for their contribution in the experimental work in Mexico.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2018.00409/full#supplementary-material

REFERENCES

Amaya, O., Ruíz, G., Espinoza, J., and Rivera, W. (2014). Saxitoxin analysis with a receptor binding assay (RBA) suggest PSP intoxication of sea turtles in El Salvador. *Harmful Algae News* 48, 6–7.

Amaya, O., Ruíz, G., Rivera, W., and Espinoza, J. (2012). Application of a receptor binding assay to the analyses of PSP toxins in four species of shellfish in El Salvador. *Harmful Algae News* 45:15.

Anderson, D. (2017). "Harmful algal blooms," in *Harmful algal Blooms (HABs) and Desalination: A Guide to Impacts, Monitoring and Management*, eds

D. M. Anderson, S. F. E. Boerlage, and M. B. Dixon (Paris: Intergovernmental Oceanographic Commission of UNESCO), 17–52.

Arbeláez-Merizalde, N., and Mancera-Pineda, J. E. (2016). Annual variability of Prorocentrum lima and abiotic factors in Chengue Bay, Tayrona National Park, Colombian Caribbean. *Harmful Algae News* 54, 8–9.

Arbeláez-Merizalde, N., Mancera-Pineda, J. E., and Reguera, B. (2017). Dinoflagelados epífitos de *Thalassia testudinum* en dos sistemas costeros del Caribe colombiano. *Bol. Invest. Mar. Cost.* 46, 9–40. doi: 10.25268/bimc.invemar.2017.46.2.725

Chow, N., Vammen, K., and Reguera, B. (2010). First report of PSP on Pacific coast of Nicaragua associated with *Pyrodinium bahamense*. *Harmful Algae News* 41, 6–7.

Clément, A., Lincoqueo, L., Saldivia, M., Brito, C. G., Muñoz, F., Fernández, C., et al. (2016). Exceptional summer conditions and HABs of Pseudochattonella in Southern Chile create record impacts on salmon farms. *Harmful Algae News* 53, 1–3.

Comisión Federal de Protección contra Riesgos Sanitarios [COFEPRIS] (2018). *Ocurrencia de Florecimientos Algales Ocurridos en el Territorio Nacional de 2003 a 2014*. Available at: https://www.gob.mx/cofepris/documentos/presencia-de-marea-roja-en-costas-nacionales-durante-2003 [accessed May 19, 2018].

Cuellar-Martinez, T., Alonso-Rodríguez, R., Ruiz-Fernández, A. C., de Vernal, A., Morquecho-Escamilla, L., Limoges, A., et al. (2018). Environmental forcing on the flux of organic-walled dinoflagellate cysts in recent sediments from a subtropical lagoon in the Gulf of California. *Sci. Total Environ.* 621, 548–557. doi: 10.1016/j.scitotenv.2017.11.269

de Vernal, A., Henry, M., and Bilodeau, G. (2010). *Micropaleontological Preparation Techniques and Analyses. Les Cahiers du GEOTOP No 3*. Available at: www.geotop.ca/upload/files/laboratoires/laboratoire-de-micropaleontologie-et-palynologie-marine-uqam/Micropal_Methods_2010.pdf [accessed May 20, 2018]

Díaz-Asencio, L., Chamero-Lago, D., Gómez-Batista, M., Vandersea, M. W., García-Chamero, A., Moreira-González, A., et al. (2016). "Progress on Ciguatera risk assessment in South Central Cuba," in *Abstract Book of the 17th International Conference on Harmful Algae*, Florianópolis, 103.

Díaz-Asencio, L., Clausing, R. J., Llorina-Rañada, M., Alonso-Hernández, C. M., and Dechraoui-Bottein, M. Y. (2018). A radioligand receptor binding assay for ciguatoxin monitoring in environmental samples: method development and determination of quality control criteria. *J. Environ. Radioact.* 192, 289–294. doi: 10.1016/j.jenvrad.2018.06.019

Espinoza, J., Amaya, O., and Quintanilla, R. (2013a). *Atlas de Fitoplancton Marino*. San Salvador: Universidad de El Salvador.

Espinoza, J., Amaya, O., Reguera, B., Alvarado, Y., Ruíz, G., and Escobar, D. (2013b). A *Cochlodinium polykrikoides* Bloom in El Salvador. *Harmful Algae News* 47, 12–13.

Harmful Algae Event Database [HAEDAT] (2018). Available at: http://haedat.iode.org/index.php [accessed June 20, 2018].

Heisler, J., Glibert, P. M., Burkholder, J. M., Anderson, D. M., Cochlan, W., Dennison, W. C., et al. (2008). Eutrophication and harmful algal blooms: a scientific consensus. *Harmful Algae* 8, 3–13. doi: 10.1016/j.hal.2008.08.006

IAEA (2013). *Detection of Harmful Algal Toxins Using the Radioligand Receptor Binding Assay a Manual of Methods (TECDOC-1729)*. Vienna: IAEA.

Loza, S., Moreira, A., Comas, A., Sánchez, M., Carmentate, M., Álamo, B., et al. (2013). A phaeocystis bloom in the cuban archipelago. *Harmful Algae News* 47, 20–21.

Malagón, A., and Perdomo, L. V. (2013). *Cochlodinium polykrikoides* Bloom in the Colombian Caribbean. *Harmful Algae News* 47, 12–13.

Moestrup, Ø, Akselmann, R., Fraga, S., Hoppenrath, M., Iwataki, M., Komárek, J., et al. (eds) (2009). *IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae*. Available at http://www.marinespecies.org/hab [accessed May 18, 2018].

Moreira-González, A., and Comas-González, A. (2014). Blooms of a *Chattonella* species (Raphidophyceae) in La Redonda Lagoon, Northeastern Cuba. *Harmful Algae News* 48, 12–13.

Moreira-González, A., Reguera, B., Alonso, C., and Comas, A. (2013). "Potentially harmful microalgae from the Southern-Central Coast of Cuba," in *Proceedings of the 14th International Conference on Harmful Algae*, eds P. Pagou and G. Hallegraeff (Paris: International Society for the Study of Harmful Algae and Intergovernmental Oceanographic Commission of UNESCO), 135–137.

Moreira-González, A., Seisdedo-Losa, M., Muñoz-Caravaca, A., Comas-González, A., and Alonso-Hernández, C. (2014). Spatial and temporal distribution of phytoplankton as indicator of eutrophication status in the Cienfuegos Bay, Cuba. *Rev. Gestão. Cost. Int.* 14, 597–609. doi: 10.5894/rgci506

Moreira-González, A. R. (2010). Dinoflagellate blooms in eutrophic zones of Bahía de Cienfuegos, Cuba. *Harmful Algae News* 41, 10–11.

Moreira-González, A. R., Comas-González, A., Valle, A., Seisdedo, M., and Fernandes, L. F. (2016a). Bloom of Vulcanodinium rugosum linked to skin lesions in Cienfuegos Bay, Cuba. *Harmful Algal News* 55, 10–11.

Moreira-González, A. R., Comas-González, A., Valle, A., Sosa, D., and Muñoz, A. (2016b). *Cochlodinium polykrikoides* blooms in southern Cuba during anomalous hot dry season. *Harmful Algae News* 54, 3–4.

Orellana-Cepeda, E. C., Granados-Machuca, C., and Serrano-Esquer, J. (2004). "*Ceratium furca*: one possible cause of mass mortality of cultured blue-fin tuna at Baja California, Mexico," in *Harmful Algae*, eds K. A. Steidinger, J. Landsberg, C. R. Tomas, and G. A. Vargo (St. Petersburg, FL: United States of America, Florida Fish and Wildlife Conservation Commission), 514–516.

Quintanilla, C. R., and Amaya, O. (2017). "First report of the epiphytic genera *Gambierdiscus* and *Ostreopsis* from the coast of El Salvador, Eastern Tropical Pacific," in *Proceedings of the 17th International Conference on Harmful Algae*, eds L. A. O. Proença and G. Hallegraeff (Florianópolis: International Society for the Study of Harmful Algae and Intergovernmental Oceanographic Commission of UNESCO), 80–83.

Reguera, B., Alonso, R., Moreira, A., and Méndez, S. (2011). *Guía Para el Diseño y Puesta en Marcha de un Plan de Seguimiento de Microalgas Productoras de Toxinas*. Paris: UNESCO, 59.

Reguera, B., Alonso, R., Moreira, A., Méndez, S., and Dechraoui-Bottein, M.-Y. (2016). "Guide for designing and implementing a plan to monitor toxin-producing microalgae," in *Proceedings of the Intergovernmental Oceanographic Commission (IOC) of UNESCO and International Atomic Energy Agency (IAEA)*, 2nd Edn, (Paris: IOC Manuals and Guides), 59.

Sanchez-Cabeza, J. A., Ruiz-Fernández, A. C., de Vernal, A., and Machain, M. (2012). Reconstruction of Pyrodinium blooms in the Tropical East Pacific (Mexico): are they related to ENSO? *Environ. Sci. Technol.* 46, 6830–6834. doi: 10.1021/es204376e

van den Bergh, J. C. J. M., Nunes, P. A. L. D., Dotinga, H. M., Kooistra, W. H. C. F., Vrieling, E. G., and Peperzak, L. (2002). Exotic harmful algae in marine ecosystems: an integrated biological–economic–legal analysis of impacts and policies. *Mar. Policy* 26, 59–74. doi: 10.1016/S0308-597X(01)00032-X

Van Dolah, F. M., Fire, S. E., Leighfield, T., Mikulski, C. M., and Doucette, G. J. (2012). Determination of paralytic shellfish toxins in shellfish by receptor binding assay: collaborative study. *J. AOAC Int.* 95, 795–812. doi: 10.5740/jaoacint.CS2011_27

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Cuellar-Martinez, Ruiz-Fernández, Alonso-Hernández, Amaya-Monterrosa, Quintanilla, Carrillo-Ovalle, Arbeláez M, Díaz-Asencio, Méndez, Vargas, Chow-Wong, Valerio-Gonzalez, Enevoldsen and Dechraoui Bottein. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Marine Science

ORIGINAL RESEARCH
published: 13 November 2018
doi: 10.3389/fmars.2018.00394

Plankton Multiproxy Analyses in the Northern Patagonian Shelf, Argentina: Community Structure, Phycotoxins, and Characterization of Toxic *Alexandrium* Strains

Valeria A. Guinder[1,2], Urban Tillmann[3], Bernd Krock[3], Ana L. Delgado[1,2], Torben Krohn[3], John E. Garzón Cardona[1,2], Katja Metfies[3,4], Celeste López Abbate[1,2], Ricardo Silva[5] and Rubén Lara[1,2]*

[1] Instituto Argentino de Oceanografía, Consejo Nacional de Investigaciones Científicas y Técnicas, Bahía Blanca, Argentina, [2] Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, Bahía Blanca, Argentina, [3] Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung, Bremerhaven, Germany, [4] Helmholtz Institute for Functional Marine Biodiversity, Oldenburg, Germany, [5] Instituto Nacional de Investigación y Desarrollo Pesquero, Mar del Plata, Argentina

OPEN ACCESS

Edited by:
Marius Nils Müller, Universidade Federal de Pernambuco, Brazil

Reviewed by:
Jelena Godrijan, Bigelow Laboratory for Ocean Sciences, United States
Carnicer Olga, Pontificia Universidad Católica del Ecuador, Ecuador

***Correspondence:**
Valeria A. Guinder
vguinder@criba.edu.ar

Specialty section:
This article was submitted to Marine Ecosystem Ecology, a section of the journal Frontiers in Marine Science

Received: *25 May 2018*
Accepted: *09 October 2018*
Published: *13 November 2018*

Citation:
Guinder VA, Tillmann U, Krock B, Delgado AL, Krohn T, Garzón Cardona JE, Metfies K, López Abbate C, Silva R and Lara R (2018) Plankton Multiproxy Analyses in the Northern Patagonian Shelf, Argentina: Community Structure, Phycotoxins, and Characterization of Toxic Alexandrium Strains. Front. Mar. Sci. 5:394. doi: 10.3389/fmars.2018.00394

The extensive Argentine continental shelf supports high plankton productivity and fish catches. In particular, El Rincón coastal area and the adjacent shelf fronts (38.5–42°S, 58.5–62°W) comprise diverse habitats and hold species of economic and ecological value. So far, studies of the microbial community present at the base of the food web remain scarce. Here, we describe the late winter plankton (5–200 μm) structure in terms of abundance, biomass, species composition, functional groups, and phycotoxin profiles in surface waters of El Rincón in September 2015. Diatoms are the most abundant and the largest contributors to carbon biomass at most stations. They dominated the coastal and inner-shelf (depths <50 m), while dinoflagellates and small flagellates (<15 μm) dominated offshore at the middle-shelf waters (depth ∼100 m). In addition, large (>20 μm) heterotrophic protists such as various ciliates and dinoflagellates species were more abundant offshore. Scanning of phycotoxins disclosed that paralytic shellfish poisoning (PSP) toxins were dominated by gonyautoxins-1/4 (GTX1/4), whereas lipophilic toxins were detected in low abundance, for example, domoic acid (DA). However, a bloom of *Pseudo-nitzschia* spp. (up to 3.6×10^5 cells L^{-1}) was detected at inner-shelf stations. Pectenotoxin-2 (PTX-2) and 13-desmethyl spirolide C (SPX-1) were the most abundant in the field. The PTX-2 co-occurred with *Dinophysis* spp., mainly *D. tripos*, while SPX-1 dominated at middle-shelf stations, where cells of *Alexandrium catenella* (1 strain) and *A. ostenfeldii* (3 strains) were isolated. The quantitative PSP profiles of the *Alexandrium* strains differed significantly from the *in situ* profiles. Moreover, the three *A. ostenfeldii* strains produced PSP and additionally, five novel spirolides. Phylogenetic analyses of these newly isolated strains

from the South Atlantic revealed a new ribotype group, suggesting a biogeographical distinction in the population. The plankton survey presented here contributes baseline knowledge to evaluate potential ecosystem changes and track the global distribution of toxigenic species.

Keywords: protists' plankton, functional groups, shelf front, phycotoxins, *Alexandrium ostenfeldii*, novel spirolides, Patagonian Shelf, SW Atlantic

INTRODUCTION

Shelf seas play a major role in ocean carbon cycling and budget, as a large part of the global marine primary production takes place in the coastal areas, and about half of this organic carbon is exported to the deep ocean (Liu et al., 2010). The Patagonian Shelf Large Marine Ecosystem (PLME) (Heileman, 2009) is one of the widest in the world, one of the most productive (Lutz et al., 2010), and hydrographically complex in the Southern Hemisphere (Palma et al., 2008; Matano et al., 2010; Paniagua et al., 2018). The extensive shelf breakfront and other shelf fronts support high phytoplankton productivity and fisheries incomes (Acha et al., 2004; Carreto et al., 2016; Carranza et al., 2017; Díaz et al., 2018). Moreover, recent studies on satellite images showed that chlorophyll *a* (chl-*a*) has notably risen over the last two decades (Marrari et al., 2017), which highlights the role of this large ecosystem in the global carbon uptake and in the regional sustainability of marine resources and stakeholder communities (Bianchi et al., 2009; Marrari et al., 2013). Hence, the identification of the phytoplankton responsible for high productivity, concerning beneficial and harmful blooms, becomes essential to track changes in the food web and manage potential risk for the biota and human health.

In particular, the area of the Argentine shelf called El Rincón extends from 38.5°S to approximately 42°S (**Figure 1**) and is especially wide at some areas, that is, ∼300 km (Lucas et al., 2005). El Rincón ecosystem, with its middle-shelf front (located at ∼100 m isobaths, Romero et al., 2006), embraces large habitat heterogeneity (Lucas et al., 2005; Palma et al., 2008) characterized by high zooplankton and fish biodiversity (*e.g.,* Marrari et al., 2004; López Cazorla et al., 2014; Acha et al., 2018). For instance, its frontal areas are the main spring reproductive habitats of the northern stock of Argentine anchovy (*Engraulis anchoita*) (Marrari et al., 2013; Díaz et al., 2018) and other valuable species (*e.g.,* Hoffmeyer et al., 2009; Marrari et al., 2013; Acha et al., 2018). So far, there has been only one study on the planktonic microorganisms of El Rincón (Negri et al., 2013) and few of the adjacent areas (Silva et al., 2009; Segura et al., 2013), and they are mainly focused on the smallest cell sized fractions. Along the Argentine shelf fronts and shelf- breakfront, spatial heterogeneity of the phytoplankton composition has been related to contrasting nutritional properties of water masses (Subantarctic Shelf waters and Malvinas Current waters) and the latitudinal and cross-shelf progression in the timing of the spring bloom (García et al., 2008; Ferreira et al., 2013; Olguín et al., 2015; Carreto et al., 2016). Analyses of satellite images of the ocean color (Romero et al., 2006; Dogliotti et al., 2009; Delgado et al., 2015; Marrari et al., 2017) in combination with field *in situ* chlorophyll estimation (Lutz et al., 2010) have detected phytoplankton blooms in spring and summer (maxima between October and February). Harmful algal blooms (HABs) have been documented in the Argentine shelf for the last 30 years, mainly associated with paralytic shellfish toxins (PSTs) produced by dinoflagellates of the genus *Alexandrium* (Carreto et al., 1981, 2001; Montoya et al., 2010; Almandoz et al., 2014; Fabro et al., 2017). Severe detrimental consequences in the food web, ecosystem services, and human health have been attributed to these toxic events (Benavides et al., 1995; Montoya et al., 1996; Gayoso and Fulco, 2006). Other toxigenic species and associated toxins were registered in the shelf and frontal areas, for instance, dinoflagellates of the genus *Dinophysis* (Sar et al., 2012; Fabro et al., 2016) and diatoms of the genus *Pseudo-nitzschia* (Negri et al., 2004; Almandoz et al., 2007, 2017). Blooms composed of various species of *Azadinium* spp. and *Amphidoma* spp., two genera known for the production of azaspiracids (AZAs) (Tillmann, 2018a), have been described from the Argentine shelf (Akselman and Negri, 2012; Tillmann et al., 2018; Tillmann and Akselman, 2016; Tillmann, 2018b), but the production potential of AZAs by the local species/populations is yet known only for *Azadinium poporum* (Tillmann et al., 2016). The analyses of phytoplankton composition and toxin production may lead to the understanding of carbon fluxes through the pelagic food web and to the benthos, where large beds of the scallop *Zygochlamys patagonica* are located (Bogazzi et al., 2005; Franco et al., 2017). The aim of this work is to characterize the plankton community in El Rincón including the detection of phycotoxins and toxic species. Particular interest is focused on the species of the dinoflagellate, *Alexandrium*, isolated at the middle-shelf, as this genus is of major concern due to its tendency to form HABs and its global biogeographical distribution (Anderson et al., 2012).

MATERIALS AND METHODS

Field Sampling

An oceanographic expedition on board the vessel *Bernardo Houssay* was carried out from the 5th to the 9th of September 2015 in the northern Patagonian shelf in the Argentine Sea. Twenty-four study stations were sampled in El Rincón (38°50′–41°S, 60–62°W) (**Figure 1**) (coastal, inner-shelf), which is an area encompassed between the coast and the isobath of 50 m depth and between the Bahía Blanca Estuary in the north and the Negro River Estuary in the south. In addition, two offshore stations, 33 and 34, located at the middle-shelf at 100 m depth (**Figure 1**), were sampled in the same expedition.

At each study station, surface water temperature (±0.2°C) and salinity (±0.06) were measured *in situ* by triplicate readings

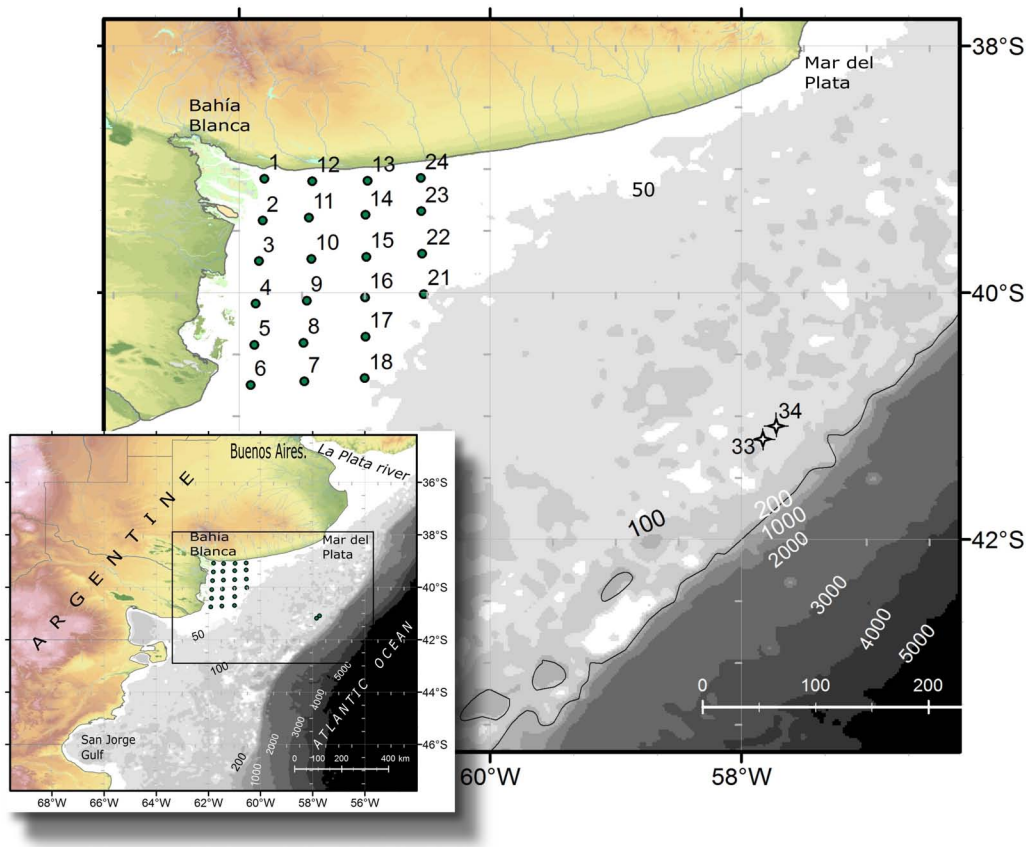


FIGURE 1 | Study area and location of the sampling stations during the oceanographic expedition in September 2015 aboard the research vessel *B. Houssay*.

with a multiparameter probe (Horiba U-10, Japan). Using Niskin bottles, surface water samples were collected for quantification of plankton and estimations of chl-*a*. For the former, 250 mL were fixed with Lugol's solution (1% final concentration) and kept in the dark until analysis under the microscope, while for chl-*a*, a volume of 200–250 mL was filtrated onboard through filter GF/C and kept at −20°C. Then, pigments were extracted using acetone:water (9:1) and the quantification was performed with a spectrofluorophotometer RF-5301PC Shimadzu calibrated with a standard of *Anacystis nidulans,* according to Holm-Hansen et al. (1965). In addition, using plankton net with a mesh of 35 μm, water samples were collected and fixed with formaldehyde (2% final concentration) for the identification of plankton species.

Chlorophyll *a* and Sea Surface Temperature (SST) From Satellite Observations

Satellite data was obtained from the Moderate Resolution Imaging Spectroradiometer (MODIS) on board the satellite Aqua. Daily level 1A data at 1 km spatial resolution, from 15th August to 30th September 2015, were downloaded from the Ocean Color website[1]. The files were processed into L2 and L3 product files using SeaDAS software, version 7.4 (Fu et al., 1998). The standard NASA atmospheric correction algorithm was applied, which provided the best performance in the optically complex waters of the studied area (Delgado et al., 2018) and other coastal waters (*i.e.*, Jamet et al., 2011; Goyens et al., 2013). The Chl-*a* and sea surface temperature (SST) products were derived from the OCx (Hu et al., 2012) and the non-linear sea surface temperature (NLSST) (Brown and Minnet, 1999) algorithms, respectively. Then, L3 products of 15 days (15th–31st August, 1st–15th September, and 16th–30th September) were obtained from the L2 (1 km) products using arithmetic averaging in Mercator projection (Campbell et al., 1995; Antoine, 2004).

Plankton Analysis

For quantitative estimations, plankton cells >5 μm were enumerated with a Wild M20 inverted microscope according to the procedures described by Hasle (1978). Depending on the seston content, subsamples of 10 mL (coastal stations) or 50 mL of seawater were collected with Niskin bottles, preserved with Lugol's solution, and were settled in Utermöhl composite sedimentation chambers during 24 h. The entire chamber was analyzed under a magnification of 400×. Plankton abundance was then expressed in cells L^{-1}. Samples of plankton at stations 4, 15, and 24 were not available. Unidentified organisms other than

[1]https://oceancolor.gsfc.nasa.gov

diatoms, dinoflagellates, and coccolithophores were classified as "flagellates" (regardless of flagella visible or not) and according to their size. Concerning dinoflagellates, species of the genera *Protoperidinium, Gyrodinium,* and some cf. *Gymnodinium*, ciliates, and the thecamoeba, *Paulinella ovalis,* were counted as heterotrophic protists. Plankton identification was performed in net haul samples under a Zeiss Standard R microscope and a Nikon Eclipse microscope, using phase contrast and differential interference contrast (DIC) and a magnification of 1000×. Local literature was consulted for the identification of plankton taxonomy from the Argentine Sea (references in the text) and general literature for identification of phytoplankton (Tomas, 1997; Hoppenrath and Debres, 2009). No cells of *Dinophysis* spp. were detected in surface bottle samples, while the associated toxin pectenotoxin-2 (PTX-2) was detected in net samples; the latter was analyzed to quantify their abundances and expressed in cells per net tow (cells NT^{-1}).

Cell dimensions were measured throughout the counting procedure using an ocular micrometer. Cell volumes of plankton (in μm^3) were calculated assigning simple geometric shapes to species according to Hillebrand et al. (1999) and transformed into carbon content (pg C $cell^{-1}$) using two different carbon-to-volume ratios, one for diatoms and one for all the other algae groups (Menden-Deuer and Lessard, 2000).

Sampling of Phycotoxins

Samples of plankton were collected by horizontal net tow hauls with a 35-μm mesh of 40-cm diameter. Net haul concentrates were adjusted to a defined volume of 2–5 L (depending on the net tow volume) using 0.2 μm filtered seawater. An aliquot of 100 mL was fixed with acidic Lugol's iodine solution (1% final concentration) for identification of the species. From the remaining volume, plankton was collected on a 20-μm mesh and transferred to a 50-mL centrifugation tube, which was adjusted to a final volume of 45 mL with filtered seawater. This sample was split into three aliquots, and each aliquot was centrifuged at a maximum speed of 3,500 × *g* in a 15-mL centrifugation tube (model 2036, Rolco, Buenos Aires, Argentina). The supernatants were discharged, pellets were resuspended in approximately 1 mL filtered sea water, transferred to a 2-mL cryovial, and centrifuged again (10 min, 3220 × *g*, model 5415 D, Eppendorf, Hamburg, Germany). Finally, supernatants were removed and cell pellets frozen at −20°C. A quantitative comparison of toxin abundances in the net tows of this expedition was not possible due to technical difficulty. Net tows could not be performed in a standardized way. No net sample could be taken at station 34.

Isolation of *Alexandrium* spp.

At stations 33 and 34, 2 L of seawater from 10 m depth were filled into different 500 mL PE flasks and kept at 4°C until inspection. Single cells of *Alexandrium* spp. were isolated from these samples by micropipette after analyzing under a stereomicroscope (SZH-ILLD, Olympus, Hamburg, Germany). Cells were transferred into individual wells of 96-well tissue culture plates (TPP, Trasadingen, Switzerland) containing 300 μL of Keller medium (Keller et al., 1987), prepared from 0.2 μm sterile-filtered natural Antarctic seawater at 1/10th of the original concentration. Isolated cells were then incubated at 15°C at a photon flux density of 50 μmol photons m^{-2} s^{-1} on a 16:8 light:dark (L:D) photocycle. After 3–4 weeks, four unialgal and clonal isolates (provisionally named H-3-D10, H-1-G8, H-3-D4, and H-2-A4), all originating from station 33, were transferred to polystyrene cell culture flasks, each containing 50 mL of ½ strength K medium and were maintained, thereafter, under the same conditions as described. Observation of live or fixed cells was carried out with a stereomicroscope (SZH-ILLD, Olympus) and with an inverted microscope (Axiovert 200 M, Zeiss, Munich, Germany) equipped with epifluorescence and DIC optics. Light microscopic examination of the thecal plate tabulation was performed on neutral lugol-fixed cells (1% final concentration) stained with calcofluor white (Fritz and Triemer, 1985). Photographs were taken with a digital camera (Axiocam MRc5, Zeiss).

For harvest of cellular DNA and analyzing of toxin, cultures of all strains were grown in 250 mL cell culture flasks at 15°C under a photon flux density of 50 μmol m^{-2} s^{-1} on a 16:8 h light:dark photocycle. In each harvest, cell density was determined by settling Lugol-fixed samples and counting cells >800 under an inverted microscope. For DNA extraction, 50 mL of culture were centrifuged as described later. For toxin analysis, 200 mL of cultures were harvested as 4 × 50 mL in 50-mL centrifugation tubes by centrifugation (Eppendorf 5810R, Hamburg, Germany) at 3,220 *g* for 10 min. Supernatant was discarded and all four pellets from one isolate were combined in a microcentrifuge tube and again centrifuged (Eppendorf 5415, 16,000 × *g*, 5 min) and stored frozen (−20°C) until use.

DNA Extraction and Phylogeny

For phylogenetic analyses of *Alexandrium* strains, genomic DNA was isolated using the E.Z.N.A Plant DNA Kit (Omega Bio-tek Inc., Norcross, GA, United States). The original isolation protocol was modified by insertion of an additional washing step using "SPW wash buffer." A NanoDrop ND-1000 system (PEQLAB, Erlangen, Germany) was used to determine the concentration and the purity of the genomic DNA. Phylogenetic inferences were based on analyses of the D1/D2 region of the 28S large subunit (LSU). The fragment of the LSU was amplified with the universal primer set D1R-F (5′ACCCGCTGAATTTAAGCATA3′) and D2C-R (5′CCTTGGTCCGTGTTTCAAGA3′) (John et al., 2014). The polymerase chain reaction (PCR) cocktail of a final volume of 50 μL contained ∼ 20 ng genomic DNA as template, 1× amplification buffer (5 Prime, Hamburg, Germany), 0.1 mM dNTPs (5 Prime), 0.1 μM of each primer, and 0.05 U of HotMaster Taq DNA Polymerase (5 Prime). The amplification protocol was performed for 2 min at 94°C, 30 s at 94°C, 30 s at 55°C, and 2 min at 68°C for 35 cycles and an extension for 10 min at 68°C. The correct size of the amplified PCR fragment was determined on a 1.5% agarose gel. The PCR product was purified with the NucleoSpin Gel and PCR cleanup kit (Macherey und Nagel, Düren, Germany) according the manufacturer's protocol and subjected to sequencing via standard BigDye Terminator v3.1 cycle sequencing chemistry (Applied Biosystems, Darmstadt, Germany). The resulting sequences were assembled using the DNASTAR software package (Lasergene,

United States). The LSU sequences obtained in this study were deposited in the GenBank database and the accession numbers are: BankIt2158787 H-2-A4 MK059419, BankIt2158787 H-1-G8 and MK059420 BankIt2158787 H-3-D4 MK059421. The sequences of the studied strains of *Alexandrium ostenfeldii* (H-2-A4, H-1-G8, and H-3-D4) were compared with 36 of other representative *A. ostenfeldii*/*A. peruvianum* strains obtained from GenBank. The LSU sequences of *Alexandrium insuetum* and *Alexandrium minutum* were used as outgroups to root the tree. The sequence of the *Alexandrium catenella* strain (H-3-D10) was compared with the LSU sequence assemblage, which is used to formally revise the *Alexandrium tamarense* species with complex taxonomy (John et al., 2014).

The MEGA7 (Kumar et al., 2016) was used for sequence alignment. The LSU sequences were aligned using ClustalW. The final alignment for the LSU phylogeny consisted of 572 positions for both *Alexandrium ostenfeldii* and *Alexandrium catenella*. The phylogenetic model was selected using the MEGA7 software package. The phylogenetic trees were represented using the maximum likelihood (ML) results, with bootstrap values from the ML method (n = 1000 replicates).

TABLE 1 | Mass spectrometric (ms) parameters used for analysis.

	PREC	EMS	CID	SRM
Curtain gas [au]	20	20	20	20
Collision gas [au]	High	Low	High	Medium
ESI voltage [V]	5500	5500	5500	5500
Temperature [°C]	650	650	650	650
Spray gas [au]	40	40	40	40
Auxiliary gas [au]	70	70	70	70
Interface heater	ON	ON	ON	ON
Declustering potential [V]	121	121	121	121
Collision energy spread [V]	–	0	0	–
Entrance potential [V]	10	5	–	10
Collision energy [V]	57	–	57	57
Collision exit potential [V]	22	–	–	22

PREC, precursor ion scan; EMS, enhanced mass scan; CID, collision-induced dissociation; SRM, selected ion monitoring; au, arbitrary units.

Analysis of Toxin of Field Samples

Cell pellets from the plankton net tows were resuspended in 500 μL of 0.03 M acetic acid for PST analysis and in 500 μL methanol for the determination of lipophilic toxins and subsequently homogenized with 0.9 g of lysing matrix D by reciprocal shaking at maximum speed (6.5 m s^{-1}) for 45 s in a Bio101 FastPrep instrument (Thermo Savant, Illkirch, France). After homogenization, samples were centrifuged at 16,100 × g at 4°C for 15 min. The supernatants were transferred to spin filters (0.45 μm pore size, Millipore Ultrafree, Eschborn, Germany) and centrifuged for 30 s at 800 × g, followed by transfer to autosampler vials.

Analysis of multiple lipophilic toxins was performed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) (AWI, Bremerhaven, Germany), as described by Krock et al. (2008). Contents of the toxin are expressed as nanograms per net tow (ng NT^{-1}). Paralytic shellfish poisoning (PSP) toxins were analyzed by ion-pair chromatography coupled with postcolumn derivatization and fluorescence detection (PCOX method) as described in detail by Van de Waal et al. (2015).

Analysis of Toxin of *Alexandrium* Cultures

In addition to a general screening of the selected variants of different groups of lipophilic phycotoxins, *A. ostenfeldii* strains were specifically analyzed for the presence of a wide variety of spiroimines. For spirolides, precursor ion experiments (PRECs) for the characteristic spirolide fragments m/z of 150, 164, and 180 were performed for the search of spirolide variants. For gymnodimines (including unknown variants), enhanced mass scans (EMSs) in the mass range of m/z 480–550 were performed. Collision-induced dissociation (CID) spectra of all positive m/z values detected with the before-mentioned experiments were recorded in the mass range of m/z 150–750. Applied mass spectrometric (MS) parameters are detailed in **Table 1**. All positively confirmed spiroimines were quantified in the selected reaction monitoring (SRM) mode using the mass transition listed in **Table 2** and calibrated against an external SPX-1 standard curve with four concentrations in the range from 1 to 1,000 pg μl^{-1} (Certified Reference Materials Program, NRC IMB, Halifax, NS, Canada). Results are expressed as SPX-1 equivalents.

Data Analyses of the Structure of Plankton

The distribution of the functional groups of plankton and potential toxic species in the studied area was analyzed by colored maps using the software Ocean Data View, version ODV 4.7.10. Non-parametric Spearman's correlation analyses were employed to determine correlations between chlorophyll concentration, phytoplankton abundance, and biomass. Cluster analysis was applied to the stations to assess the spatial structure of plankton, where the abundance (in cells L^{-1}) was log-transformed to normalize data. A matrix of similarities between each pair of sampling station was calculated using the Bray-Curtis similarity index. Then, the groups that were revealed by SIMPROF test in the cluster analysis were pointed out to in the map. The organisms with higher contribution to differences among groups were identified by means of SIMPER (similarity percentages) test. Statistical analysis was performed using the software PRIMER (Clarke and Gorley, 2006).

RESULTS

SST, Salinity, and Chlorophyll *a*

Surface temperature was measured *in situ* in the coastal, inner-shelf (21 stations with depth <50 m), and it varied between 10.0 and 11.3°C with a mean value of 10.4 ± 1.0°C, whereas lowest values of 7.5 and 7.2°C were detected at middle-shelf stations viz. 33 and 34. Salinity values ranged between 32.6 and

TABLE 2 | Mass transitions (Q1 and Q3 masses) used for the screening and quantification of spiroimines and corresponding spiroimines.

Q1 mass (m/z)	Q3 mass (m/z)	Spiroimine	Q1 mass (m/z)	Q3 mass (m/z)	Spiroimine
508	490	GYM A	666	180	Unspecified
510	492	desMe-GYM D	674	164	Unspecified
522	504	12-Methyl-GYM A	678	150	Unspecified
524	506	GYM A/B/D	678	164	13,19-didesMethyl-spirolide C/13/19,31-didesMethyl-spirolide C
526	508	oxo-desMe-GYM D	686	164	Unspecified
534	150	Unspecified	692	150	Spirolide A
536	150	Unspecified	692	164	13-desMethyl-spirolide C spirolide G
540	164	Unspecified	692	178	27-oxo-13,19-didesMethyl-spirolide C
540	522	Unspecified	694	150	Spirolide B
542	524	Unspecified	694	164	13-desMethyl-spirolide D Pinnatoxin G
548	530	Unspecified	694	180	27-hydroxy-13,19-didesMethyl-spirolide C
552	150	Unspecified	696	164	Unspecified
564	546	Unspecified	698	164	Unspecified
582	564	Unspecified	706	164	Spirolide C 20-Methyl-spirolide G
592	164	Spirolide M	708	164	Spirolide D
618	164	Compound 1	708	180	27-hydroxy-13-desMethyl-spirolide C
628	150	Unspecified	710	150	Unspecified
640	164	Unspecified	710	164	Compound 2
644	164	Unspecified	718	164	Compound 3
650	164	Spirolide H	720	164	Unspecified
652	164	Spirolide I	722	164	Unspecified
658	150	Unspecified	722	180	Unspecified
658	164	Unspecified	766	164	Pinnatoxin F
666	164	Unspecified	784	164	Pinnatoxin E

35 with a mean of 34.1 ± 0.7, displaying lower values at coastal stations 2–10 and at stations 33 and 34. The satellite estimations of the three 15-days means of SST (**Figures 2A–C**) and chl-*a* distribution (**Figures 2D–F**) showed that SST increased from August to September (**Figures 2A–C**) in the coastal and the inner-shelf (8–13°C), while they remained rather constant and cooler (∼5–6°C) in the middle-shelf. Conversely, chl-*a* displayed larger variability in the middle-shelf (**Figures 2D–F**), with higher values during the first half of September (**Figure 2E**) when the expedition was performed. The *in situ* chl-*a* ranged from 0.2 to 1.9 $\mu g\ L^{-1}$ (**Figure 3A**), with a mean of 0.8 ± 0.48 $\mu g\ L^{-1}$.

Total Abundance, Biomass, and Composition of Plankton

The abundance of photosynthetic protists ("phytoplankton") >5 μm was found between 0.21 × 10^5 cells L^{-1} and 6.47 × 10^5 cells L^{-1} (**Figure 3A**), with values more than twofold the mean of 1.5 × 10^5 cells L^{-1} occurring only at a few stations: 1, 5, 22, 23, and 34 (**Figure 3A**). Biomass of phytoplankton varied between 3.8 and 103.1 $\mu g\ C\ L^{-1}$ (**Figure 3A**) and both the total abundance and biomass of plankton displayed weak linear correlation with chl-*a* concentration (R^2 ∼0.56, n = 21). Abundance of heterotrophic protists was found between 3.5 × 10^3 cells L^{-1} and 1.8 × 10^5 cells L^{-1} and showed larger values in the middle-shelf (**Figure 3B**), mainly caused by high abundance of dinoflagellates. A frequent heterotrophic flagellate identified in the study area was *Leucocryptos* sp. (length 7–13 μm), and the thecamoeba *Paulinella ovalis* was seen frequently at the coastal stations (1–14). Naked ciliates ∼20 μm were observed commonly offshore (max. 5.95 × 10^4 cells L^{-1} at station 34), while tintinnids were dominant at the coastal stations close to the Bahía Blanca Estuary (station 1, 2, and 12), with low abundance (max. 7 × 10^3 cells L^{-1} at station 2).

Diatoms were the dominant group in the study area, achieving more than 50% of the abundance and the biomass of total phytoplankton at most of the stations (**Figures 4**, **5**), with the exception of stations 7, 8, 14, 16, 18, and 34, where the groups flagellates and/or dinoflagellates were more abundant (**Figures 4**, **5**). Diatoms showed the highest concentrations at the coastal stations (**Figure 5**), mainly represented by *Cymatosira belgica, Asterionellopsis glacialis*, *Paralia sulcata,* and *Thalassionema nitzschioides*, each species with abundances between 1.3 × 10^5 cells L^{-1} and 2.3 × 10^5 cells L^{-1} (list of species in **Supplementary Table 1**). In particular, diatoms displayed high concentrations at stations 22 and 23 (**Figure 5**) due to a bloom of *Pseudo-nitzschia* spp. with abundances up to 3.6 × 10^5 cells L^{-1} (**Figure 6**). At station 33, the diatom *Eucampia cornuta* reached a high concentration (8.0 × 10^4 cells L^{-1}). Two silicoflagellates were common in the study area, *Dictyocha speculum* and *D. fibula*, with maximal abundance at station 34 (3.7 × 10^4 cells L^{-1}). Dinoflagellates were more abundant at stations 33 and 34, followed by stations 21 and 22 (**Figure 5**), with dominance of heterotrophic dinoflagellates such as cf. *Gymnodinium*, *Protoperidinium* spp., *Torodinium robustum, Gyrodinium spirale,* and *Amphidinium* spp. Among the photosynthetic dinoflagellates, the most frequent were *Prorocentrum* spp., *Oxytoxum* sp., *Tripos* spp., and *Scrippsiella* sp. Small photosynthetic thecate dinoflagellates (length ∼10–18 μm) were present as well such as *Heterocapsa* sp. and species of the family Amphidomataceae (e.g., *Amphidoma parvula*, Tillmann et al., 2018), which will be further presented in detail in a companion paper (Tillmann et al. in prep). Cells of the genus *Alexandrium* were found at relatively low abundances, up to 1.0 × 10^3 cells L^{-1}, and *Dinophysis* was not detected in bottle samples, but recorded in net samples with up to 3.6 × 10^3

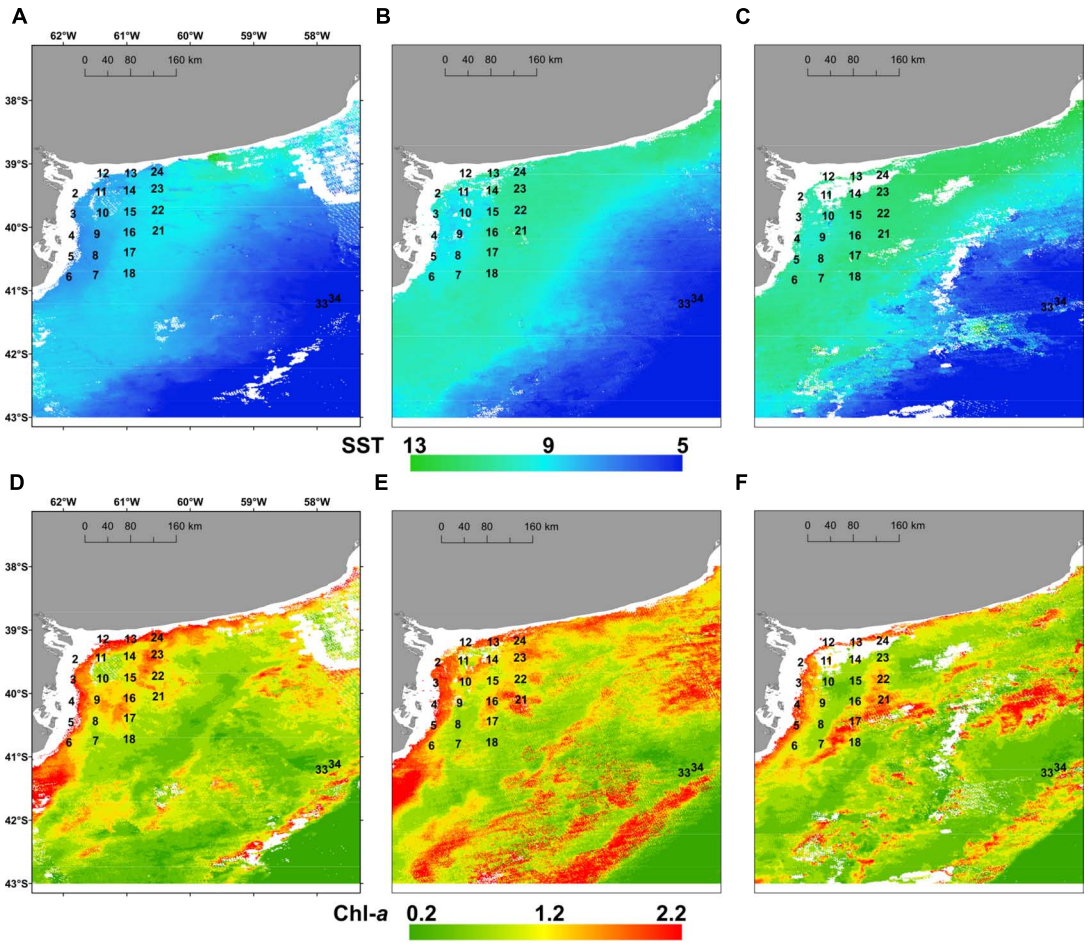


FIGURE 2 | Mean sea surface temperature, SST (°C) (upper panels) and mean surface chlorophyll *a*, chl-*a* (μg L^{-1}) (lower panels) registered by satellite images in the study area for the periods: **(A,D)** 15th–31st August 2015; **(B,E)** 1st–15th September 2015 and **(C,F)** 16th–30th September 2015.

cells NT^{-1} (**Figure 6**). The group of coccolithophores was mainly represented by *Gephyrocapsa oceanica* (5–10 μm), with the highest concentration at station 12 (3.1×10^4 cells L^{-1}), followed by *Emiliania huxleyi* (3–7 μm) at the offshore stations (up to 7.4×10^3 cells L^{-1} at station 33). The Xanthophyceae *Ophiocytium* sp. at the coastal stations and the mixotrophic ciliate *Mesodinium rubrum* at station 34 notably contributed to the total carbon biomass (**Figure 4**) due to their large cell size. The analysis of the plankton structure in the study area displayed spatial differentiation across the coastal and inner-shelf to middle-shelf stations (**Figure 7**), with decreasing ratios of diatoms to dinoflagellates and a rising trend of heterotrophic protists (**Figures 3**, **5**).

Description of the *Alexandrium* spp. Strains

Based on thecal plate pattern determined under the fluorescence microscope, three of the strains (H-1-G8, H-3-D4, and H-2-A4) were identified as *A. ostenfeldii,* whereas the fourth strain (H-3-D10) was of the *tamarense/catenella* morphotype. Cells of the strain H-3-D10, during exponential growth in culture, mainly occurred in short two-cell chains, but occasionally were present in four-cell chains. For all strains of *A. ostenfeldii*, pairs of cells probably representing late stages of cell division were regularly observed.

Cells of H-3-D10 had a typical *Alexandrium* outline with a conical epitheca and a more trapezoidal hypotheca (**Figures 8A–I**). Cells sizes ranged from 26.4 to 42.9 μm in length (mean 35.4 ± 3.1, n = 30) and from 30.0 to 39.7 μm in width (mean 35.0 ± 2.2, n = 30). The number, size, and shape of thecal plates (**Figures 8C–G**) conformed to the species description of *Alexandrium catenella*. The first apical plate consistently had a small ventral pore (**Figure 8D**). A large connecting pore was occasionally seen on both the pore plate (**Figure 8E**) and the posterior sulcal plate (**Figure 8H**).

Cells of *A. ostenfeldii* were variable in size and shape (**Figures 8J–R**), with most cells being round to slightly ellipsoid (**Figures 8J,K**). Cell length ranged from 27.7 to 43.1 μm (mean 35.4 ± 4.5, n = 30) and width from 27.4 to 42.6 μm (mean 34.7 ± 4.2, n = 30). The plate pattern (**Figures 8L–P**) and the typical shape of the first apical plate and the large ventral pore

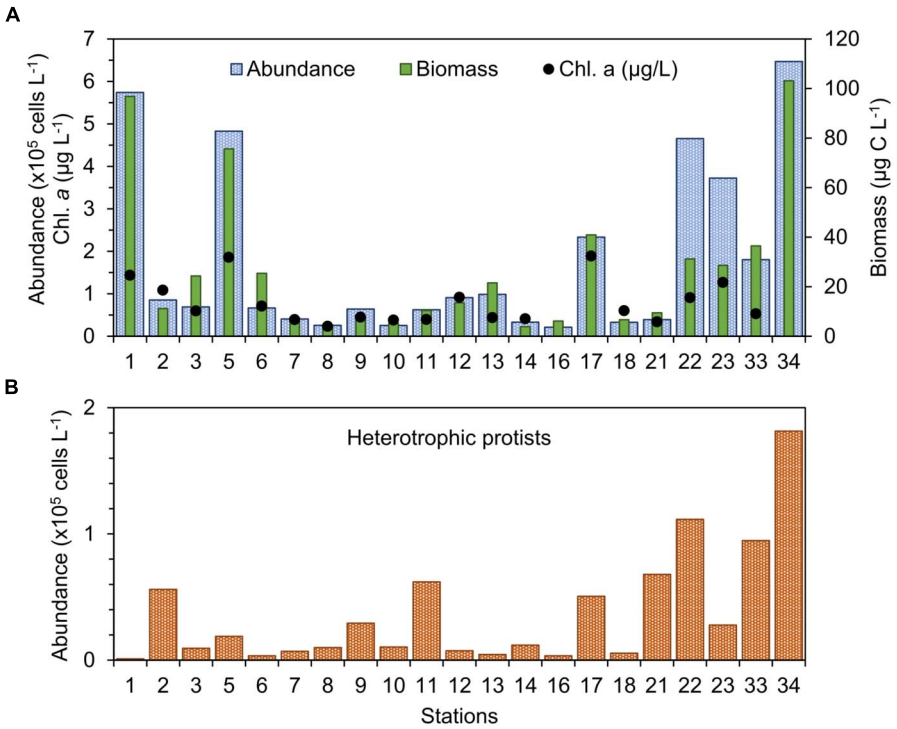


FIGURE 3 | (A) Abundance, biomass, and chlorophyll *a* concentration of phytoplankton and **(B)** abundance of heterotrophic protists in the study stations in September 2015.

(**Figures 8L,M**) clearly identified all three strains as *A. ostenfeldii*. The right anterior margin of plate 1' was straight. The shape of the anterior sulcal plate sa was slightly variable within all three strains. The majority of cells had a "door-latch" shaped sa plate but more "A-shaped" sa plates were also present (**Figures 8Q,R**).

Phylogeny

The phylogenetic analysis for *Alexandrium ostenfeldii* including three strains isolated in this study (H-2-A4, H-1-G8, and H-3-D4) revealed a tree with seven distinct phylogenetic groups. All three strains isolated in this study from the Argentine Sea formed a well-supported (ML 96%) monophyletic group within a larger cluster of sequences that consists of the groups 5, 6, and 7 (**Figure 9**). The three strains of *Alexandrium ostenfeldii* were identical in the sequence of the LSU-fragment analyzed. The dissimilarity with the next neighbors in the tree (group 5) was ∼0.5%. All other groups displayed a higher dissimilarity peaking at 2% for the isolate from the Bohai Sea in China. The phylogenetic placement of H-3-D10 (not shown) identified this strain as *Alexandrium catenella* (=group I of the former *A. tamarense/A. fundyense/A. catenella* species complex). The dissimilarity of H-3-D10 with group II of the former *A. tamarense/A. fundyense/A. catenella* species complex was in the range of 5%.

Phycotoxins in Field Samples

Paralytic shellfish poisoning toxins were detected in plankton net tows at stations 4, 15, 17, 21, and 33 at relatively low abundances ranging between 114.4 ng NT^{-1} at station 33 and 2593.8 ng NT^{-1} at station 17 (**Figure 10**). The toxin profiles were dominated by gonyautoxin-1 and -4 (GTX1/4) at stations 15, 17, and 21. In addition to GTX1/4, C1/2, and GTX2/3 were detected at almost the same relative abundances at all five stations (**Figure 10**).

Lipophilic toxins were present at low abundances during the expedition [**Figure 6**, gymnodimine A (GYM) is not shown]. Domoic acid (DA) was found only at stations 15 and 17 at abundances of 2.0 and 6.1 ng NT^{-1}, respectively. The second-least abundant phycotoxin was GYM, found only at station 3 at an abundance of 45.4 ng NT^{-1}. SPX-1 was found at stations 17, 21, and 33 at abundances ranging from 0.5 ng NT^{-1} at station 21 to 143.6 ng NT^{-1} at station 33. The most-dominant lipophilic toxin in terms of geographic distribution and abundance was PTX-2, which was detected at stations 4, 5, 15, 17, 21, and 33 at abundances ranging from 15.6 ng NT^{-1} at station 5 to 5684.4 ng NT^{-1} at station 21.

Toxin Profiles of *Alexandrium* Isolates

The toxin profile of *Alexandrium catenella* strain H-3-D10 was dominated by C1/2 (72%), followed by GTX1/4 (15%), neosaxitoxin (NEO, 9%), GTX2/3 (2%), and saxitoxin (STX, 2%) (**Figure 11**). Also, the three *A. ostenfeldii* strains H-1-G8, H-3-D4, and H-2-A4 were producers of PSP toxin with a quantitatively different PSP profile than *A. catenella* H-3-D10. In contrast to

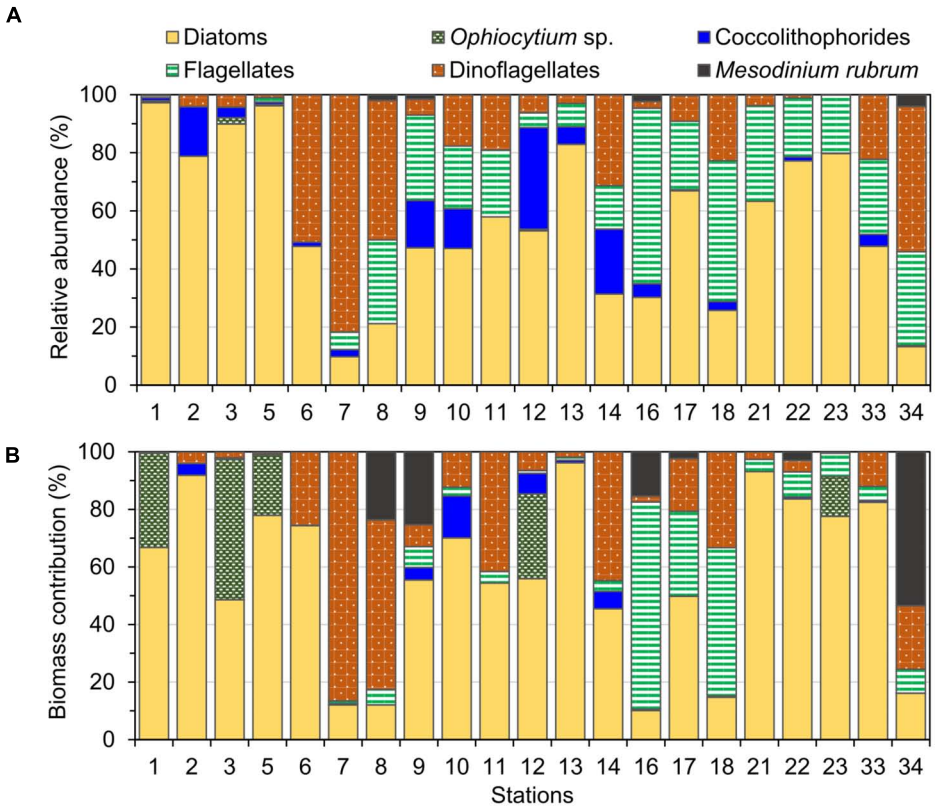


FIGURE 4 | Relative **(A)** abundance and **(B)** biomass of the main phytoplankton groups identified at the study stations in September 2015.

A. catenella, the most abundant toxins of the three *A. ostenfeldii* strains were GTX2/3 followed by C1/2 and STX (**Figure 11**).

In addition to PSP toxins, the three *A. ostenfeldii* strains also produced spirolides (**Figure 12**). The most-abundant spirolide among all three strains was SPX-1 ranging between 83 and 93% of total spirolides, followed by a novel spirolide with the pseudo-molecular $[M + H]^+$ ion *m/z* 592 ranging between 3.5 and 11.5%, which was named here spirolide M. All three *A. ostenfeldii* strains additionally contained five further spirolides as minor compounds: 27-hydroxy-13-desmethyl spirolide C and four, yet, uncharacterized spirolides named compounds (1) to (4) (**Figures 11**, **12**).

DISCUSSION

Environmental Conditions and Distribution of Plankton

The variability of hydrographic conditions in El Rincón has been ascribed to the seasonal influence of water mass currents along the shelf and shelf breakfronts, river discharges into the shallow area, and anthropogenic activities along the coastline (e.g., Lucas et al., 2005; Palma et al., 2008). In this study, the lower temperature and salinity registered at middle-shelf resulted from the influence of colder Subantarctic shelf waters diluted by low salinity discharges from the Magellan Strait (Palma and Matano, 2012), while the higher water temperatures in the shallow area of El Rincón were due to the northward outflow of high salinity and warmer waters from the San Matías Gulf into the inner-shelf (Palma et al., 2008). The freshwater discharges of the Negro and the Colorado Rivers (Lucas et al., 2005) were detected *in situ* at the coastal stations near their deltas, while the influence of the highly eutrophic and hypersaline Bahía Blanca Estuary (Guinder et al., 2010; López Abbate et al., 2015) was notable at stations 1 and 12, where the *in situ* salinities reached the maxima. A particular characteristic of El Rincón area is the formation of an anticyclone gyre in the center during winter, which looses intensification when nearing spring and summer (Palma et al., 2008). Lower concentrations of phytoplankton were found in the center of El Rincón, where the anticyclone gyre developed. Conversely, the growth of large diatoms with high carbon biomass at most coastal stations might be related with nutrient inputs from land such as the Bahía Blanca Estuary (Guinder et al., 2010; Koppprio et al., 2017; López Abbate et al., 2017) as well as with the mixed and turbid water column (Litchman and Klausmeier, 2008) characteristic of this shallow area (Guinder et al., 2009; Delgado et al., 2015). In eutrophic estuaries and adjacent coastal shelves, diatoms commonly dominate the phytoplankton, and this group is of high nutritional quality and facilitates efficient trophic transfer to support the secondary production (Winder et al., 2017). Dinoflagellates, the second-most abundant phytoplankton group in the area and dominant at the stratified middle-shelf

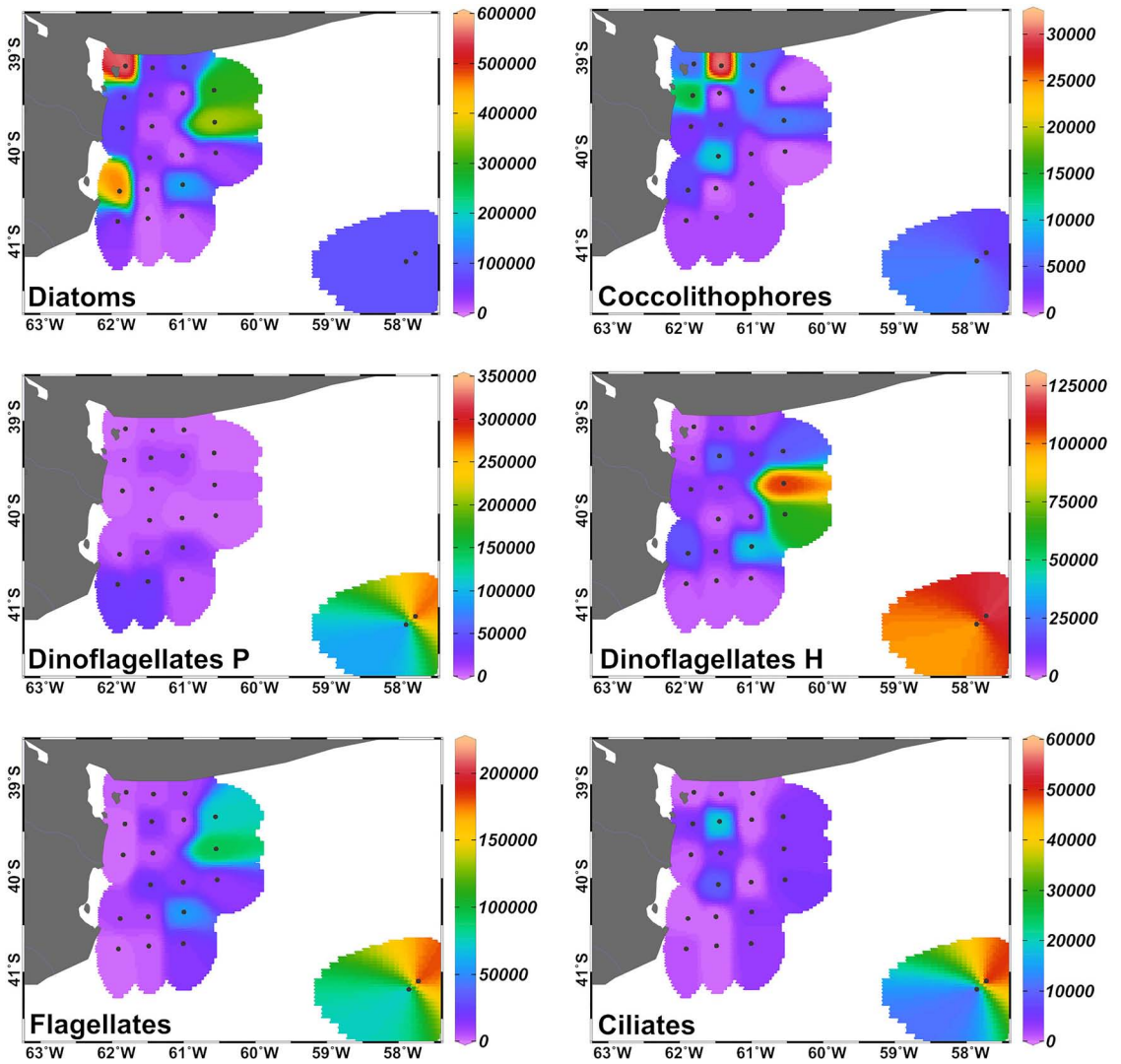


FIGURE 5 | Distribution of the abundance (in cells L^{-1}) of the main protists' groups (cells >5 μm) in the inner-shelf and at the two middle-shelf stations in September 2015. Dinoflagellates P: phototrophic, dinoflagellates H: heterotrophic.

stations (e.g., Carreto et al., 2016) also represent high food quality for higher trophic levels (Winder et al., 2017), except if they are toxigenic species (see section Lipophilic Toxins and Potential Producers in Field Samples). The high concentrations of heterotrophic protists (dinoflagellates and ciliates) at the middle-shelf might be related with an earlier phytoplankton bloom followed by zooplankton grazing (García et al., 2008; Carreto et al., 2016). Some particular species of dinoflagellates such as *Scrippsiella* sp. and *Heterocapsa* sp. were abundant at the coastal stations close to the Bahía Blanca Estuary (stations 1 and 2) and sandy beaches (stations 12–13), where these species have been commonly registered and associated with mixed and highly turbid waters (e.g., Guinder et al., 2010; Delgado et al., 2018). Similarly, the dinoflagellate *Prorocentrum minimum* was frequently found at several shallow stations of El Rincón, in agreement with the detection of this species blooming in weak stratified subantarctic waters (Gómez et al., 2011). Further, coccolithophore species, tintinnids, and naked ciliates also displayed differential distribution across the coastal middle-shelf transition, likely related to their specific ecological traits under different environmental conditions (*e.g.*, Le Quéré et al., 2005; Litchman and Klausmeier, 2008).

Considering that remote sensing chlorophyll data are often used as a proxy of phytoplankton biomass (Delgado et al., 2015), it is worth noting that in this study, abundance (in cells L^{-1}) and biomass (cell carbon content in μg C L^{-1}) of phytoplankton were only weakly correlated with chlorophyll. This might be related with the relatively high abundance of pico- and ultra-phytoplankton (<5 μm) characteristic of El Rincón (Negri et al., 2013), but it was not measured in this study and with the adjustment of the cell content of chlorophyll to fluctuating turbidity and underwater light conditions typical

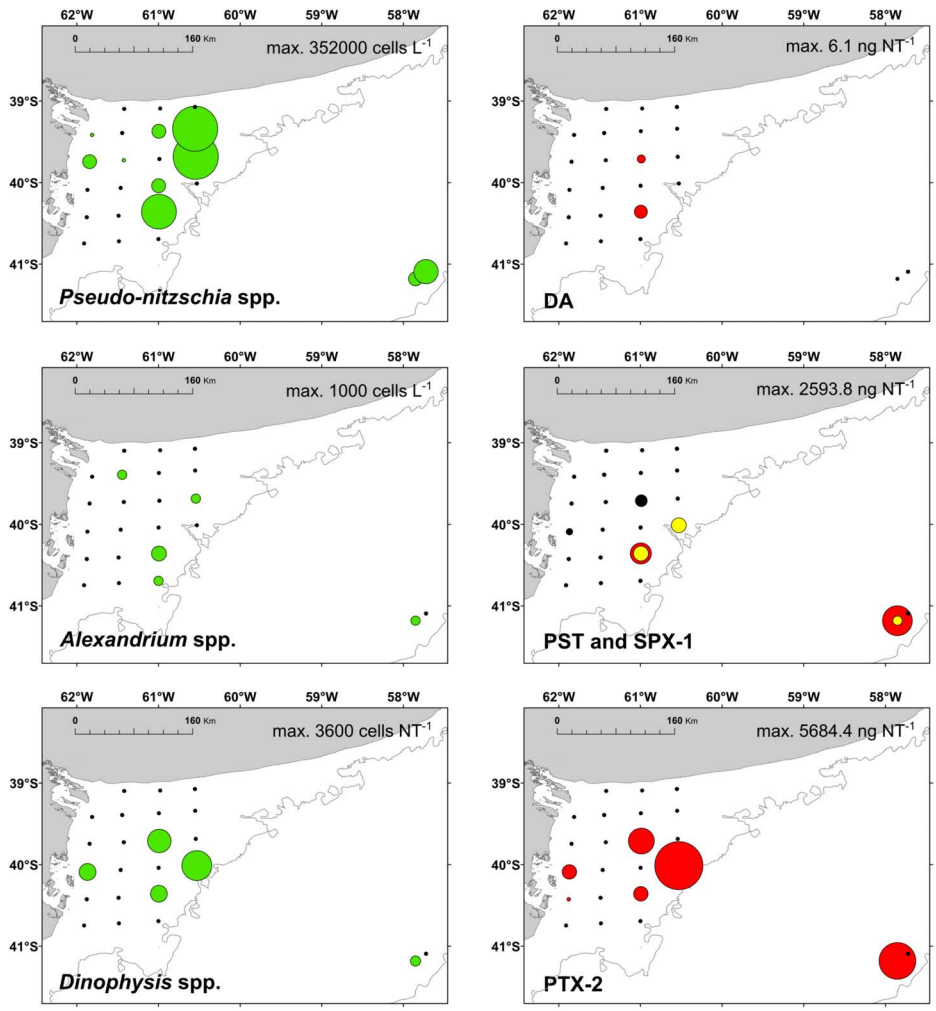


FIGURE 6 | Left panels: distribution of the potential toxigenic diatom *Pseudo-nitzschia* spp. and the dinoflagellate *Alexandrium* spp; and the ciliate *Mesodinium rubrum* (prey of *Dinophysis* spp.) (green); right panels: distribution of the respective phycotoxins in September 2015 (red). DA, domoic acid; PST, paralytic shellfish toxins; SPX-1, 13-desmethyl spirolide C (yellow); and PTX-2, pectenotoxin-2 (produced by *Dinophysis*).

of this coastal area (Guinder et al., 2009; Delgado et al., 2018).

Lipophilic Toxins and Potential Producers in Field Samples

In the present study, in agreement with historical records (Carreto et al., 1981), the detection of phycotoxins and their producers indicates the occurrence of HABs in a highly productive area of the Argentine shelf causing potential risk for marine biota and human welfare. The spatial heterogeneity of El Rincón seems to provide multiple niches for the development of diverse phytoplankton ecological traits including the production of toxins. Even in late winter, a number of lipophilic toxins were detected during the cruise. The amino acid domoic acid (DA) is produced by several species of the diatom of genera *Nitzschia* and *Pseudo-nitzschia* (Trainer et al., 2012). Here, blooms of *Pseudo-nitzschia* spp. were identified, but the low detection of domoic acid in the field could be related to the high species richness of this genus in the Argentine Sea (∼39–47°S), where half of them are non-toxigenic species (four of a total of eight described species; Almandoz et al., 2017). Identification of *Pseudo-nitzschia* up to species level by light microscopy is hardly possible and thus a correlation of *Pseudo-nitzschia* and DA is difficult. Furthermore, environmental factors influence the production of DA in *Pseudo-nitzschia*. A recent study has shown that DA production in a *Pseudo-nitzschia* species previously regarded as non-toxic was induced by the presence of herbivorous copepods (Harðardóttir et al., 2015). Additionally, mismatch between *Pseudo-nitzschia* and DA in the field samples could rely also on the sampling scheme. Samples for toxin analysis were obtained by horizontal plankton net tows, while cell counts were performed on Niskin bottle samples from discrete depth.

The most abundant toxin was PTX-2, which is produced by several species of the genus *Dinophysis* (Reguera et al., 2014). No cells of *Dinophysis* were detected in bottle samples

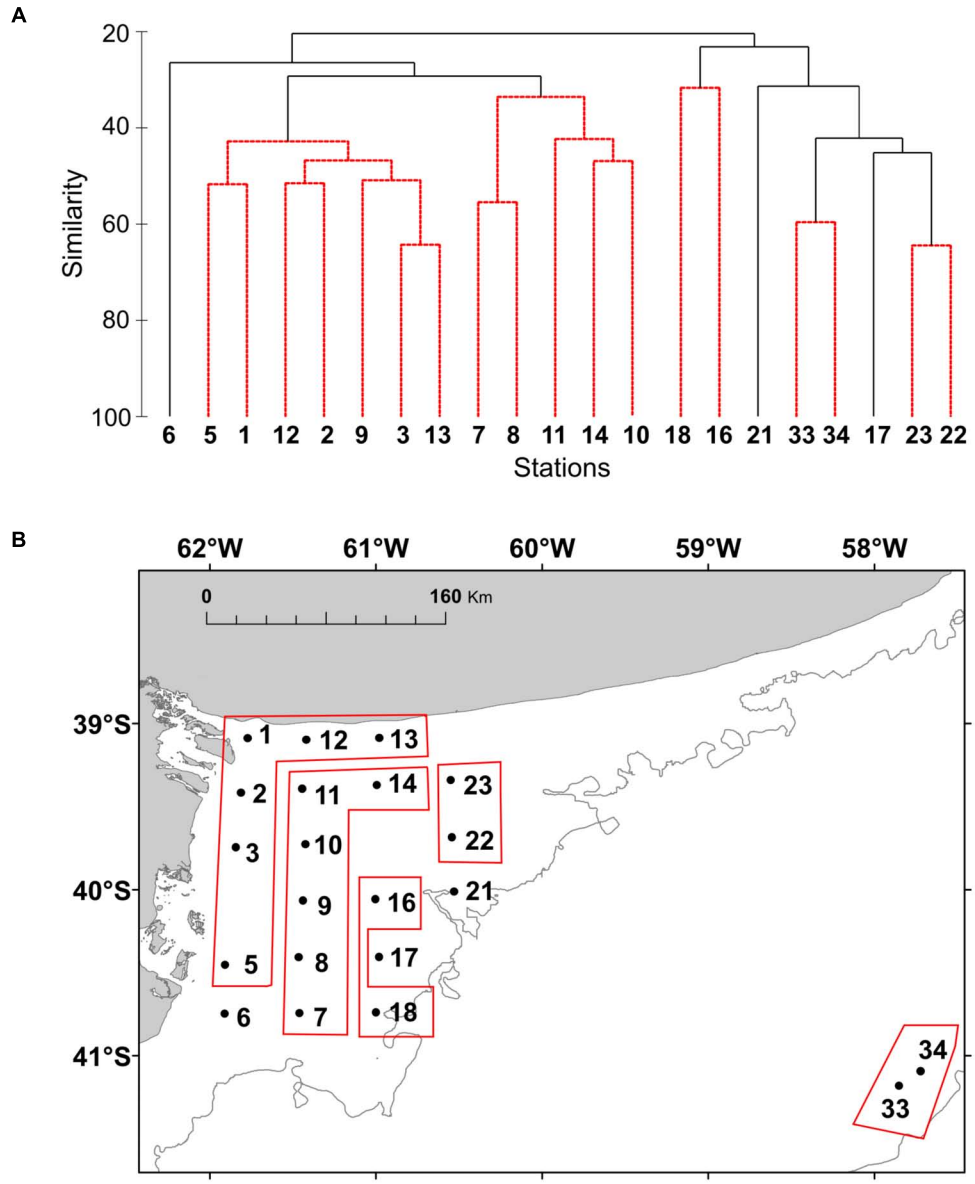


FIGURE 7 | (A) Cluster analysis based on Bray-Curtis similarity index of the plankton community at the study stations of El Rincón in September 2015.The abundance of the species (cells L^{-1}) was transformed using log (x + 1). **(B)** The resulted groups from the SIMPROF are shown in the map of the study area.

indicating an overall abundance below the detection limits of the sedimentation counting method. In the horizontal net samples, however, cells of *D. tripos*, and some of *D. acuminata* only at station 15, were found in co-occurrence with PTX-2 (**Figure 6**). A comparable link of *Dinophysis tripos* with PTX-2 was found by Fabro et al. (2015) in the Argentine Sea (∼38–56°S) in late summer, albeit at higher cell abundances and lower concentration of PTX-2 compared to this study. In late winter, potential predators of *Dinophysis,* such as *Noctiluca scintillans* and *Gyrodinium* spp., were also found abundant in net samples, especially at stations 17 and 21. Moreover, *Dinophysis* generally co-occurred with the ciliate *Mesodinium rubrum*, the obligate prey of this dinoflagellate (Park et al., 2006), which indicates potentially good growth conditions for *Dinophysis*. The dinoflagellate genus *Alexandrium* was found in relatively low abundance in the field. Cells of *Alexandrium* spp. were found in the shallow area of El Rincón and at the two middle-shelf stations. This is in agreement with previous studies that documented a wide distribution of the genus under contrasting physical conditions at a wide latitudinal range of the Argentine shelfbreak front (Carreto et al., 1998; Montoya et al., 2010; Almandoz et al., 2014; Fabro et al., 2017). The presence of cells of *Alexandrium* spp. in the field was related with the detection of PSP toxins, in particular, *A. ostenfeldii* and the spirolide SPX-1 (**Figure 6**). Furthermore, the most abundant toxins produced by the *Alexandrium* isolates (C1/2,

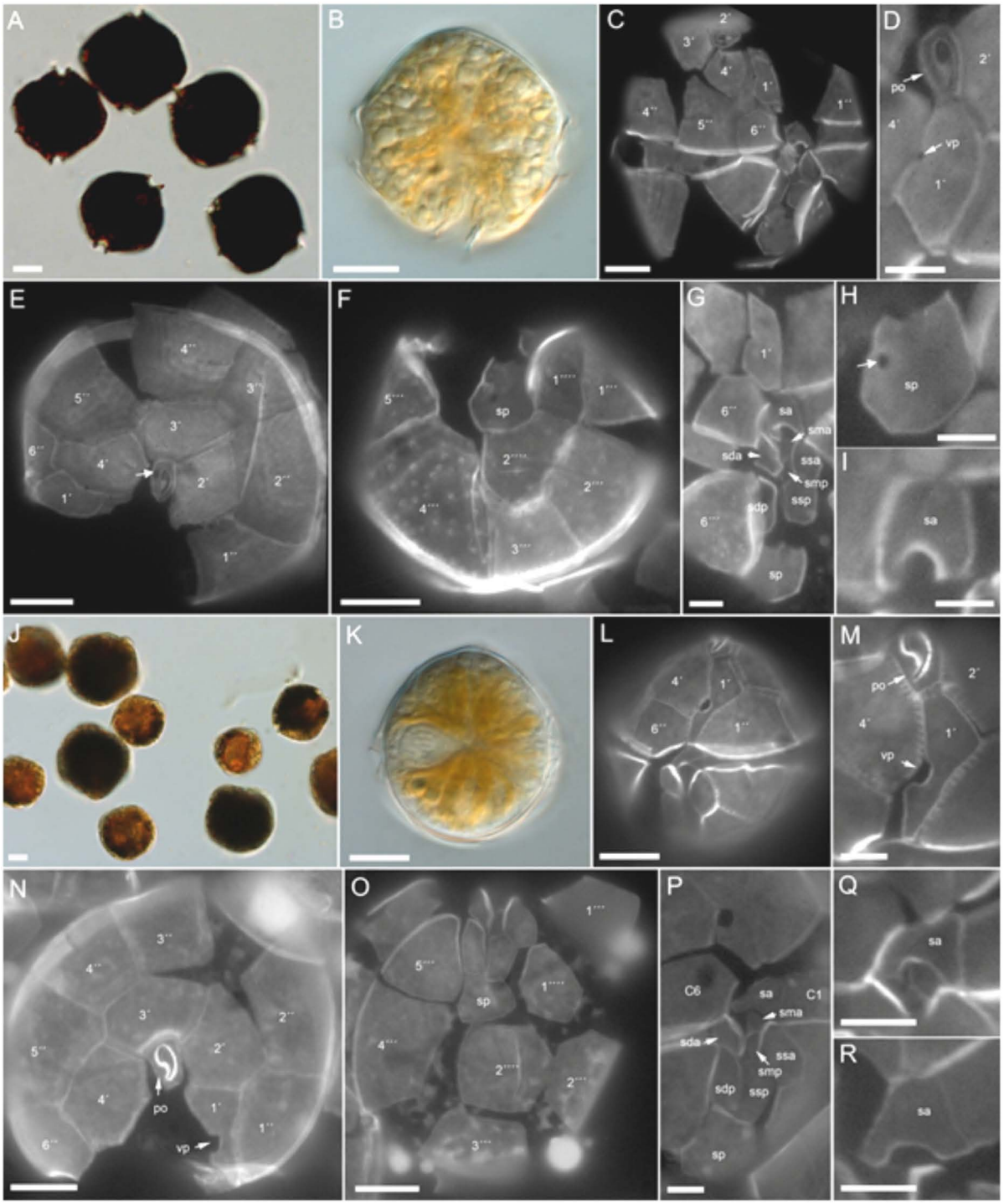


FIGURE 8 | *Alexandrium* spp. morphology. **(A–I)** *A. catenella* (H3-D10). Bright field images of **(A)** lugol-stained or **(B)** living cell. Epifluorescence images of calcofluor-stained cells showing **(C–G)** the plate pattern, and **(H)** details of the posterior and **(I)** anterior sulcal plate. Note in **(D)** the ventral pore (vp) and in **(E)** the attachment pore on the pore plate (white arrow). **(J–R)** *A. ostenfeldii* (H-3D4). Bright field images of **(J)** lugol-stained or **(K)** living cell. Epifluorescence images of calcofluor-stained cells showing **(L–P)** the plate pattern, and **(Q,R)** details of the anterior sulcal plate. Vp, ventral pore; Po, pore plate. Plate labels according to the Kofoidean system. Sulcal plates abbreviations: sp, posterior sulcal plate; ssp, left posterior sulcal plate; sdp, right posterior sulcal plate; smp, median posterior sulcal plate; sda, right anterior sulcal plate; ssa, left anterior sulcal plate; sma, median anterior sulcal plate; sa, anterior sulcal plate. Scale bars = 10 μm **(A–C,E,F,J–L,N,O)** or 5 μm **(D,G–I,M,P–R)**.

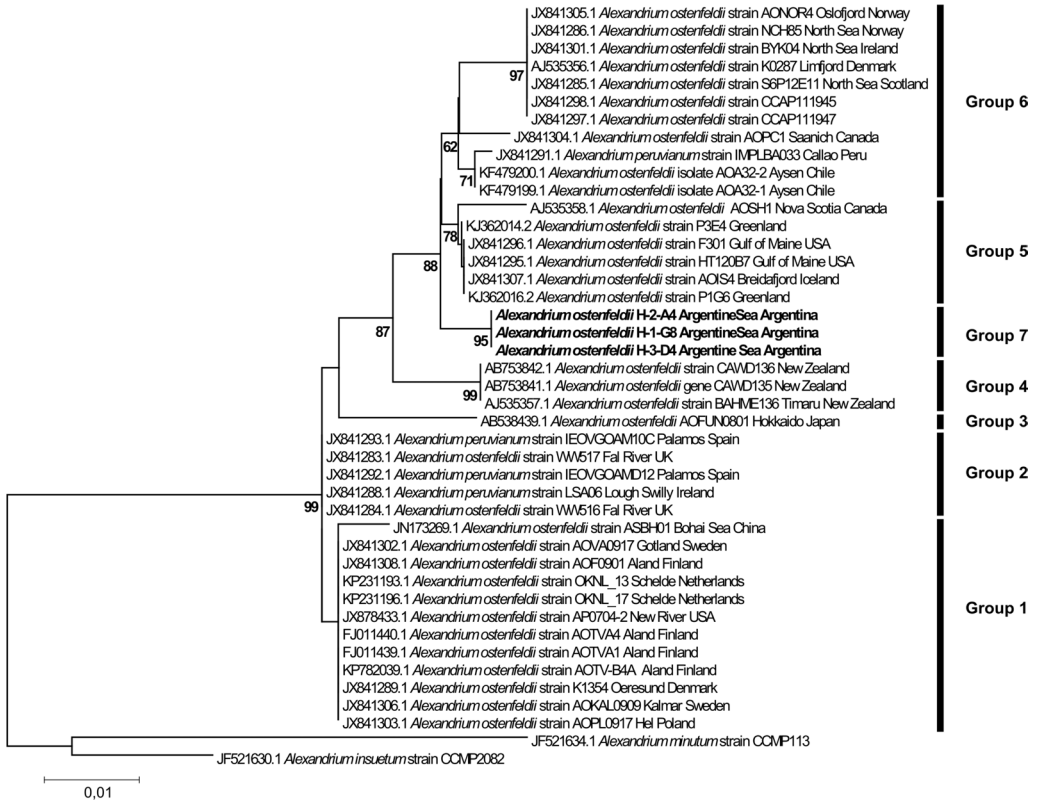


FIGURE 9 | Phylogenetic tree of *Alexandrium ostenfeldii*.

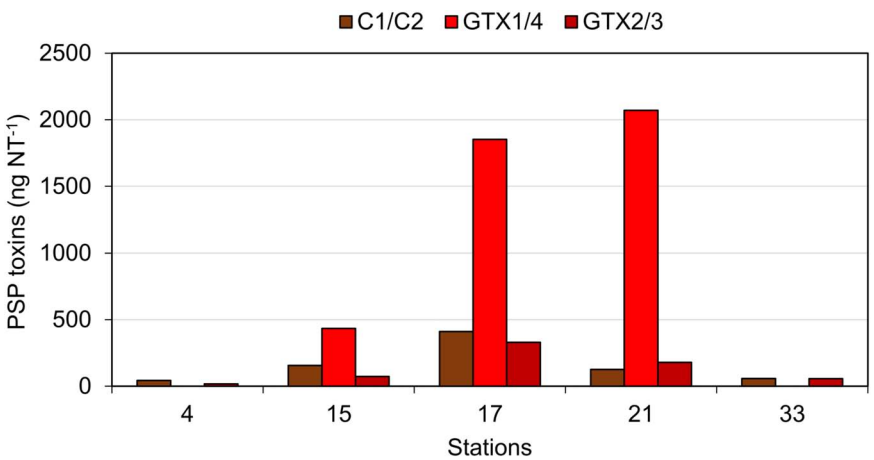


FIGURE 10 | The PSP toxins detected in net hauls of plankton. Note that the water columns sampled at different stations were not normalized and quantitative comparisons among stations are not possible.

GTX2/3, and SPX-1) were also detected in the samples of plankton.

Phylogeny of *Alexandrium* Strains

In the Argentine Sea, the genus *Alexandrium* has been registered in a wide latitudinal area (∼37–55.5°S) either near the coast, middle-shelf, and shelfbreak front, not only mainly in spring, but also in autumn and summer (e.g., Balech, 1995; Carreto et al., 1998; Gayoso and Fulco, 2006; Montoya et al., 2010). Most of the species identified by microscopy and/or molecular analyses belong to the *A. tamarense/catenella/fundyense* complex (Fabro et al., 2017 and references therein). In previous studies, there was only one strain of the *A. tamarense/catenella/fundyense* species complex isolated from the Argentine shelf that has

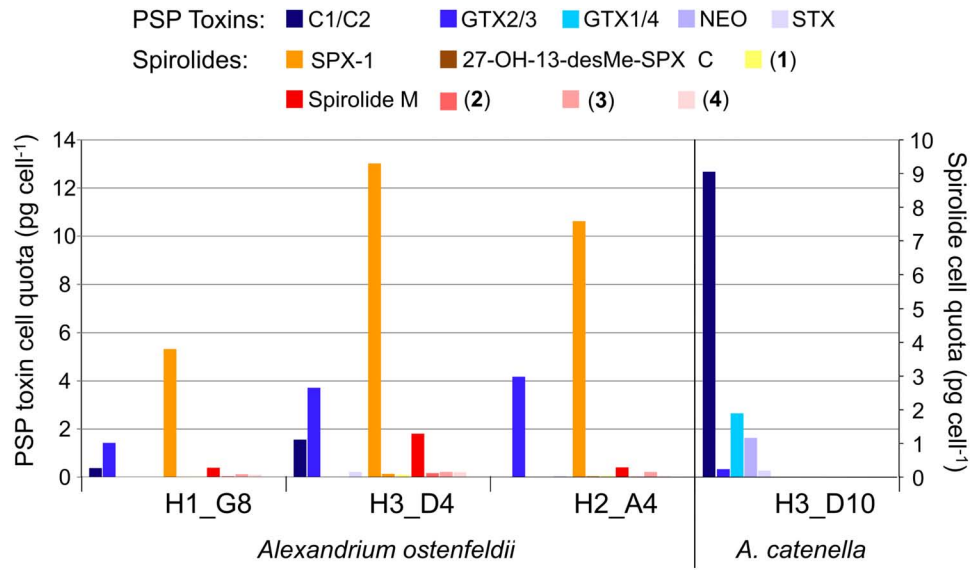


FIGURE 11 | Profiles of the toxin of four *Alexandrium* isolates.

been genetically characterized and identified as ribotype group I (which now in the new nomenclature of the genus is *A. catenella*), that is, the strain MDQ1096 isolated from the coast of Mar del Plata (∼38°S), 500 km north of the Bahía Blanca Estuary (Carreto et al., 2001; Penna et al., 2008). In addition, the sequence analyses of the two strains, H5 and H7, isolated from the San Jorge Gulf (∼45–47.5°S) in autumn 2012, and reported in the old morphological terminology as *A. tamarense* by Krock et al. (2015), are now confirmed to be *A. catenella*, that is, ribotype group I of the *A. tamarense/catenella/fundyense* species complex (Metfies and Tillmann, unpublished). Genetic analysis has shown that the strain MDQ1096 has the same D1–D2 LSU rRNA gene sequences as the *A. catenella* from Chilean waters and from the Southeast Pacific (Lilly et al., 2007; Aguilera-Belmonte et al., 2011). All these studies carried out in the Argentine continental shelf over the last decades support the apparent dominance of *A. catenella* over other *Alexandrium* species in that area and further suggest that its population reaches wide distribution in the Southern Hemisphere.

The new sequence data of the Argentine Sea *Alexandrium ostenfeldii* strains in the LSU phylogenetic analysis added a new and well-supported ribotype group to the six groups identified previously (Kremp et al., 2014; Salgado et al., 2015; Van de Waal et al., 2015). The general clustering of the sequences is in agreement with previous phylogenetic analyses based on concatenated rDNA (Orr et al., 2011; Kremp et al., 2014) with two main clusters, where groups 1 and 2 are forming one cluster and the other groups are forming another cluster. Groups 1 and 2 subsume strains from various parts of the globe with all strains from group 1 originating from low-salinity environments. Strains of group 2 originate from coastal embayments and estuaries of Ireland, the United States, and the Spanish Mediterranean. In contrast, grouping of strains in groups 3–7 seems to match their geographic distribution, with group 3 representing Japan, group 4 from New Zealand, and group 5 with strains from the North Atlantic up to the Greenland coast. For strains currently unified in group 6, a further substructure into a clade with strains from the North Sea (including adjacent fjords as Oslofjord and Limfjord) and another clade with strains from the South Pacific (Chile, Peru) is indicated, but only weakly statistically supported by a bootstrap value of 61. The separate placement of our first Argentinean *A. ostenfeldii*, representing the first data from the South Atlantic, perfectly fits into such a picture of geographically separated *A. ostenfeldii* populations.

PSP Toxin Profiles in Field Samples and Toxin Profiles of *Alexandrium* Isolates

It is noteworthy that the quantitative field profiles of the PSP toxin did not match the relative abundance of PSP toxins of the *Alexandrium* strains isolated during this expedition. The PSP profiles of all three *A. ostenfeldii* strains were dominated by GTX2/3, whereas the PSP profile of the *A. catenella* strain was dominated by C1/2. In contrast, the profiles of the toxin of stations 15, 17, and 21 were dominated by GTX1/4. The GTX1/4 were not detected at station 33, but only C1/2 and GTX2/3 were detected (**Figure 10**). However, it is reasonable to assume that GTX1/4 was present, but not detected as its limit of detection (LOD) is much higher than the LOD of C1/2 and GTX2/3. The GTX1/4 were only produced by the *A. catenella* strain, but at a lower abundance as C1/2. This toxin profile is consistent with profiles previously reported of *A. catenella* in the region (Montoya et al., 2010; Krock et al., 2015). Interestingly, the discrepancy between the profiles of the PSP toxin of isolated strains and that of the field samples of the plankton in the Argentine Sea observed here (**Figures 10**, **11**) has been seen previously (Montoya et al., 2010) and may indicate either a

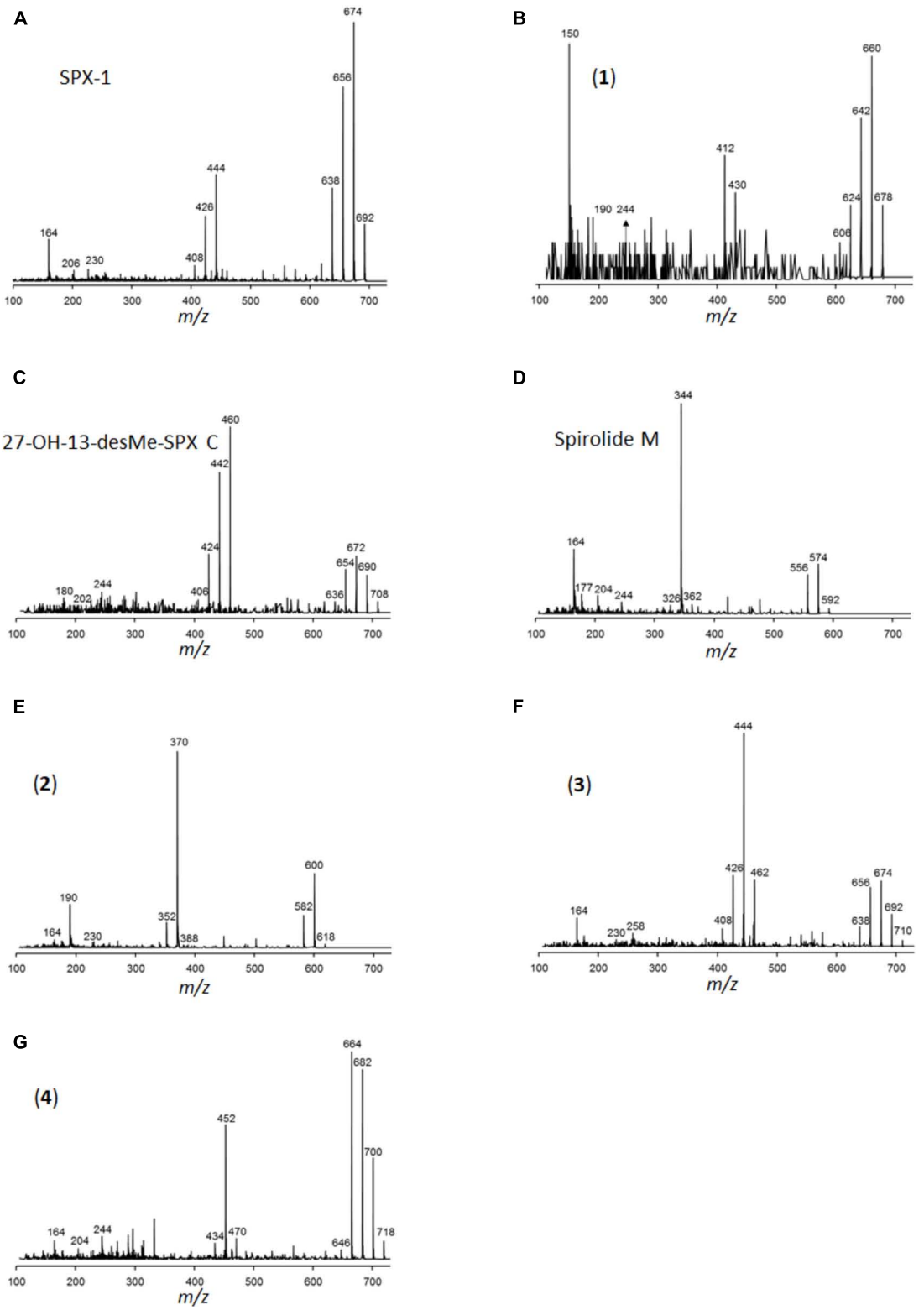


FIGURE 12 | Collision-induced dissociation (CID) spectra of the spirolides detected in *A. ostenfeldii*: **(A)** SPX-1 (13-desMethyl-spirolide C), identical with spectrum shown in Sleno and Volmer (2005), **(B)** 13/19,31-didesMethyl-spirolide C, **(C)** 27-Hydroxy-13-desMethyl-spirolide C, **(D)** Spirolide M, **(E)** compound 2, **(F)** compound 3, and **(G)** compound 4.

change in production of toxins in relation to growth conditions of the culture or may be related with an unexplored diversity of PSP-producing organisms in the SW Atlantic. There are few previous reports of the toxin profiles of *A. ostenfeldii* of the SW Atlantic. The presence of *A. ostenfeldii* at higher latitudes of the Patagonian shelf has been well known for a long time (Balech, 1995), and this has been recently reaffirmed (Fabro et al., 2017), but no strains were isolated for toxinological studies. The only available strain to compare our data with is an *A. ostenfeldii* strain isolated from the Beagle Channel (54° S) (Almandoz et al., 2014) that did not produce PSP toxins, but only spirolides. In addition to the lack of production of PSP of the strain from the Beagle Channel (BC), the profiles of the spirolide of the BC strain and the three strains of this expedition were quite different: the BC strain was reported to produce SPX-1, 20-methyl spirolide-G and an unknown spirolide with *m/z* 592, whereas the three strains investigated in this work produced SPX-1 [retention time (RT) 12.3 min], 27-hydroxy-13-desmethyl spirolide-C (RT 12,6 min), spirolide-M (RT 12.7 min), and four undescribed spirolides (**Figure 11**). The only common spirolides in strains from both areas are SPX-1 and spirolide-M with *m/z* 592 (see in the following). The CID spectrum of the unknown spirolide *m/z* 592 reported by Almandoz et al. (2014) and spirolide-M show identical fragments (**Figure 12D**; Almandoz et al., 2014). Moreover, the chromatographic behavior of spirolide-M and the unknown *m/z* 592 is consistent as both compounds elute after SPX-1, which in addition to the mass spectrometric data is a strong evidence that spirolide-M is also present in the BC strain.

Description of Novel Spirolides

The three *A. ostenfeldii* strains isolated from the Argentine shelf produced five novel spirolides, which highlights the large structural variability of this class of toxin. Compound (1) (*m/z* 678, RT 11.9 min) shares its molecular mass and most fragments of the CID spectrum with 13,19-didesmethyl spirolide C (13,19-didesMe SPX C). An obvious difference between both CID spectra is the cycloimine fragment, which is *m/z* 164 in the case of 13, 19-didesMe SPX C and *m/z* 150 in the case of compound (**1**). The fragment *m/z* 150 is typical for A-type spirolides that are lacking the methyl group at C31 (Hu et al., 2001). Given this evidence, it is reasonable to assume that the missing methyl group at C31 is located at a different position such as C13 or C19 that are often methylated in spirolides. However, the exact position of the shifted methyl group cannot be determined by mass spectrometry alone.

With the pseudo-molecular $[M + H]^+$ ion *m/z* 592, spirolide M (SPX M) is the smallest yet reported spirolide. The difference of the mass in comparison with the next bigger spirolide (spirolide H, *m/z* 650; Roach et al., 2009) is 58 Da. In comparison with the CID spectrum of spirolide H (SPX H), only two water losses from the pseudo-molecular ion are observed (*m/z* 574 and 556) instead of three in the CID spectrum of SPX H. The difference in the mass between the $[M + H]^+$ ion and the midmass fragment of SPX M is 248 Da (*m/z* 592 > 344), which is typical for spirolides of the C-type and SPX H. The loss of 248 Da is clear evidence that the C1-C11 part of SPX M must be identical to SPX H. Additionally, the spectrum of SPX M shows the identical small fragments of the cycloimine moiety (*m/z* 164, 177, and 204), which leads to the conclusion that the cycloimine ring with its five adjacent C atoms is also identical to SPX H. However, the CID spectrum of SPX M displays a fragment *m/z* 244 that is 14 Da higher than the fragment *m/z* 230 of SPX H. These mass spectral elements are consistent with a structure identical to SPX H with the difference of SPX M containing a dimethylated monospiroketal ring instead of the dispiroketal ring system of SPX H. However, most spirolides possess trispiroketal ring systems; only SPX H and I have dispiroketal ring systems. In contrast, SPX M is the first spirolide to be reported to have only a monospiroketal ring.

The difference in mass of compound **(2)** (*m/z* 618, RT 13, 6 min) to the closest known spirolide in terms of molecular mass SPX H is 32 Da. Similar to SPX M, compound (**2**) also eliminates 248 Da from its $[M + H]^+$ ion, which indicates a conserved structure in the C1-C11 part of the spirolide such as SPX C and H. However, the CID spectrum of compound (**2**) shows a fragment *m/z* 190, which has not been observed in other spirolides. Most spirolides form a *m/z* 164 fragment or a *m/z* 180 fragment in the case of a 27-hydroxylation. However, a fragment *m/z* 190 has not been observed in spirolides and thus it is difficult to interpret without additional information.

Compound **(3)** has the pseudo-molecular ion of *m/z* 710 (RT 12.50 min) and differs from the previous spirolides by a mass difference of 266 Da (instead of 248 Da) between the $[M + H]^+$ ion and the midmass fragment *m/z* 462. This cleavage has not been detected in other spirolides, but can be explained by a hydroxylation and a reduction of a double bond between positions C1-C11. These hypothetical changes applied to SPX-1 would in fact result in a pseudo-molecular ion *m/z* 710. Nevertheless, there still must be other structural differences between SPX-1 and compound (**3**), because compound (**3**) produces the fragments m/z 230 and 258, which are not observed in the CID spectrum of SPX-1. Fragment *m/z* 258 is also formed by 20-methyl spirolide G, which has a 6-6-5 trispiroketal ring system instead of the 6-5-5 trispiroketal ring system of most other spirolides. An exact structure of compound (**3**) cannot be assigned by mass spectral data alone.

Compound **(4)** shows a pseudo-molecular ion of *m/z* 718 (RT 13.0 min) differing from compounds (**1–3**) by a mass difference of 266 Da between the $[M + H]^+$ ion and the midmass fragment *m/z* 452. Accordingly, it can be deduced that compounds (**3**) and (**4**) share the same structural element between positions C1-C11. These changes applied to SPX-1 would result in a hypothetical pseudo-molecular ion of *m/z* 710, which is 8 Da less than the actual observed ion *m/z* 718. Since the cycloimine fragments *m/z* 164 and 204 are conserved in (**4**), identical structures of the cycloimine moiety between SPX-1 and compound (**4**) can be deduced. This means that an additional structural difference between SPX-1 and compound (**4**) must be located in the trispiroketal ring system. Full structural elucidation of the novel compounds can only be achieved by NMR spectroscopy that was not applicable in this case, because of the need for purified compounds in the microgram range, which was impossible to achieve within this study.

FINAL REMARKS

So far, this is the first study in the shelf area of El Rincón that contemplates in detail the composition and the structure of the plankton community >5 μm, including phototrophic and heterotrophic protists. Under the growing need for the global plankton register to assess community changes and their underlying drivers, our study provides baseline information of plankton state in a highly productive area of the Global Ocean. Moreover, our work highlights the necessity of combining complementary approaches in plankton studies to address the diversity and distribution of the potential toxic species. Morphological differentiation between the species of the former *Alexandrium tamarense/fundyense/catenella* complex is impossible and molecular sequencing is inevitable in this case to facilitate species identification. Further integrative studies of toxin production are needed to fully evaluate the discrepancy between phycotoxin profiles quantified in the field and in isolated cells grown in the laboratory. Our findings reinforce the presence of toxigenic species in the Argentine shelf, indicating a possible hazard for fisheries and human well-being. The first molecular characterization of *A. ostenfeldii* for the SW Atlantic and the detection of novel spirolides suggest that the Patagonian Shelf is still an extensive underexplored area of the Global Ocean.

AUTHOR CONTRIBUTIONS

VG, UT and BK designed the study. VG contributed to sampling, microscopic identifications and counting, data analyses and drafting the work. UT contributed to sampling, isolation, culturing, LM, and taxonomy of *Alexandrium* spp. BK and TK contributed to the LC-MS/MS analysis and interpretation of phycotoxins profiles. AD contributed to satellite image process and interpretation. JGC contributed to sampling and chlorophyll data. KM contributed to molecular analyses and interpretation. CLA and RS supported for microscopic identifications and editing. RL contributed to logistic support for sampling. All authors contributed with the interpretation of the results and have approved the final version.

FUNDING

This study was supported by the bilateral project MINCYT-DAAD (Ministerio de Ciencia, Tecnología e Innovación Productiva, Argentina and Deutscher Akademischer Austauschdienst, Germany), code DA/13/04; two research projects of Agencia Nacional de Promoción Científica y Tecnologica (ANPCyT), codes PICT-1681-2013 and PICT-1241-2013; the binational project MINCyT-BMBF (AL/11/03-ARG 11/021) and the Helmholtz-Gemeinschaft Deutscher Forschungszentren through the research program PACES of the Alfred-Wegener-Institut, Helmholtz-Zentrum für Polar- und Meeresforschung. Financial support for open-access publication was given by BMBF (Post Grant Fund # 16PGF0042).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2018.00394/full#supplementary-material

REFERENCES

Acha, E. M., Ehrlich, M. D., Muelbert, J. H., Pájaro, M., Bruno, D., Machinandiarena, L., et al. (2018). "Ichthyoplankton associated to the frontal regions of the Southwestern Atlantic," in *Plankton Ecology of the Southwestern Atlantic, From Subtropical to the Subantarctic Realm*, eds M. Hoffmeyer, M. E. Sabatini, F. Brandini, D. Calliari, and N. H. Santinelli (Berlin: Springer), 219–246.

Acha, E. M., Mianzan, H. W., Guerrero, R. A., Favero, M., and Bava, J. (2004). Marine fronts at the continental shelves of austral South America. *J. Mar. Syst.* 44, 83–105. doi: 10.1016/j.jmarsys.2003.09.005

Aguilera-Belmonte, A., Inostroza, I., Franco, J. M., Riobó, P., and Gómez, P. I. (2011). The growth, toxicity and genetic characterization of seven strains of *Alexandrium catenella* (Whedon and Kofoid) Balech 1985 (Dinophyceae) isolated during the 2009 summer outbreak in southern Chile. *Harmful Algae* 12, 105–112. doi: 10.1016/j.hal.2011.09.006

Akselman, R., and Negri, R. M. (2012). Blooms of Azadinium cf. *spinosum* Elbrächter et Tillmann (Dinophyceae) in northern shelf waters of Argentina. Southwestern Atlantic. *Harmful Algae* 19, 30–38. doi: 10.1016/j.hal.2012.05.004

Almandoz, G. O., Fabro, E., Ferrario, M., Tillmann, U., Cembella, A., and Krock, B. (2017). Species occurrence of the potentially toxigenic diatom genus *Pseudo-nitzschia* and the associated neurotoxin domoic acid in the Argentine Sea. *Harmful Algae* 63, 45–55. doi: 10.1016/j.hal.2017.01.007

Almandoz, G. O., Ferrario, M. E., Ferreyra, G. A., Schloss, I. R., Esteves, J. L., and Paparazzo, F. E. (2007). The genus *Pseudo-nitzschia* (Bacillariophyceae) in continental shelf waters of Argentina (Southwestern Atlantic Ocean, 38-55 S). *Harmful Algae* 6, 93–103. doi: 10.1016/j.hal.2006.07.003

Almandoz, G. O., Montoya, N. G., Hernando, M. P., Benavides, H. R., Carignan, M. O., and Ferrario, M. E. (2014). Toxic strains of the *Alexandrium ostenfeldii* complex in southern South America (Beagle Channel, Argentina). *Harmful Algae* 37, 100–109. doi: 10.1016/j.hal.2014.05.011

Anderson, D. M., Alpermann, T. J., Cembella, A. D., Collos, Y., Masseret, E., and Montresor, M. (2012). The globally distributed genus *Alexandrium*: multifaceted roles in marine ecosystems and impacts on human health. *Harmful Algae* 14, 10–35. doi: 10.1016/j.hal.2011.10.012

Antoine, D. (2004). *Guide to the Creation and Use of Ocean-Colour, Level-3, Binned Data Products*. Reports of the International Ocean-Colour Coordinating Group No.4. Dartmouth, NS: IOCCG.

Balech, E. (1995). *The Genus Alexandrium Halim (Dinoflagellata)*. Cork: Sherkin Island, Co., 151.

Benavides, H., Prado, L., Diaz, S., and Carreto, J. I. (1995). "An exceptional bloom of *Alexandrium catenella* in the Beagle channel, Argentina," in *Harmful Marine Algal Blooms*, eds P. Lassus, G. Arzul, E. Erard-le Denn, P. Gentien, and C. Marcaillou-Le Baut (Paris: Lavoisier Intercept Ltd.), 113–119.

Bianchi, A. A., Ruiz Pino, D., Perlender, H. G. I., Osiroff, A. P., Segura, V., Lutz, V., et al. (2009). Annual balance and seasonal variability of sea-air CO2 fluxes in the Patagonia Sea: their relationship with fronts and chlorophyll distribution. *J. Geophys. Res.* 114:C03018. doi: 10.1029/2008JC004854

Bogazzi, E., Baldoni, A. N., Rivas, A., Martos, P., Reta, R. A., Orensanz, J. M., et al. (2005). Spatial correspondence between areas of concentration of Patagonian scallop (*Zygochlamys patagonica*) and frontal systems in the southwestern Atlantic. *Fish. Oceanogr.* 14, 359–376. doi: 10.1111/j.1365-2419.2005.00340.x

Brown, O. B., and Minnet, P. J. (1999). *MODIS Infrared Sea Surface Temperature Algorithm*. Technical Report No ATBD25. Coral Gables, FL: University of Miami.

Campbell, J. W., Blaisdell, J. M., and Darzi, M. (1995). "Level-3 SeaWiFS data products: spatial and temporal binning algorithms," in *NASA Technical Memorandum 1995-104566*, Vol. 32, eds S. B. Hooker, E. R. Firestone, and J. G. Acker (Greebelt, MD: SeaWiFS Technical Report Series; NASA-GSFC), 1–73.

Carranza, M. M., Gille, S. T., Piola, A. R., Charo, M., and Romero, S. I. (2017). Wind modulation of upwelling at the shelf-break front off Patagonia: observational evidence. *J. Geophys. Res. Oceans* 122, 2401–2421. doi: 10.1002/2016JC012059

Carreto, J. I., Akselman, R., Montoya, N. G., Negri, R. N., Benavides, H., Carignan, M. O., et al. (1998). "*Alexandrium tamarense* blooms and shellfish toxicity in the Argentine Sea: a retrospective view," in *Proceedings of the VIII International Conference on Harmful Algae*, eds B. Reguera, J. Blanco, M. L. Fernández, and T. Wyatt (Vigo), 131–134.

Carreto, J. I., Carignan, M. O., and Montoya, N. G. (2001). Comparative studies on mycosporine-like amino acids, paralytic shellfish toxins and pigment profiles of the toxic dinoflagellates *Alexandrium tamarense*, *A. catenella* and *A. minutum*. *Mar. Ecol. Progr. Ser.* 223, 49–60. doi: 10.3354/meps223049

Carreto, J. I., Lasta, M., Negri, R. M., and Benavides, H. R. (1981). *Los Fenómenos de Marea Roja y Toxicidad de Moluscos Bivalvos en el Mar Argentino*. Mar del Plata: INIDEP, 399.

Carreto, J. I., Montoya, N. G., Carignan, M. O., Akselman, R., Acha, E. M., and Derisio, C. (2016). Environmental and biological factors controlling the spring phytoplankton bloom at the Patagonian shelf-break front-Degraded fucoxanthin pigments and the importance of microzooplankton grazing. *Prog. Oceanogr.* 146, 1–21. doi: 10.1016/j.pocean.2016.05.002

Clarke, K. R., and Gorley, R. N. (2006). *PRIMER v6: User Manual/Tutorial*. Plymouth: PRIMER E.

Delgado, A. L., Loisel, H., Jamet, C., Vantrepotte, V., Perillo, G. M., and Piccolo, M. (2015). Seasonal and inter-annual analysis of chlorophyll-a and inherent optical properties from satellite observations in the inner and mid-shelves of the south of buenos aires province (Argentina). *Remote Sens.* 7, 11821–11847. doi: 10.3390/rs70911821

Delgado, A. L., Guinder, V. A., Dogliotti, A., Zapperi, G., and Pratolongo, P. (2018) Consistency of MODIS-aqua(absorption)by phytoplankton coefficient in Southern Atlantic Ocean coastal optically complex waters: analysis and validation. Accepted in continental shelf Research.

Díaz, M. V., Marrari, M., Casa, V., Gattás, F., Pájaro, M., and Macchi, G. J. (2018). Evaluating environmental forcing on nutritional condition of *Engraulis anchoita* larvae in a productive area of the Southwestern Atlantic Ocean. *Prog. Oceanogr.* 168, 13–22. doi: 10.1016/j.pocean.2018.09.007

Dogliotti, A. I., Schloss, I. R., Almandoz, G. O., and Gagliardini, D. A. (2009). Evaluation of SeaWiFS and MODIS chlorophyll-a products in the argentinean patagonian continental shelf (38°-55°S). *Int. J. Remote Sens.* 30, 251–273. doi: 10.1080/01431160802311133

Fabro, E., Almandoz, G. O., Ferrario, M., John, U., Tillmann, U., Toebe, K., et al. (2017). Morphological, molecular, and toxin analysis of field populations of *Alexandrium* genus from the Argentine Sea. *J. Phycol.* 53, 1206–1222. doi: 10.1111/jpy.12574

Fabro, E., Almandoz, G. O., Ferrario, M., Tillmann, U., Cembella, A., and Krock, B. (2016). Distribution of *Dinophysis* species and their association with lipophilic phycotoxins in plankton from the Argentine Sea. *Harmful Algae* 59, 31–41. doi: 10.1016/j.hal.2016.09.001

Fabro, E., Almandoz, G. O., Ferrario, M. E., Hoffmeyer, M. S., Pettigrosso, R. E., Uibrig, R., et al. (2015). Co-occurrence of *Dinophysis tripos* and pectenotoxins in Argentinean shelf waters. *Harmful Algae* 42, 25–33. doi: 10.1016/j.hal.2014.12.005

Ferreira, A., Stramski, D., García, C. A., García, V. M., Ciotti, A. M., and Mendes, C. R. (2013). Variability in light absorption and scattering of phytoplankton in Patagonian waters: role of community size structure and pigment composition. *J. Geophys. Res. Oceans* 118, 698–714. doi: 10.1002/jgrc.20082

Franco, B. C., Palma, E. D., Combes, V., and Lasta, M. L. (2017). Physical processes controlling passive larval transport at the patagonian shelf break front. *J. Sea Res.* 124, 17–25. doi: 10.1016/j.seares.2017.04.012

Fritz, L., and Triemer, R. E. (1985). A rapid simple technique utilizing calcofluor white M2R for the visualization of dinoflagellate thecal plates. *J. Phycol.* 21, 662–664. doi: 10.1111/j.0022-3646.1985.00662.x

Fu, G., Baith, K. S., and McClain, C. R. (1998). "SeaDAS: the SeaWiFS data analysis system," in *Proceedings of the 4th Pacific Ocean Remote Sensing Conference*, (Qingdao), 73–79.

García, V. M. T., García, C. A. E., Mata, M. M., Pollery, R. C., Piola, A. R., Signorini, S. R., et al. (2008). Environmental factors controlling the phytoplankton blooms at the Patagonia shelf-break in spring. *Deep Sea Res. Part I* 55, 1150–1166. doi: 10.1016/j.dsr.2008.04.011

Gayoso, A. M., and Fulco, K. (2006). Occurrence patterns of *Alexandrium tamarense* (Lebour) Balech populations in the Golfo Nuevo (Patagonia, Argentina), with observations on ventral pore occurrence in natural and cultured cells. *Harmful Algae* 5, 233–241. doi: 10.1016/j.hal.2004.12.010

Gómez, M. I., Piola, A. R., Kattner, G., and Alder, V. A. (2011). Biomass of autotrophic dinoflagellates under weak vertical stratification and contrasting chlorophyll levels in subantarctic shelf waters. *J. Plankton Res.* 33, 1304–1310. doi: 10.1093/plankt/fbr031

Goyens, C., Jamet, C., and Schroeder, T. (2013). Evaluation of four atmospheric correction algorithms for MODIS-aqua images over contrasted coastal waters. *Remote Sens. Environ.* 131, 63–75. doi: 10.1016/j.rse.2012.12.006

Guinder, V. A., Popovich, C. A., Molinero, J. C., and Perillo, G. M. E. (2010). Long-term changes in the composition, occurrence, timing and magnitude of phytoplankton blooms in the Bahía Blanca estuary. Argentina. *Mar. Biol.* 157, 2703–2716. doi: 10.1007/s00227-010-1530-5

Guinder, V. A., Popovich, C. A., and Perillo, G. M. E. (2009). Particulate suspended matter concentrations in the Bahía Blanca Estuary, Argentina: implication for the development of phytoplankton blooms. *Estuar. Coast. Shelf Sci.* 85, 157–165. doi: 10.1016/j.ecss.2009.05.022

Harðardóttir, S., Pančić, M., Tammilehto, A., Krock, B., Møller, E. F., Nielsen, T. G., et al. (2015). Dangerous relations in the Arctic marine food web: interactions between toxin producing *Pseudo-nitzschia* diatoms and calanus copepodites. *Mar. Drugs* 13, 3809–3835. doi: 10.3390/md13063809

Hasle, G. (1978). "Concentrating phytoplankton. Settling. The inverted microscope method," in *Phytoplankton Manual. Monographs on Oceanographic Methodology*, ed. A. Sournia (Paris: UNESCO), 88–96.

Heileman, S. (2009). "XVI-55 patagonian shelf LME in the UNEP large marine ecosystem report: a perspective on changing conditions in LMES of the world's regional seas," in *UNEP Regional Seas Report and Studies N° 182. United Nations Environment Programme*, eds K. Sherman and G. Hempel (Nairobi: UNDP), 735–746.

Hillebrand, H., Dürselen, C. D., Kirschtel, D., Pollingher, U., and Zohary, T. (1999). Biovolume calculation for pelagic and benthic microalgae. *J. Phycol.* 35, 403–424. doi: 10.1046/j.1529-8817.1999.3520403.x

Hoffmeyer, M. S., Menéndez, M. C., Biancalana, F., Nizovoy, A. M., and Torres, E. R. (2009). Ichthyoplankton spatial pattern in the inner shelf off Bahía Blanca Estuary, SW Atlantic Ocean. *Estuar. Coast. Shelf Sci.* 84, 383–392. doi: 10.1016/j.ecss.2009.07.017

Holm-Hansen, O., Lorenzen, C. J., Holmes, R. W., and Strickland, J. D. H. (1965). Fluorometric determination of chlorophyll. *J. Mar. Sci.* 30, 3–15. doi: 10.1093/icesjms/30.1.3

Hoppenrath, M., and Debres, G. (2009). *Marine Phytoplankton. Selected Microplankton Species From the North Sea Around Helgoland and Sylt*. Stuttgart: Schweitzerbarth Verlag, 264.

Hu, C., Lee, Z., and Franz, B. (2012). Chlorophyll a algorithms for oligotrophic oceans: a novel approach based on three-band reflectance difference. *J. Geophys. Res.* 117:C01011. doi: 10.1029/2011JC007395

Hu, T., Burton, I. W., Cembella, A. D., Curtis, J. M., Quilliam, M. A., Walter, J. A., et al. (2001). Characterization of Spirolides A, C, and 13-Desmethyl C, new marine toxis isolated from toxic plankton and contaminated shellfish. *J. Nat. Prod.* 64, 308–312. doi: 10.1021/np000416q

Jamet, C., Loisel, H., Kuchinke, C. P., Ruddick, K., Zibordi, G., and Feng, H. (2011). Comparison of three SeaWiFS atmospheric correction algorithms for turbid waters using AERONET-OC measurements. *Remote Sens. Environ.* 115, 1955–1965. doi: 10.1016/j.rse.2011.03.018

John, U., Litaker, R. W., Montresor, M., Murray, S., Brosnahan, M. L., and Anderson, D. M. (2014). Formal revision of the *Alexandrium tamarense* species complex (Dinophyceae) taxonmomy: the introduction of five species with emphasis on molecular-based (rDNA) classification. *Protist* 165, 779–804. doi: 10.1016/j.protis.2014.10.001

Keller, M. D., Selvin, R. C., Claus, W., and Guillard, R. R. L. (1987). Media for the culture of oceanic ultraphytoplankton. *J. Phycol.* 23, 633–638. doi: 10.1111/j.1529-8817.1987.tb04217.x

Kopprio, G. A., Streitenberger, M. E., Okuno, K., Baldini, M., Biancalana, F., Fricke, A., et al. (2017). Biogeochemical and hydrological drivers of the dynamics of *Vibrio* species in two Patagonian estuaries. *Sci. Total Environ.* 579, 646–656. doi: 10.1016/j.scitotenv.2016.11.045

Kremp, A., Tahvanainen, P., Litaker, W., Krock, B., Suikkanen, S., Leaw, C. P., et al. (2014). Phylogenetic relationships, morphological variation, and toxin pattern in the *Alexandrium ostenfeldii* (Dinopyhceae) complex: implications for species boundaries and identities. *J. Phycol.* 50, 81–100. doi: 10.1111/jpy.12134

Krock, B., Borel, C. M., Barrera, F., Tillmann, U., Fabro, E., Almandoz, G. O., et al. (2015). Analysis of the hydrographic conditions and cyst beds in the San Jorge Gulf, Argentina, that favor dinoflagellate population development including toxigenic species and their toxins. *J. Mar. Syst.* 148, 86–100. doi: 10.1016/j.jmarsys.2015.01.006

Krock, B., Tillmann, U., John, U., and Cembella, A. D. (2008). LC-MS-MS aboard ship: tandem mass spectrometry in the search for phycotoxins and novel toxigenic plankton from the North Sea. *Anal. Bioanal. Chem.* 392, 797–803. doi: 10.1007/s00216-008-2221-7

Kumar, S., Stecher, G., and Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33, 1870–1874. doi: 10.1093/molbev/msw054

Le Quéré, C., Harrison, S. P., Prentice, I. C., Buitenhuis, E. T., Aumont, O., Bopp, L., et al. (2005). Ecosystem dynamics based on plankton functional types for global ocean biogeochemistry models. *Glob. Change Biol.* 11, 2016–2040.

Lilly, E. L., Halanich, K. M., and Anderson, D. M. (2007). Species boundaries and global biogeography of the *Alexandrium tamarense* complex (Dinophyceae). *J. Phycol.* 43, 1329–1338. doi: 10.1111/j.1529-8817.2007.00420.x

Litchman, E., and Klausmeier, C. A. (2008). Trait-based community ecology of phytoplankton. *Annu. Rev. Ecol. Evol. S.* 39, 615–639. doi: 10.1146/annurev.ecolsys.39.110707.173549

Liu, K.-K., Atkinson, L., Quinones, R. A., and Talaue-McManus, L. (2010). "Biogeochemistry of continental margins in a global context," in *Carbon and Nutrient Fluxes in Continental Margins. A Global Synthesis*, eds K.-K. Liu, L. Atkinson, R. A. Quinones, and L. Talaue-McManus (Berlin: Springer), 3–24. doi: 10.1007/978-3-540-92735-8_1

López Abbate, M. C., Molinero, J. C., Guinder, V. A., Dutto, M. S., Barría de Cao, M. S., Ruiz Etcheverry, L. A., et al. (2015). Microplankton dynamics under heavy anthropogenic pressure. The case of the Bahía Blanca Estuary, southwestern Atlantic Ocean. *Mar. Pollut. Bull.* 95, 305–314. doi: 10.1016/j.marpolbul.2015.03.026

López Abbate, M. C., Molinero, J. C., Guinder, V. A., Perillo, G. M., Freije, R. H., Sommer, U., et al. (2017). Time-varying environmental control of phytoplankton in a changing estuarine system. *Sci. Total Environ.* 609, 1390–1400. doi: 10.1016/j.scitotenv.2017.08.002

López Cazorla, A., Molina, J. M., and Ruarte, C. (2014). The artisanal fishery of *Cynoscion guatucupa* in Argentina: exploring the possible causes of the collapse in Bahía Blanca estuary. *J. Sea Res.* 88, 29–35. doi: 10.1016/j.seares.2013.12.016

Lucas, A. J., Guerrero, R. A., Mianzán, H. W., Acha, M. E., and Lasta, C. A. (2005). Coastal oceanographic regimes of the Northern Argentine continental Shelf (34-43°S). *Estuar. Coast. Shelf Sci.* 65, 405–420. doi: 10.1016/j.ecss.2005.06.015

Lutz, V. A., Segura, V., Dogliotti, A. I., Gagliardini, D. A., Bianchi, A., and Balestrini, C. E. (2010). Primary production in the Argentine Sea during spring estimated by field and satellite models. *J. Plankton Res.* 32, 181–195. doi: 10.1093/plankt/fbp117

Marrari, M., Delia Viñas, M., Martos, P., and Hernández, D. (2004). Spatial patterns of mesozooplankton distribution in the Southwestern Atlantic Ocean (34–41°S) during austral spring: relationship with the hydrographic conditions. *ICES J. Mar. Sci.* 61, 667–679. doi: 10.1016/j.icesjms.2004.03.025

Marrari, M., Piola, A. R., and Valla, D. (2017). Variability and 20-year trends in satellite-derived surface chlorophyll concentrations in large marine ecosystems around South and Western Central America. *Front. Mar. Sci.* 4:372. doi: 10.3389/fmars.2017.00372

Marrari, M., Signorini, S., McClain, C. R., Pájaro, M., Martos, P., Viñas, M. D., et al. (2013). Reproductive success of the Argentine anchovy, *Engraulis anchoita*, in relation to environmental variability at a mid-shelf front (Southwestern Atlantic Ocean). *Fish. Oceanogr.* 22, 247–261. doi: 10.1111/fog.12019

Matano, R. P., Palma, E. D., and Piola, A. R. (2010). The influence of the Brazil and Malvinas currents on the southwestern Atlantic shelf. *Ocean Sci.* 6, 983–995. doi: 10.5194/os-6-983-2010

Menden-Deuer, S., and Lessard, E. J. (2000). Carbon to volume relationships for dinoflagellates, diatoms, and of the protist plankton. *Limnol. Oceanogr.* 45, 569–579. doi: 10.4319/lo.2000.45.3.0569

Montoya, N. G., Akselman, R., Franco, J., and Carreto, J. I. (1996). "Paralytic shellfish toxins and mackerel (Scomber Japonicus) mortality in the Argentine Sea," in *Harmful and Toxic Algal Blooms Intergovernmental Oceanographic Commission (IOC) of UNESCO*, eds T. Yasumoto, Y. Oshima, and Y. Fukuyo (Paris: UNESCO), 417–420.

Montoya, N. G., Fulco, V. K., Carignan, M. O., and Carreto, J. I. (2010). Toxin variability in cultured and natural populations of *Alexandrium tamarense* from southern South America - evidences of diversity and environmental regulation. *Toxicon* 56, 1408–1418. doi: 10.1016/j.toxicon.2010.08.006

Negri, R. M., Montoya, N., Carreto, J. I., Akselman, R., and Inza, D. (2004). "*Pseudo-nitzschia* australis, *Mytilus edulis*, *Engraulis anchoita* and domoic acid in the Argentine Sea," in *Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO*, (St. Petersburg), 139–141.

Negri, R. M., Silva, R. I., Segura, V., and Cucchi Colleoni, D. (2013). Estructura de la comunidad del fitoplancton en el área de "El Rincón", Mar Argentino (Febrero 2011). *Rev. Invest. Desarr. Pesq.* 23, 7–22.

Olguín, H. F., Brandini, F., and Boltovskoy, D. (2015). Latitudinal patterns and interannual variations of spring phytoplankton in relation to hydrographic conditions of the southwestern Atlantic Ocean (34-62 S). *Helgoland Mar. Res.* 69:177. doi: 10.1007/s10152-015-0427-6

Orr, R. J. S., Stuken, A., Rundberget, T., Eikrem, W., and Jakobsen, K. S. (2011). Improved phylogenetic resolution of toxic and non-toxic *Alexandrium* strains using a concatenated rDNA approach. *Hamful Algae* 10, 676–688. doi: 10.1016/j.hal.2011.05.003

Palma, E. D., and Matano, R. P. (2012). A numerical study of the Magellan plume. *J. Geophys. Res.* 117, 1–16. doi: 10.1029/2011JC007750

Palma, E. D., Matano, R. P., and Piola, A. R. (2008). Numerical study of the Southwestern Atlantic Shelf circulation: stratified ocean response to local and offshore forcing. *J. Geophys. Res.* 113, 1–24. doi: 10.1029/2007JC004720

Paniagua, G. F., Saraceno, M., Piola, A. R., Guerrero, R., Provost, C., Ferrari, R., et al. (2018). Malvinas current at 408S–418S: first assessment of temperature and salinity temporal variability. *J. Geophys. Res. Oceans* 123, 5323–5340. doi: 10.1029/2017JC013666

Park, M. G., Kim, S., Kim, H. S., Myung, G., Kang, Y. G., and Yih, W. (2006). First successful culture of the marine dinoflagellate *Dinophysis acuminata*. *Aquat. Microb. Ecol.* 45, 101–106. doi: 10.3354/ame045101

Penna, A., Fraga, S., Masó, M., Giacobbe, M. G., Bravo, I., Garcés, E., et al. (2008). Phylogenetic relationships among the Mediterranean *Alexandrium* (Dinophyceae) species based on sequences of 5.8 S gene and Internal Transcript Spacers of the rRNA operon. *Eur. J. Phycol.* 43, 163–178. doi: 10.1080/09670260701783730

Reguera, B., Riobó, P., Rodríguez, F., Díaz, P. A., Pizarro, G., Paz, B., et al. (2014). Dinophysis toxins: causative organisms, distribution and fate in shellfish. *Mar. Drugs* 12, 394–461. doi: 10.3390/md12010394

Roach, J. S., LeBlanc, P., Lewis, N. I., Munday, R., Quilliam, M. A., and MacKinnon, S. L. (2009). Characterization of a dispiroketal spirolide subclass from *Alexandrium ostenfeldii*. *J. Nat. Prod.* 72, 1237–1240. doi: 10.1021/np800795q

Romero, S. I., Piola, A. R., Charo, M., and Garcia, C. A. E. (2006). Chlorophyll-a variability off Patagonia based on SeaWiFS data. *J. Geophys. Res. Oceans* 111, C05021. doi: 10.1029/2005JC003244

Salgado, P., Riobó, P., Rodríguez, F., Franco, J. M., and Bravo, I. (2015). Differences in the toxin profiles of *Alexandrium ostenfeldii* (Dinophyceae) strains isolated from different geographic origins: evidence of paralytic toxin, spirolide, and gymnodimine. *Toxicon* 103, 85–98. doi: 10.1016/j.toxicon.2015.06.015

Sar, E. A., Sunsen, I., Goya, A. B., Lavigne, A. S., Tapias, E., García, C., et al. (2012). First report of diarrheic shellfish toxins in mollusks from buenos aires province (Argentina) associated with *Dinophysis* spp.: evidence of okadaic acid, dinophysis toxin-1 and their acyl derivatives. *Bol. Soc. Argent. Bot.* 47, 5–14.

Segura, V., Lutz, V. A., Dogliotti, A., Silva, R. I., Negri, R. M., Akselman, R., et al. (2013). Phytoplankton types and primary production in the Argentine Sea. *Mar. Ecol. Prog. Ser.* 491, 15–31. doi: 10.3354/meps10461

Silva, R. I., Negri, R., and Lutz, V. (2009). Summer succession of ultraphytoplankton at the EPEA coastal station (northern Argentina). *J. Plankton Res.* 31, 447–458. doi: 10.1093/plankt/fbn128

Sleno, L., and Volmer, D. A. (2005). Toxin screening in phytoplankton: detection and quantitation using MALDI triple quadruploe mass spectrometry. *Anal. Chem.* 77, 1509–1517. doi: 10.1021/ac0486600

Tillmann, U. (2018a). "Amphidomataceae," in *Harmful Algae Blooms, a Compendium Desk Reference*, eds S. E. Shumway, J. A. Burkholder, and S. L. Morton (Hoboken, NJ: Wiley-Blackwell). doi: 10.1002/9781118994672.ch16b

Tillmann, U. (2018b). Electron microscopy of a 1991 spring plankton sample from the Argentinean Shelf reveals the presence of four new species of the Amphidomataceae (Dinophyceae). *J. Phycol. Res.* 66, 269–290. doi: 10.1111/pre.12225

Tillmann, U., and Akselman, R. (2016). Revisiting the 1991 algal bloom in shelf waters off Argentina: *Azadinium luciferelloides* sp nov. (Amphidomataceae, Dinophyceae) as the causative species in a diverse community of other amphidomataceans. *Phycol. Res.* 64, 160–175. doi: 10.1111/pre.12133

Tillmann, U., Borel, C. M., Barrera, F., Lara, R., Krock, B., Almandoz, G., et al. (2016). *Azadinium poporum* from the Argentine Continental Shelf, Southwestern Atlantic, produces azaspiracid-2 and azaspiracid-2 phosphate. *Harmful Algae* 51, 40–55. doi: 10.1016/j.hal.2015.11.001

Tillmann, U., Gottschling, M., Guinder, V., and Krock, B. (2018). *Amphidoma parvula* (Amphidomataceae), a new planktonic dinophyte from the Argentine Sea. *Eur. J. Phycol.* 53, 14–28. doi: 10.1080/09670262.2017.1346205

Tomas, C. R. (ed.) (1997). *Identifying Marine Phytoplankton*. Amsterdam: Elsevier, 858.

Trainer, V. L., Bates, S. S., Lundholm, N., Thessen, A. E., Cochlan, W. P., Adams, N. G., et al. (2012). *Pseudo-nitzschia* physiological ecology, phylogeny, toxicity, monitoring and impacts on ecosystem health. *Harmful Algae* 14, 271–300. doi: 10.1016/j.hal.2011.10.025

Van de Waal, D. B., Tillmann, U., Martens, H., Krock, B., van Scheppingen, Y., and John, U. (2015). Characterization of multiple isolates from an *Alexandrium ostenfeldii* bloom in the Netherlands. *Harmful Algae* 49, 94–104. doi: 10.1016/j.hal.2015.08.002

Winder, M., Carstensen, J., Galloway, A. W. E., Jakobsen, H. H., and Cloern, J. E. (2017). The land-sea interface: a source of high-quality phytoplankton to support secondary production. *Limnol. Oceanogr.* 62, S258–S271. doi: 10.1002/lno.10650

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Guinder, Tillmann, Krock, Delgado, Krohn, Garzón Cardona, Metfies, López Abbate, Silva and Lara. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Marine Science

REVIEW
published: 15 November 2018
doi: 10.3389/fmars.2018.00421

The Genus *Alexandrium* (Dinophyceae, Dinophyta) in Brazilian Coastal Waters

Mariângela Menezes[1]*, *Suema Branco*[1], *Maria Cecília Miotto*[2] and *Catharina Alves-de-Souza*[3]

[1] Laboratório de Ficologia, Departamento de Botânica, Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [2] Laboratório de Biologia Molecular, Universidade do Vale do Itajaí, Itajaí, Brazil, [3] Algal Resources Collection, MARBIONC, University of North Carolina Wilmington, Wilmington, DE, United States

A review of the dinoflagellate genus *Alexandrium* occurring in Brazilian coastal waters is presented based on both published information and new data. Seven *Alexandrium* species have been recorded from Brazil so far: *Alexandrium catenella*, *Alexandrium fraterculus*, *Alexandrium gaardnerae*, *Alexandrium kutnerae*, *Alexandrium tamiyavanichi*, *Alexandrium tamutum*, and *Alexandrium* sp. While *A. gaardnerae* and *A. kutnerae* were identified based only on morphological characteristics, phylogenetic analysis (ITS and LSU rDNA) were performed for the remaining species based on cultures and/or field populations. Monoclonal cultures of the analyzed species were isolated from field samples obtained from Bahia (*A. tamiyavanichi*, two strains), Rio de Janeiro (*A. tamutum*, three strains; *Alexandrium* sp., *two* strains), Santa Catarina (*A. fraterculus*, one strain), and Rio Grande do Sul (*Alexandrium tamarense*, three strains). This is the first record of *A. tamutum* for the South Atlantic. In addition, molecular data for Brazilian strains of *A. fraterculus* are presented for the first time, as well as sequences from the ITS region for *A. catenella* (previously reported as *A. tamarense*) from Brazilian coastal waters. The morphological characters of the three species corresponded to those typically recorded in the literature and their identification was confirmed by molecular analysis. Based on the LSU rDNA and ITS regions, the three strains of *A. catenella* showed a high degree of similarity with strains from Southern Chile and North America. The implications and limitations of these findings for the monitoring protocols within the global and regional context are discussed.

Keywords: *Alexandrium catenella* (group I), *Alexandrium tamutum*, phenotypic plasticity, taxonomy, phylogeny, paralytic shellfish poisoning

OPEN ACCESS

Edited by:
Jorge I. Mardones, Instituto de Fomento Pesquero (IFOP), Chile

Reviewed by:
Satoshi Nagai, National Research Institute of Fisheries Science, Japan Fisheries Research and Education Agency, Japan
F. Leonardo Guzmán, Instituto de Fomento Pesquero (IFOP), Chile

***Correspondence:**
Mariângela Menezes
menezes.mariangela@gmail.com

Specialty section:
This article was submitted to Marine Ecosystem Ecology, a section of the journal Frontiers in Marine Science

Received: *01 August 2018*
Accepted: *23 October 2018*
Published: *15 November 2018*

Citation:
Menezes M, Branco S, Miotto MC and Alves-de-Souza C (2018) The Genus Alexandrium (Dinophyceae, Dinophyta) in Brazilian Coastal Waters. Front. Mar. Sci. 5:421. doi: 10.3389/fmars.2018.00421

INTRODUCTION

The dinoflagellate genus *Alexandrium* Halim currently encompasses more than 30 species (Anderson et al., 2012), some of them known worldwide as the causative agents of blooms and/or production of neurotoxins associated to the Paralytic Shellfish Poisoning (PSP) syndrome (Wang, 2008; Etheridge, 2010). Recognized as the most geographically widespread algal-related shellfish poisoning syndrome, PSP constitutes a serious human illness caused by the ingestion of seafood contaminated with saxitoxin (STXs) and its derivatives (Deeds et al., 2008; Anderson et al., 2012). PSP symptoms include spreading numbness and tingling sensations, headache and nausea, and in more severe cases may cause paralysis of the muscles of the chest and abdomen leading to death

(Etheridge, 2010). Besides the evident relevance to human health, accumulation of PSP toxins in filter-feeding bivalves constitutes an additional issue for the shellfish industry and fisheries activities (Etheridge, 2010; Rhodes and Munday, 2016).

Many factors have been suggested as the cause of the observed increase in the worldwide extension and frequency of *Alexandrium* blooms and other Harmful Algal Bloom (HAB) species, such as ocean currents, climate change, pollution, ballast water dispersal, and colonization of newly generated niches (Nagai et al., 2007; Matsuyama et al., 2010; Anderson et al., 2012, 2015; Anderson, 2017; Gobler et al., 2017). It has also been suggested that the higher number of blooms reported nowadays is a direct result of increased monitoring activities and the implementation of more sensitive tools to detect and prevent the negative effects of HABs worldwide, particularly in aquaculture areas (Anderson, 2007; Anderson et al., 2015). Both toxic and non-toxic blooms of *Alexandrium* species have been reported from distinct geographic regions, e.g., Japan (Kodama, 2010), Mediterranean Sea (Karydis and Kitsiou, 2012), China (Lu et al., 2014), Argentina Sea (Fabro et al., 2017), Australia (Ajani et al., 2017), and the Bering Sea (Natsuike et al., 2017).

In Brazil, records of HABs related to human intoxications in coastal areas increased in the last 20 years, becoming a national issue with socio-economic repercussions (Silva et al., 2013). Particularly, reports of *Alexandrium* species in the Brazilian coast have been intensifying (**Figure 1**). To date, five species were previously identified based on light microscopy: *Alexandrium garderae* Nguyen-Ngoc & Larsen [= *Alexandrium concavum* (Gaarder) Balech], *Alexandrium fraterculus* (Balech) Balech, *Alexandrium kutnerae* (Balech) Balech, *Alexandrium catenella* (Whedon & Kofoid) Balech [= *Alexandrium tamarense* (Lebour) Balech], and *Alexandrium tamiyavanichi* Balech (Balech, 1995; Persich et al., 2006; Menezes et al., 2010). STX production has been confirmed for isolates of *A. catenella* (Persich et al., 2006) and *A. tamiyavanichi* (Menezes et al., 2010). A toxic bloom of *Alexandrium* sp. (previously identified as *Alexandrium minutum* Halim by Menezes et al., 2007) and a non-toxic bloom of *Alexandrium frateculus* were recorded, respectively, in beaches at Rio de Janeiro (Menezes et al., 2007) and Santa Catarina (Omachi et al., 2007). Cysts of *A. catenella* and *A.* cf. *minutum* were additionally detected in sediments from Patos lagoon and Sepetiba bay, respectively (Persich and Garcia, 2003; Juliano and Garcia, 2006). Among these studies, only Persich et al. (2006) and Menezes et al. (2010) applied molecular techniques to study the phylogeny and biogeography of the identified species.

Here, we present a review of the dinoflagellate genus *Alexandrium* in Brazilian coastal waters, based both on available published information and new morphological and molecular data (28S rDNA e ITS) for *A. fraterculus*, *A. catenella*, *Alexandrium* sp., and *Alexandrium tamutum* Montresor, Beran & John (the latter being the first record for the South Atlantic).

MATERIALS AND METHODS

This review was based on previously published information for *A. garderae* (Balech, 1995), *A. kutnerae* (Balech, 1995), *A. tamyavanichi* (Menezes et al., 2010), and *A. catenella* (formerly identified as *A. tamarense* by Persich et al., 2006). New morphological and molecular data were obtained from the analysis of monoclonal strains of *A. tamutum* and *Alexandrium* sp. (same species identified as *A. minutum* by Menezes et al., 2007) isolated from Rio de Janeiro, and *A. fraterculus* isolated from Santa Catarina (**Figure 1**). Cells were grown using in K medium at 16 and 24 PSU (*A. tamutum*), K medium at 30 PSU (*Alexandrium* sp.), and F/2 medium at 34 PSU (*A. fraterculus*). Cultures were maintained at 20–23°C with photon flux density of 70–136 $\mu E\ m^{-2}.s^{-1}$ and a 12:12-h light:dark photoperiod. We also performed the morphological characterization and ITS rDNA sequencing for three of the *A. catenella* strains (ATBR1A7, ATBR2E, ATBR3D) used by Persich et al. (2006), and obtained from the Woods Hole Oceanographic Institution. Finally, the morphology of *Alexandrium* sp. was further re-analyzed by scanning electron microscopy (SEM) using environmental Lugol fixed samples obtained from the bloom recorded in 2007 from Rio de Janeiro by Menezes et al. (2007). The precedence of all strains/samples used here is informed in **Table 1**.

FIGURE 1 | Distribution of *Alexandrium* species along the Brazilian coast. BA, Bahia; RJ, Rio de Janeiro; RS, Rio Grande do Sul; SC, Santa Catarina.

Morphological Analysis

The tabulation of the species was characterized by the addition of calcofluor MR2 (10 ng ml^{-1}) under an Olympus BX51 epifluorescence microscope (Fritz and Triemer, 1985). For SEM, cells were harvested by filtration on 0.45 µm pore acetate membranes (Millipore). Filters were immediately fixed for 2 h in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH

TABLE 1 | Records of *Alexandrium* species in Brazilian coast.

Species	Years	Location	Coordinates	Source	Strains	References
Alexandrium catenella	1997 1999	Patos lagoon estuary mouth (Rio Grande do Sul)	32°81′00′′ to 32°82′20′′S; 51°85′10′′ to 51°85′50′′W	Cysts in sediment samples	ATBRA ATBR2B ATBR2C ATBR2D **ATBR2E** ATBR2F ATBR2G ATBR3A ATBR3C **ATBR3D** ATBR1A7 **ATBR1A10** ATBRC6	Persich et al., 2006
Alexandrium catenella		Cassino beach (Rio Grande do Sul)	32°14′56′′S 52°9′36′′W	Bloom sample (2×10^5 cells L^{-1})		Odebrecht et al., 1997
Alexandrium fraterculus	2010	Alegre beach (Santa Catarina)	26°46′21′′S, 48°39′31′′W	Plankton samples	**AF#53**	This study
Alexandrium fraterculus	2004	Itajaí (Santa Catarina)	26°57′03′′S, 48°45′44′′W	Bloom sample (8.8×10^4 cells L^{-1})		Omachi et al., 2007
Alexandrium gaarderae	Unknown	Rio Grande do Sul	Unknown	Plankton sample		Balech, 1995
Alexandrium kutnerae	Unknown	Rio Grande do Sul	Unknown	Plankton sample		Balech, 1995
Alexandrium tamiyavanichi	2004 2006	Porto Seguro inner coastal platform	16°36′S, 39°05′W	Plankton samples	A1PSA B2PSA PSII	Menezes et al., 2010
Alexandrium tamutum	2013 2014	Guanabara bay, (Rio de Janeiro)	22°81′16′′S 43°15′45′′W	Plankton samples (1×10^6 cells L^{-1})	**UFRJ-MN03** **UFRJ-MN04** **RJ_BG**	This study
Alexandrium sp.	2007	Leblon beach (Rio de Janeiro)	22°54′14′′S 43°13′16′′W	Bloom samples (4.3×10^5 L^{-1})		Menezes et al., 2007
Alexandrium sp.	2014	Guanabara bay (Rio de Janeiro)	22°81′16′′S 43°15′45′′W	Plankton samples (6.7×10^5 cells L^{-1})	**UFRJ-MN01** **UFRJ-MN02**	This study

Strains analyzed in this study are depicted in bold.

7.2). After that, they were washed three times (10 min) using the same buffer and postfixed in 1% osmium tetroxide in sodium cacodylate buffer (pH 7.2). Filters were finally dehydrated in an ascending ethanol series of 10 min each 35, 50, 70, 90, and 100%, followed by two washes for 10 min each in 100% ethanol, critical-point dried (CPD 020 Balzers Union). Dried filters were mounted on stubs, coated with gold (Balzers/Union FL-9496), and viewed with a JEOL 5310 scanning electron microscope (Akishima, Tokyo, Japan). The metric data that represented outliers for minimal and maximal cell dimensions were shown in parentheses.

PCR Amplification and DNA Sequencing

DNA from *A. tamutum* and *Alexandrium* sp. was extracted from exponentially growing cultures using NucleoSpin® Plant II extraction kit (Macherey-Nagel GmbH & Co., KG, Germany) following the manufacturer's protocol. The ITS (including 5.8S rDNA gene) and LSU (D1–D3) regions of rDNA were amplified using primers ITSA/ITSB (Sato et al., 2011) and D3F/D1R (Scholin et al., 1994; Litaker et al., 2003), respectively. The PCR mix (25 μl final volume) contained 1 U GoTaq® DNA polymerase (Promega, Madison, WI, USA), 1X GoTaq® Flexi buffer (Promega), 1.25 mM $MgCl_2$ solution (Promega), 0.16 mM dNTPs (Thermo Scientific Inc., USA), 8 pmol of each primer, 0.2 μg of Bovine Serum Albumin (BSA) (New England Biolabs Inc.), and ∼15 ng genomic DNA. The PCR protocol was as follows: initial denaturation for 5 min at 94°C, followed by 35 cycles of 1 min denaturation at 94°C, 1 min annealing at 50 and 55°C (ITS and LSU rDNA, respectively), and 1 min extension at 72°C, plus a final extension of 5 min at 72°C. The PCR products were purified and sequenced by Macrogen (Seoul, Korea) in both directions using the PCR primers. The sequences obtained were deposited in GenBank (**Supplementary Tables S1, S2**).

DNA extraction from *A. fraterculus* strain was performed using a modified CTAB protocol (Lebret et al., 2012), whereas DNA extracts from fixed samples of *A. catenella* strains were obtained using a modified guanidinium isothiocyanate protocol (Alves-de-Souza et al., 2011). Both ITS and LSU (D1–D3) regions of rDNA were obtained for *A. fraterculum* whereas only ITS rDNA sequences were obtained for *A. catenella* using the same primer mentioned above. For both species, the PCR mix (15 mL final volume) contained 1–6 mL of the

DNA extract, 330 mM of each deoxynucleoside triphosphate (dNTP), 2.5 mM of $MgCl_2$, 1.25 U of GoTaq1 DNA polymerase (Promega Corporation), 0.17 mM of both primers, 1X reaction buffer (Promega Corporation). PCR protocol included an initial denaturating step at 95°C for 5 min, 35 cycles at 95°C for 1 min, annealing at 55°C for 45 s, extension at 72°C for 1 min 15 s, with a final extension step at 72°C for 7 min. PCR products were purified using the ExoSAP-IT kit (USB) following the manufacturer's recommendations and directly sequenced on an ABI Prism 3100 automatic sequencer (Applied Biosystems).

Phylogenetic analyses were performed for each rDNA region (ITS and LSU) individually. *Alexandrium* sequences obtained from this study and from GenBank (**Supplementary Tables S1, S2**) were aligned using the online package MAFFT version 7 (https://mafft.cbrc.jp/alignment/server/) followed by manual editing. The Maximum Likelihood (ML) analyses were conducted using the software MEGA 7.0 (Kumar et al., 2016). The evolutionary models used were the Hasegawa-Kishino-Yano model (HKY) for ITS rDNA and the Tamura-Nei model (TN93) for LSU rDNA, both with gamma distribution. These models were the best available for the tree of the rDNA region data (ITS and LSU). All positions containing gaps and missing data were eliminated. The robustness of the inferred topology was tested by bootstrap resampling (1,000 replicates). Bayesian inferences (BI) were obtained with the software MrBayes 3.2 (Ronquist et al., 2012). Rather than selecting a nucleotide substitution model by *a priori* model selection, we used the "lset nst = mixed" Markov Chain Monte Carlo procedure, consisting of two independent trials with four chains. The chains were run for 5,000,000 generations and sampled every 100th cycle. Posterior probability (PP) values for the resulting 50% majority rule consensus tree were estimated after discarding the first 25% of the trees as burn-in. All trees were rooted using *Alexandrium lee* and *Alexandrium diversaporum* sequences as outgroup.

RESULTS

Based on both published information and the new data, seven *Alexandrium* species have been identified from Brazilian coastal waters so far (**Figure 1**, **Table 1**). Except for *A. catenella* and *A. fraterculus,* which were reported for different locations in Rio Grande do Sul and Santa Catarina (southern Brazil), the other five species were recorded only once for the northeast (*A. tamiyavanichi*), southeast (*Alexandrium* sp., *A. tamutum*), and south (*Alexandrium gaardnerae* and *A. kutnerae*) parts of the country. This is the first report of *A. tamutum* for the South Atlantic.

Morphology

Alexandrium catenella (Figures 2A,B)

Cells isodiametric (40–50 μm long, 30–50 μm wide). The first apical plate (1′) was irregularly rhomboidal connected direct or indirectly to the apical pore (Po). The third apical plate (3′) was asymmetrical and the ventral pore (v.p.) was always located along the margin between plate 1′ and plate 4′. The posterior sulcal plate (s.p.) was always pentagonal without a connecting pore.

The morphology of the three isolates agreed with the description reported by Balech (1995) for the morphospecies *A. tamarense*. No morphological variability regarding presence/absence of the both v.p. and s.p. connecting pore. Although previously identified as *A. tamarense* (Persich et al., 2006), these three isolates along with others from South and North America (*A. tamarense* complex group I, sensu John et al., 2014) should be currently named as *A. catenella* (Prud'homme van Reine, 2017). Besides the three isolates analyzed in this study, Persich et al. (2006) analyzed the toxin content of ten more strains isolated from the same locality (Patos Lagoon estuary) (**Table 1**). These isolates showed toxicity from 7.07 to 65.92 pg STX eq $cell^{-1}$ and total toxin content ranged from 42 to 199 fmol $cell^{-1}$. Toxin C1,2 was predominant in most of the strains, comprising up to 78.4% of the total toxins (Persich et al., 2006). More recently, high concentrations of PSP toxins (601.13 to 3.199.6 μg STX eq.kg^{-1}) were detected in bivalves (*Perna perna* and *Crassostera gigas*) on October 2017 from several coastal locations in Santa Catarina (http://www.cidasc.sc.gov.br/defesasanitariaanimal/). These PSP levels were associated with the presence of *A. tamarense* morphospecies complex in the plankton (7,350–11,750 cells L^{-1}) and lead to the closure of all mariculture farms in the area for ∼2 months. Although several toxic *Alexandrium* species have been previously reported in Brazil, this is the first record of high PSP toxins in shellfish associated to a species of this genus.

Alexandrium fraterculus (Figures 2C–E)

Chains formed by of 2–8 isodiametric, pentagonal cells (30–40 μm long, 30–40 μm wide). The first apical plate (1′) was directly attached to the Po. The ventral pore (v.p.) was located in the middle of the right margin, in the suture with the fourth apical plate (4′). The posterior sulcal plate (s.p.) was isodiametric, rhomboidal with a large connecting pore located in the center of the plate. The anterior sulcal plate (s.a.) was slightly longer than it was wide, with a deep posterior sinus.

No toxicity was found in the analyzed strain (L.A.O. Proença, personal communication).

Alexandrium gaarderae

This species was reported by Balech (1995) based on environmental samples from the Brazilian coast as *A. concavum* (locality not informed), along with a description but no illustrations. Cells were 50–81 μm long, 45–64.5 μm wide. The first apical plate (1′) was rhomboidal, connected indirectly or directly to the apical pore (Po). A ventral pore (v.p.) occurred enclosed within the right margin of 1′. The posterior sulcal plate (s.p.) was longer than wide, symmetrical.

Alexandrium kutnerae (Figure 2F)

This species was described by Balech (1995) as having rounded cells, 51–65 long, 41–57 wide. The first apical plate (1′) is connected directly or indirectly to the apical pore (Po). A ventral pore (v.p.) occurs inside of the first apical plate (1′). The anterior sulcal plate (s.a.) had a quadrangular or hexagonal precingular part (p.pr.). The posterior sulcal plate (s.p.) was longer than wide, symmetrical, without an attachment pore.

Alexandrium tamiyavanichi (Figures 2G–K)

This species was characterized by Menezes et al. (2010) as having small and rounded cells, sometimes slightly wider than long, 31–41 μm long and 26–35 μm width. A small ventral pore (v.p.) occurred within the right margin on the first apical plate (1′). The anterior sulcal plate (s.a.) showed a triangular precingular part (p.pr). The posterior sulcal plate (s.p.) was longer than wide, symmetrical, with an attachment pore.

Six PSP toxins have been identified for one *A. tamiyavanichi* strain (A1PSA): STX, Neo-STX, GTX4, GTX3, dcGTX2, and dcGTX3. STX was the main toxin (16.85 fmol. cell^{-1}), accounting for 67.06%) of the overall cell toxin content (Menezes et al., 2010).

Alexandrium tamutum (Figures 3A–J)

Cells were ovate to spherical, 20.1–35.0 μm long, and 19.2–33.6 μm wide. The thecae surface was smooth and very thin. The first apical plate (1′) was rhomboidal with a direct connection to the APC and posterior margin straight. A ventral pore was present on the anterior right margin of the 1′ plate. The sixth precingular (6′′) plate was pentagonal, longer than wide or wider than long. Apical pore complex (APC) with comma-shaped. The anterior sulcal plate (s.a.) was longer than wide with anterior margin straight and posterior margin concave, often extending to the epitheca. The posterior sulcal (s.p.) plate was wider than long, asymmetrical, and without a posterior attachment pore.

The three analyzed strains presented cell morphology matching with the original description of the species (Montresor et al., 2004).

Alexandrium sp. (Figure 2L)

Cells were usually solitary, sometimes forming short chains of up to four ovate to irregularly elliptical cells (17.1)19–31.2(32.3) μm long, (15.2)17.1–31.2(32.3) μm wide. The sixth precingular (6′′) plate was generally pentagonal, longer than wide, sometimes as long as wide. The first apical plate (1′) showed a clear ventral pore and connected directly or through a channel with the Po plate. Epitheca surface was smooth whereas hypotheca surface showed strong reticulation.

This species was at first identified as *A. minutum* by Menezes et al. (2007) based on samples obtained from a bloom in 2007 from the Rio de Janeiro coast. However, DNA sequencing (see Phylogenetic Analysis section) of strains recently isolated from Guanabara bay depicting the same unusual theca ornamentation pattern (epitheca smooth and hypotheca strongly reticulated) indicated that both populations belong to a new *Alexandrium* species, currently under description. Four analogous of STX have been found in the cells from the 2007's bloom: Neo-STX, STX, GTX4, and GTX2 (Menezes et al., 2007). Neo-STX was the dominant toxin, accounting to 83.47% of STX equivalents (Menezes et al., 2007).

Phylogenetic Analyses

Maximum-likelihood (ML) and BI analyses based on ITS and LSU rDNA resulted in phylogenetic trees with similar topologies (**Figures 4**, **5**). All phylogenies exhibited two main clades: (i) a clade composed of the *A. tamarense* complex (e.g., *A. catenella*, *Alexandrium fundyense, Alexandrium mediterraneum*, *A. tamarense*, *Alexandrium australiense*, *Alexandrium pacificum*), *A. tamiyavanichi*, *A. fraterculus*, and *Alexandrium affine* sequences (ML = 86% and BI = 1.0 for ITS rDNA; ML = 100% and BI = 1.0 for LSU rDNA), and (ii) a clade composed of *Alexandrium insuetum*, *A. minutum*, *A. ostenfeldii*, *A. tamutum*, and *Alexandrium* sp. sequences (ML = 72% and BI = 0.98 for ITS rDNA; ML and BI < 0.5 for LSU rDNA).

The *A. tamarense* complex formed a large monophyletic group made up by sequences of *A. catenella*, *A. mediterraneum*, *A. tamarense*, *A. australiense*, and *A. pacificum* (ML = 99% and BI = 1.0 for ITS; ML = 100% and BI = 1.0 for LSU rDNA). *Alexandrium catenella* sequences from Brazil grouped with sequences of this species from USA and Chile, North America (USA and Canada), and Japan (ML = 99% and BI = 1.0 for ITS rDNA; ML = 100% and BI = 1.0 for LSU rDNA). Two subclades were formed inside the *A. catenella* clade in trees based on ITS rDNA: a clade composed only by sequences from Chile (ML = 99% and BI = 1.0) and a clade composed with sequences from Canada, USA and Norway (ML = 98% and BI = 1.0). The latter subclade was also recognized inside the *A. catenella* clade formed in the trees based on LSU rDNA (ML = 100% and BI = 1.0).

Alexandrium tamiyavanichi formed a monophyletic clade consisting of two different subclades. The first subclade was formed only by sequences from Brazil (ML = 92% and BI = 0.96 for ITS rDNA; ML = 96% and BI = 0.89 for LSU rDNA rDNA) whereas the second subclade included sequences from Japan and Malaysia (ML = 99% and BI = 1.0 for ITS rDNA; ML = 96% and BI = 1.0 for LSU rDNA). *Alexandrium tropicale* formed a sister group of *A. tamiyavanichi* in the trees based on LSU rDNA (ML = 100% and BI = 1.0). The *A. fraterculus* clade grouped with the *A. tamiyavanichi* clade (ML = 75% and BI = 1.0 ITS rDNA trees; ML = 98% and BI = 1.0 for LSU rDNA) and included sequences from Brazil, New Zealand, Yellow Sea, Japan, and Korea (ML = 100% and BI = 1.0 for ITS and LSU rDNA).

Alexandrium tamutum sequences formed a monophyletic group (ML = 80% and BI = 0.84 for ITS; ML = 74% and BI = 0.9 for LSU). A subclade with a small branch composed of sequences from Italy was observed inside the *A. tamutum* clade in trees based on ITS rDNA (ML = 97% and BI = 1.0) whereas Brazilian strains grouped with sequences from Italy, Greenland, and United Kingdom (ML = 99% and BI = 0.83). *Alexandrium* sp. sequences formed a monophyletic group (ML = 99% and BI = 1.0 for ITS; ML = 100% and BI = 1.0 for LSU) clearly separated from other *Alexandrium* clades.

DISCUSSION

Alexandrium species reported from Brazilian coastal waters so far have been identified based mostly on traditional morphological features, such as plate patterns, cell size and shape, presence and localization of the ventral pore, presence of a sulcal pore, and

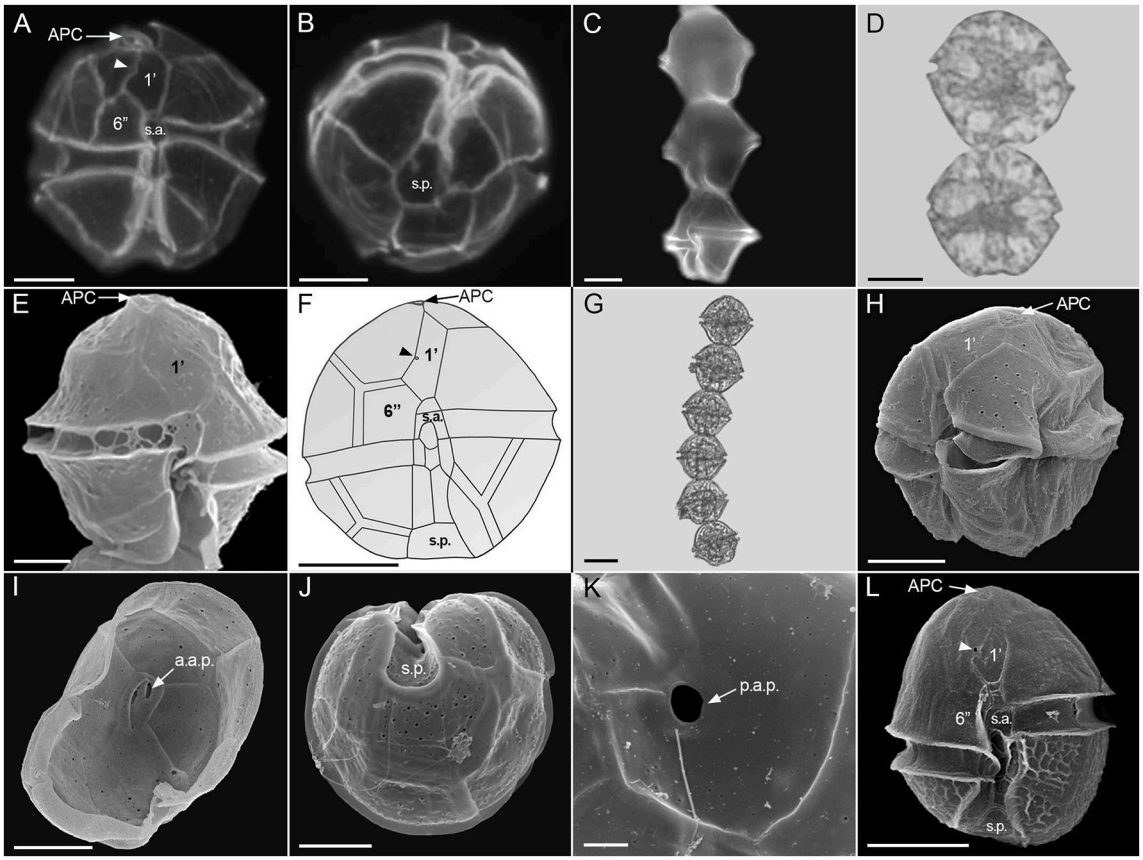


FIGURE 2 | *Alexandrium* species recorded from Brazil. Epifluorescence micrographs **(A–C)**, light micrographs **(D,G)**, scanning electron micrographs **(E,H–L)**, and drawing **(F)**. *A. catenella* in ventral view showing ventral pore (arrowhead) **(A)** and antapical view **(B)** *A. fraterculus* chains, **(C,D)** and cell in ventral view **(E)**, *A. kutnerae* in ventral view showing ventral pore (arrowhead) **(F)**, *A. tamiyavanichi* chain **(G)**, latero-ventral view **(H)**, epitheca inside showing apical pore complex with anterior attachment pore (a.a.p.) **(I)**, antapical view **(J)**, posterior sulcal plate with a large posterior attachment pore (p.a.p.) **(K)**, *Alexandrium* sp. in ventral view showing ventral pore (arrowhead) **(L)**. Scale = 10 μm **(A,B,E,F,H–J,L)**, 20 μm **(C,D,G)**, 2 μm **(K)**. APC, apical pore complex; 1′, first apical plate; 6′′, sixth precingular plate; s.a., anterior sulcal plate; s.p., posterior sulcal plate; a.a.p., anterior attachment pore; p.a.p, posterior attachment pore. The drawing of *A. kutnerae* was based on Balech (1995).

chain formation. These morphological traits, however, are widely variable often with overlapping or transitional forms between species and therefore are shown to be less robust for species discrimination (e.g. Lilly et al., 2005; Anderson et al., 2012).

Alexandrium gaarderae and *A. kutnerae* have been reported only once from Brazilian coastal waters (Balech, 1995). In addition to Brazil, *A. gaarderae* has been recorded from Argentina, Gulf of Mexico, the Caribbean Sea, and Vietnam (Balech, 1995; Larsen and Nguyen-Ngoc, 2004), whereas *A. kutnerae* has records for Argentina and Western Mediterranean (Balech, 1995; Bravo et al., 2006). While *A. gaarderae* has a distinctive cell shape characterized by a conical epitheca (Larsen and Nguyen-Ngoc, 2004), the general cell appearance of *A. kutnerae* (**Figure 2F**) reminds as observed in the *A. tamarense* complex. The presence of a pre-cingular part in the sulcal plate (s.p.) in *A. kutnerae* is the main morphological characteristic separating this species from those of the *A. tamarense* complex. However, it is not clear if this feature is discriminative enough to keep the former apart from representatives of the *A. tamarense* complex. As a matter of fact, phylogenetic analysis including ITS sequences of an isolate of *A. kutnerae* from the Catalan Sea (Spain) indicated this species belongs to the *A. pacificum* clade (Group IV, sensu John et al., 2014), with a high support value (Gu, 2011).

Taxonomic and phylogenetic reviews of the genus *Alexandrium* revealed several cryptic species in this genus, not only invalidating some of the described species in the literature but also precluding the application of a morphospecies concept for the species circumscriptions (Lilly et al., 2007; Miranda et al., 2012; John et al., 2014; Wang et al., 2014). An example of that was observed in the *A. tamarense* complex (formed by the morphospecies *A. tamarense, A. catenella,* and *A. fundyense*), where species were segregated into five genetic distinct clades, each one corresponding to a different cryptic species: *A. catenella* (Group I), *A. mediterraneum* (Group II), *A. tamarense* (Group III), *A. pacificum* (Group IV), and *A. australiense* (Group V) (Lilly et al., 2007; Miranda et al., 2012; John et al., 2014; Wang et al., 2014).

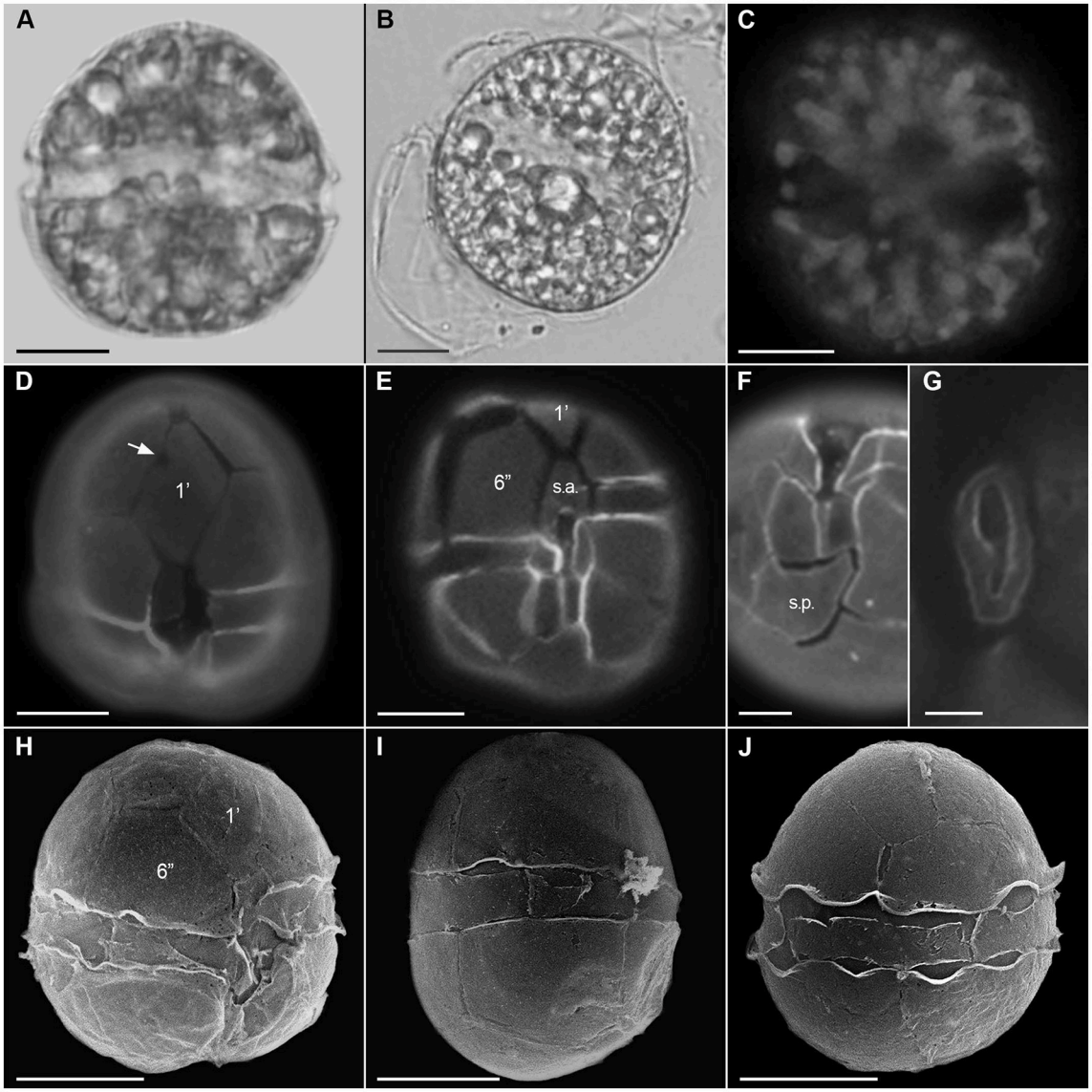


FIGURE 3 | *Alexandrium tamutum*. Light micrographs **(A,B)**, epifluorescence micrographs **(C–G)**, and scanning electron micrographs **(H–J)**. Live cell **(A)**, cyst **(B)**, chloroplast autofluorescence **(C)**, ventral view **(D,E)**, showing the firth apical plate (1′) with ventral pore (arrow), sixth precingular plate (6′′), and anterior sulcal plate (s.a.), antapical view showing posterior sulcal plate (s.p.) **(F),** apical pore complex **(G)**, ventral view showing the firth apical plate (1′) and large sixth precingular plate (6′′); lateral view **(I)**, and dorsal view **(J)**. Scale = 10 μm **(A–E,H–J)**, 5 μm **(F)**, 2 μm **(G)**.

In our morphological analysis, strains of *A. catenella* (Group I sensu John et al., 2014; formerly identified as the morphospecies *A. tamarense*) presented cells always with a ventral pore, which is consistent with the *A. tamarense* morphospecies description provided by Balech (1995). According to this author, the presence of a ventral pore in the morphospecies *A. tamarense* constitutes a diacritic character separating this morphospecies from *A. catenella* and *A. fundyense* (the latter two morphospecies lacking this feature). Stability in the presence of a ventral pore has been observed in strains of the morphospecies *A. tamarense* from Australia (MacKenzie et al., 2004), Europe (Thau Lagoon) (Genovesi et al., 2011), and from western Greenland (Tillmann et al., 2016), the former one corresponding to Group IV (now *A. pacificum*) and the latter two to Group III (now *A. tamarense*). In contrast, many studies based on wild populations and cultures have demonstrated intermediate morphology between *A. catenella*, *A. fundyense,* and *A. tamarense* morphospecies, regarding the presence/absence of a ventral pore and/or chain formation (for more details see Orlova et al., 2007; Wang et al., 2008, 2014; John et al., 2014).

Phenotypic plasticity and genetic variability has been reported for *A. catenella* strains (Group I) from Chile, Mediterranean Sea, and Japan (Penna et al., 2005; Cruzat et al., 2018). Alpermann et al. (2009) reported phenotypic and genetic variability in *A.*

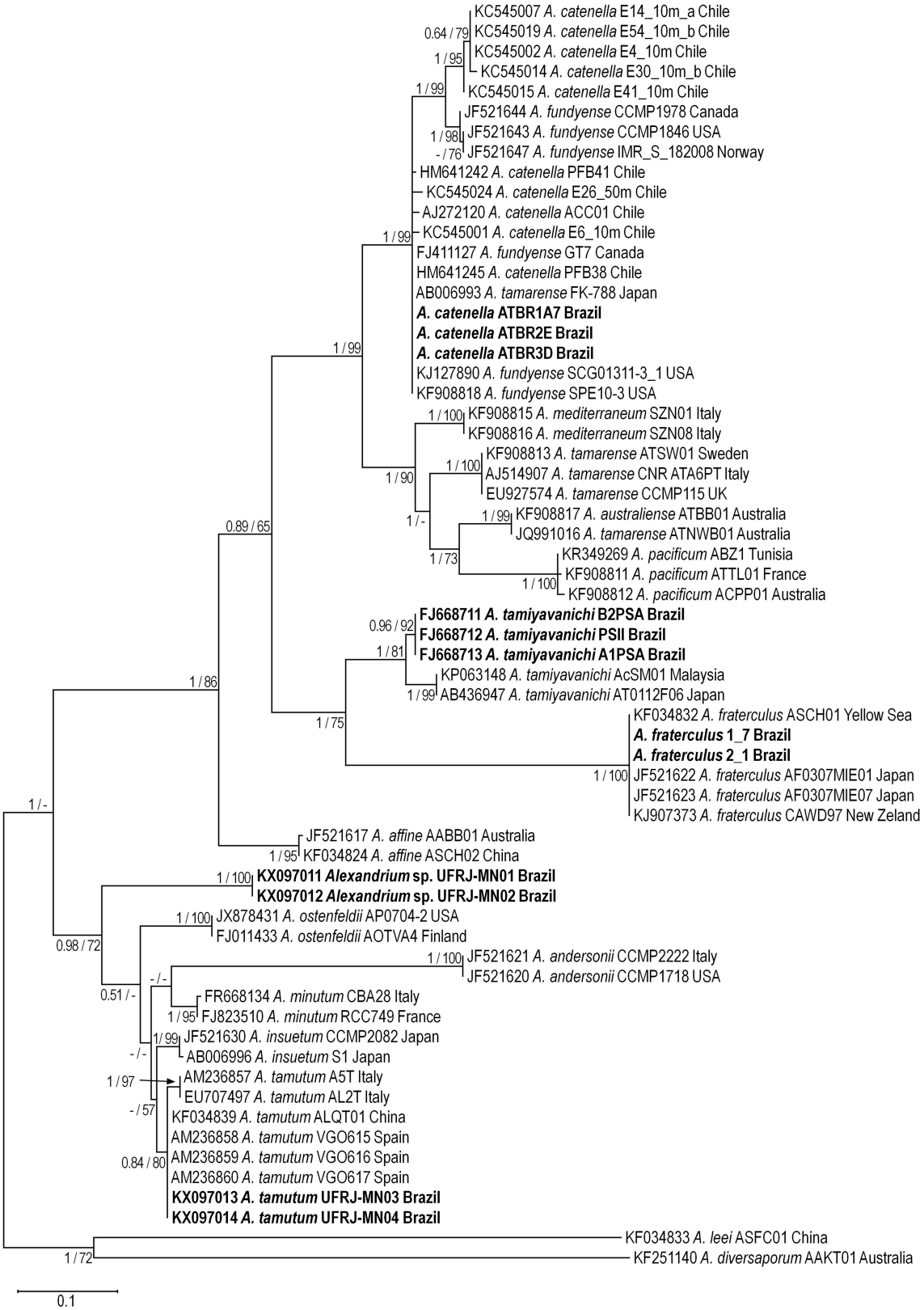


FIGURE 4 | Maximum Likelihood tree based on ITS rDNA sequences. *Alexandrium* sequences from Brazil are shown in bold. Numbers at nodes are bootstrap and posterior probabilities values for Maximum Likelihood and Bayesian Inference analysis, respectively. Only values >50% (ML) and 0.50 (BI) are shown.

FIGURE 5 | Maximum Likelihood tree based on LSU rDNA sequences. *Alexandrium* sequences from Brazil are shown in bold. Numbers at nodes are bootstrap and posterior probabilities values for Maximum Likelihood and Bayesian Inference analysis, respectively Only values >50% (ML) and 0.50 (BI) are shown.

tamarense isolates (Group III) from the North Sea coast of Scotland that were interpreted, respectively, as having strong potential for adaptability of the population and evidence for a lack of strong selective pressure on respective phenotypic traits at the time the population was sampled. Logares et al. (2007, 2009) reported almost identical DNA sequences for the dinoflagellate morphospecies *Peridinium aciculiferum* and *Scripsiella hangoei*, which are well-differentiated based on morphology (Logares et al., 2007, 2009). Similar results were reported for *P. aciculiferum, Peridinium euryceps,* and *Peridinium baicalense* by Annenkova et al. (2015). These authors hypothesized that identical sequences do not necessarily mean the same microbial species, and these taxa are not a case of phenotypic plasticity but originated via recent rapid diversification (radiation) into several

species followed by dispersion to environments with different ecological conditions (Logares et al., 2007, 2009; Annenkova et al., 2015).

Phenotypic plasticity is a single genotype's ability to produce variable phenotypes in response to environmental conditions thereby enabling it to buy additional time for adaptation to occur (Sunday et al., 2014) and provide a mechanism for adaptation to occur rapidly (Pigliucci et al., 2006; Ghalambor et al., 2015). Although it is recognized that phenotypic plasticity can increase organism survival under specific conditions, there is no consensus on its effects on the organisms: whether they drive the evolution of novel traits and promote taxonomic diversity, or whether they act more on acceleration or evolutionary changes (Pfennig and Pfennig, 2010). Little is known about the extent of the phenotypic plasticity in *Alexandrium*. Studies on the phenotypic plasticity on this genus should be addressed aiming to respond to questions such as: Could this phenotypic plasticity be a pre-adaptation or genetic shift after an invasive process of this genus to different localities? Could the acclimatization process cause definitive change in morphology?

The phylogenetic analysis of ITS and LSU rDNA sequences corroborated that the isolates of *A. catenella* from southern Brazil belong to the clade formed by *A. catenella* (Group I, sensu John et al., 2014), along with other isolates from Chile, Japan, Norway and North America, as already pointed out by Persich et al. (2006). The phylogeny resulted from ITS rDNA sequences also confirmed the existence of genetic diversity within this clade (Cruzat et al., 2018) with a subclade formed by some isolates from Chile and a second subclade formed by isolates from the United States, Canada and Norway. The presence of the Group I in South America has been hypothesized as being a result of ballast water introduction (invasive species) or natural dispersion (see Lilly et al., 2007).

According to John et al. (2003), the ancestral population of *Alexandrium* was globally distributed, diverging into the ancestors of Groups I and II that occurred in waters off North America and in the tropical Atlantic. Posteriorly, Group II evolved in the tropical Atlantic, becoming distinct from the Group I populations and later colonizing the Mediterranean (John et al., 2003). There is evidence that Group I populations were transported to the west coast of South America by a natural dispersal mechanism during a period in which the weather was substantially cooler than nowadays, e.g., as in one of the recent ice ages (Persich et al., 2006; Lilly et al., 2007). However, the presence of Group I on the east Atlantic coast of South America is hypothesized to have resulted recently from Chilean populations, given the similarity of the LSU rDAN sequences (D1–D2) among the isolates from both sites (Persich et al., 2006; Lilly et al., 2007), as corroborated by the our phylogeny results based on ITS rDNA. Although Persich et al. (2006) suggested the possibility that *A. catenella* (Group I) is spreading northward by current ocean circulation patterns, environmental changes due to global warming would likely explain the recent spread of *A. catenella* to the east coast of South America (Lilly et al., 2007).

Alexandrium tamiyavanichi from northeastern Brazilian coast was included in a distinct clade along with isolates from Japan and other Asian locations with medium support value, with the Brazilian population diverging earlier (Menezes et al., 2010). One of the hypotheses that seems plausible to explain the presence of the species in Brazil is due to an invasion event that could not be recent or occurred several times (Menezes et al., 2010). *Alexandrium tamiyavanichi* is a tropical species but it also occurs in warm-temperate regions such as Japan, Baja California, and South Africa (Sierra-Beltrán et al., 1998; Ruiz-Sebastián et al., 2005; Nagai et al., 2011). The origin of this species in Brazil could be explain by closure of Tethys Ocean and the emergence of the Isthmus of Panama that formed a land bridge between the two American continents and its final closure when there was a final interruption of the Atlantic and Pacific oceans' connection disrupting the flow of genes of many species (Hou and Li, 2018). On the other hand, after the closure of Tethys Ocean the dispersal between the Atlantic and Indo-Pacific could have occurred via southern Africa during interglacial periods (Bowen et al., 2006). These same hypotheses could be applied to *A. fraterculus* that segregated with isolates from Japan, Korea, China, and New Zealand, forming a sister clade of *A. tamiyavanichi.*

Data on the geographical distribution of *A. tamutum* are still scarce. Previously recorded as *Alexandrium* sp. for the northwest Pacific (Yoshida, 2002), *A. tamutum* was formally described based on material isolated from the Mediterranean (Montresor et al., 2004). Since then, the species has been reported in morphological studies of populations from Russia, Malaysia, and Scotland (Selina and Morozova, 2005; Hii et al., 2012; Swan and Davidson, 2014) and in morphological and genetic studies of populations from Ireland, Shetland, China, Malaysia, Greenland (Touzet et al., 2008; Brown et al., 2010; Gu et al., 2013; Kon et al., 2013; Tillmann et al., 2016). Our results demonstrated a genetic variability within the *A. tamutum* clade, which was formed by some subclades, including the Brazilian subclade. Genetic variability between isolates from different geographical areas of *A. tamutum* based on LSU and ITS rDNA has been reported by Montresor et al. (2004), Penna et al. (2008) and Tillmann et al. (2016). However, more sequences from distinct locations are necessary to confirm the occurrence of a geographical partitioning of *A. tamutum* species. The two isolates of *Alexandrium* sp. formed a monophyletic clade, however it is necessary for more sequences to make any inference about its geographical distribution. These populations have been found in Guanabara Bay and constitute a new species for the science (currently under description), related to *A. minutum* and *A. tamutum*.

This review constitutes the first approach on the occurrence of the *Alexandrium* genus in Brazilian coastal waters. The number of species recorded was very incipient considering the 7,491 km of the Brazilian coast, with the new record of *A. tamutum* and the discovery of a new species (*Alexandrium* sp.) indicating a wide gap in knowledge on the genus diversity in the country. Brazil has a Program for the National Sanitary Control of Bivalve Molluscs (PNCMB) established in 2011 designed to monitor for the presence of toxins in seafood and the presence of harmful algae in the coastal area. Although it is a national program, until now it has been implemented

only in Santa Catarina. The presence of toxic *Alexandrium* strains in other areas on the country points out to the need of expand the current coverage of the PNCMB and improve our ability to identify causative species of HABs through training courses and international technical and research collaborations.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, No. 503443/2012-3 and 471303/2013-5), by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, No. 562283/2010-2), and Marbionc Program at UNC Wilmington (State of North Carolina, USA).

ACKNOWLEDGMENTS

To Dr. Don Anderson (Woods Hole Oceanographic Institution) who kindly provided the three *A. catenella* strains used in this study. To the Institute of Biophysics Carlos Chagas Filho/UFRJ for facilitating access to their scanning electron microscope.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2018.00421/full#supplementary-material

REFERENCES

Ajani, P., Harwood, D. T., and Murray, S. A. (2017). Recent trends in marine phycotoxins from Australian coastal waters. *Mar.Drugs* 15, 1–33. doi: 10.3390/md15020033

Alpermann, T. J., Tillmann, U., Beszteri, B., Cembella, A. D., and John, U. (2009). Phenotypic variation and genotypic diversity in a planktonic population of the toxigenic marine dinoflagellate *Alexandrium tamarense* (Dinophyceae) 1. *J. Phycol.* 46, 18–32. doi: 10.1111/j.1529-8817.2009.00767.x

Alves-de-Souza, C., Cornet, C., Nowaczyk, A., Gasparini, S., and Guillou, L. (2011). *Blastodinium* spp. infect copepods in the ultra-oligotrophic marine waters of the Mediterranean sea. *Biogeosci. Disc.* 8, 1–12. doi: 10.5194/bgd-8-2563-2011

Anderson, C. R., Moore, S. K., Tomlinson, M. C., Silke, J., and Cusack, C. K. (2015). "Living with harmful algal blooms in a changing world: strategies for modeling and mitigating their effects in coastal marine ecosystems," in *The Coastal and Marine Hazards, Risks, and Disasters*, eds J. F. Shroder, J. T. Ellis, and D. J. Sherman (Amsterdan: Elsevier), 495–561.

Anderson, D. M. (2007). *The Ecology and Oceanography of Harmful Algal Blooms: Multidisciplinar Approaches to Research and Management*. IOC Bruun Memorial Lectures, Technical Series 10. Paris: UNESCO.

Anderson, D. M. (2017). "Harmful algal blooms", in *Harmful Algal Blooms (HABs) and Desalination: A Guide to Impacts, Monitoring and Management*, IOC Manuals and Guides No. 78, eds D. M. Anderson, S. F. E. Boerlage, and M. Dixon (Paris: Intergovernmental Oceanographic Commission of UNESCO), 17–52.

Anderson, D. M., Cembella, A. D., and Hallegraeff, G. M. (2012). Progress in understanding harmful algal blooms: paradigm shifts and new technologies for research, monitoring, and management. *Annu. Rev. Mar. Sci.* 4, 143–176. doi: 10.1146/annurev-marine-120308-081121

Annenkova, N. V., Hansen, G., Moestrup, Ø., and Rengefors, K. (2015). Recent radiation in a marine and freshwater dinoflagellate species flock. *The ISME J.* 9, 1821–1834. doi: 10.1038/ismej.2014.267

Balech, E. (1995). *The Genus Alexandrium Halim (Dinoflagellata)*. Cork: Sherkin Island Marine Station.

Bowen, B. W., Muss, A., Rocha, L. A., and Grant, W. S. (2006). Shallow mtDNA coalescence in Atlantic pygmy angelfishes (genus Centropyge) indicates a recent invasion from the Indian Ocean. *J. Hered.* 97, 1–12. doi: 10.1093/jhered/esj006

Bravo, I., Garcés, E., Diogène, J., Fraga, S., Sampedro, N., and Figueroa, R. I. (2006). Resting cysts of the toxigenic dinoflagellate genus *Alexandrium* in recent sediments from the Western Mediterranean coast, including the first description of cysts of *A. kutnerae* and *A. peruvianum*. *Eur. J. Phycol.* 41, 293–302. doi: 10.1080/09670260600810360

Brown, L., Bresnan, E., Graham, J., Lacaze, J. P., Turrell, E., and Collins, C. (2010). Distribution, diversity and toxin composition of the genus *Alexandrium* (Dinophyceae) in Scottish waters. *Eur. J. Phycol.* 45, 375–393. doi: 10.1080/09670262.2010.495164

Cruzat, F. A., Muñoz, C., González-Saldía, R. R., Inostroza, A., and Andree, K. B. (2018). High genetic variability of *Alexandrium catenella* directly detected in environmental samples from the Southern Austral Ecosystem of Chile. *Mar. Pollut. Bull.* 127, 437–444. doi: 10.1016/j.marpolbul.2017.12.022

Deeds, J., Landsberg, J., Etheridge, S., Pitcher, G., and Longan, S. (2008). Non-traditional vectors for paralytic shellfish poisoning. *Mar. Drugs* 6, 308–348. doi: 10.3390/md6020308

Etheridge, S. M. (2010). Paralytic shellfish poisoning: seafood safety and human health perspectives. *Toxicon* 56, 108–122. doi: 10.1016/j.toxicon.2009.12.013

Fabro, E., Almandoz, G. O., Ferrario, M., John, U., Tillmann, U., Toebe, K., et al. (2017). Morphological, molecular, and toxin analysis of field populations of Alexandrium genus from the Argentine Sea. *J. Phycol.* 53, 1206–1222. doi: 10.1111/jpy.12574

Fritz, L., and Triemer, R. E. (1985). A rapid simple technique utilizing Calcofluor white M2R for the visualization of dinoflagellate thecal plates. *J. Phycol.* 21, 662–664. doi: 10.1111/j.0022-3646.1985.00662.x

Genovesi, B., Shin-Grzebyk, M.-S., Grzebyk, D., Laabir, M., Gagnaire, P.-A., Vaquer, A., et al. (2011). Assessment of cryptic species diversity within blooms and cyst bank of the *Alexandrium tamarense* complex (Dinophyceae) in a Mediterranean lagoon facilitated by semi-multiplex PCR. *J. Plank. Res.* 33, 405–414. doi: 10.1093/plankt/fbq127

Ghalambor, C. K., Hoke, K. L., Ruell, E. W., Fischer, E. K., Reznick, D. N., and Hughes, K. A. (2015). Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. *Nature* 525, 372–375. doi: 10.1038/nature15256

Gobler, C. J., Doherty, O. M., Hattenrath-Lehmann, T. K., Griffith, A. W., Kang, Y., and Litaker, R. W. (2017). Ocean warming since 1982 has expanded the niche of toxic algal blooms in the North Atlantic and North Pacific oceans. *Proc. Natl. Acad. Sci. U.S.A.* 114, 4975–4980. doi: 10.1073/pnas.1619575114

Gu, H. (2011). Morphology, phylogenetic position, and ecophysiology of *Alexandrium ostenfeldii* (Dinophyceae) from the Bohai Sea, China. *J. Syst. Evol.* 49, 606–616. doi: 10.1111/j.1759-6831.2011.00160.x

Gu, H., Zeng, N., Liu, T., Yang, W., Müller, A., and Krock, B. (2013). Morphology, toxicity, and phylogeny of *Alexandrium* (Dinophyceae) species along the coast of China. *Harmful Algae* 27, 68–81. doi: 10.1016/j.hal.2013.05.008

Hii, K.-S., Tan, T. H., Leaw, C. P., Chai, H. C., Takata, Y., and Lim, P. T. (2012). "Occurence of three Alexandrium species, *A. affine*, *A. tamutum* and *A. tamiyavanichii* in kuching waters," in *Sustaining Tropical Natural Resources Through Innovations, Technologies and Practice*, *Proceedings NTrop4*, eds M. E.

Wasli, H. Sani, F. B. Ahmed, S. L. P. Mohamad Teen, L. K. Soon, and M. Sidi (Sarawaka), 165–171.

Hou, Z., and Li, S. (2018). Tethyan changes shaped aquatic diversification. *Biol. Rev.* 93, 874–896. doi: 10.1111/brv.12376

John, U., Fensome, R. A., and Medlin, L. K. (2003). The application of a molecular clock based on molecular sequences and the fossil record to explain biogeographic distributions within the *Alexandrium tamarense* "species complex"(Dinophyceae). *Mol. Biol. Evol.* 20, 1015–1027. doi: 10.1093/molbev/msg105

John, U., Litaker, R. W., Montresor, M., Murray, S., Brosnahan, M. L., and Anderson, D. M. (2014). Formal revision of the *Alexandrium tamarense* species complex (Dinophyceae) taxonomy: the introduction of five species with emphasis on molecular-based (rDNA) classification. *Protist* 165, 779–804. doi: 10.1016/j.protis.2014.10.001

Juliano, V. B., and Garcia, V. M. T. (2006). Cysts of potentially harmful dinoflagellates, with emphasis of the genus *Alexandrium*, in sepetiba bay (Brazil) during a port survey of Globallast. *Afr. J. Mar. Sci.* 28, 299–303. doi: 10.2989/18142320609504166

Karydis, M., and Kitsiou, D. (2012). Eutrophication and environmental policy in the Mediterranean Sea: a review. *Environ. Monit. Assess.* 184, 4931–4984. doi: 10.1007/s10661-011-2313-2

Kodama, M. (2010). Paralytic shellfish poisoning toxins: biochemistry and origin. *Aqua Biosci. Monogr.* 3, 1–38. doi: 10.5047/absm.2010.00301.0001

Kon, N. F., Hii, K. S., Tan, T. H., Yek, L. H., Lim, P. T., and Leaw, C. P. (2013). First record of marine dinoflagellate, *Alexandrium tamutum* (Dinophyceae) from Malaysia. *Malays. J. Sci.* 32, 81–88. doi: 10.22452/mjs.vol32no3.7

Kumar, S., Stecher, G., and Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33, 1870–1874. doi: 10.1093/molbev/msw054

Larsen, J., and Nguyen-Ngoc, L. (2004). Potentially toxic macroalgae of Vietnamese waters. *Opera Bot.* 140, 5–216.

Lebret, K., Kritzberg, E. S., Figueroa, R., and Rengefors, K. (2012). Genetic diversity within and genetic differentiation between blooms of a microalgal species. *Environ. Microbiol.* 14, 2395–2404. doi: 10.1111/j.1462-2920.2012.02769.x

Lilly, E. L., Halanych, K. M., and Anderson, D. M. (2005). Phylogeny, biogeography, and species boundaries within the *Alexandrium minutum* group. *Harmful Algae* 4, 1004–1020. doi: 10.1016/j.hal.2005.02.001

Lilly, E. L., Halanych, K. M., and Anderson, D. M. (2007). Species boundaries and global biogeography of the *Alexandrium tamarense* complex (Dinophyceae). *J. Phycol.* 43, 1329–1338. doi: 10.1111/j.1529-8817.2007.00420.x

Litaker, R. W., Vandersea, M. W., Kibler, S. R., Reece, K. S., Stokes, N. A., Steidinger, K. A., et al. (2003). Identification of *Pfiesteria piscicida* (Dinophyceae) and *Pfiesteria*-like organisms using internal transcribed spacer-specific PCR assays. *J. Phycol.* 39, 754–761. doi: 10.1046/j.1529-8817.2003.02112.x

Logares, R., Bråte, J., Bertilsson, S., Clasen, J. L., Shalchian-Tabrizi, K., and Rengefors, K. (2009). Infrequent marine-freshwater transitions in the microbial world. *Trends Microbiol.* 17, 414–422. doi: 10.1016/j.tim.2009.05.010

Logares, R., Rengefors, K., Kremp, A., Shalchian-Tabrizi, K., Boltovskoy, A., Tengs, T., et al. (2007). Phenotypically different microalgal morphospecies with identical ribosomal DNA: a case of rapid adaptive evolution. *Microb. Ecol.* 53, 549–561. doi: 10.1007/s00248-006-9088-y

Lu, D., Qi, Y., Gu, H., Dai, X., Wang, H., Gao, Y., et al. (2014). Causative species of harmful algal blooms in Chinese coastal waters. *Algol. Stud.* 145, 145–168. doi: 10.1127/1864-1318/2014/0161

MacKenzie, L., de Salas, M., Adamson, J., and Beuzenberg, V. (2004). The dinoflagellate genus Alexandrium (Halim) in New Zealand coastal waters: comparative morphology, toxicity and molecular genetics. *Harmful Algae* 3, 71–92. doi: 10.1016/j.hal.2003.09.001

Matsuyama, Y., Nishitani, G., and Nagai, S. (2010). "Direct detection of harmful algae from the oyster spat and live fish transporting trailers," in *Proceedings of XIII International Conference on Harmful Algae*, eds M. Kin-Chung Ho, J. Zhou and Y. Z. Qi (Hong Kong), 185–189.

Menezes, M., Branco, S., Proença, L. A. O., and Schramm, M. A. (2007). Bloom of *Alexandrium minutum* Halim on Rio de Janeiro coast: occurrence and toxicity. *Harmful Algae News* 34, 7–9.

Menezes, M., Varela, D., de Oliveira Proença, L. A., da Silva Tamanaha, M., and Paredes, J. (2010). Identification of the toxic alga *Alexandrium tamiyavanichi* (Dinophyceae) from Northeastern Brazil: acombined morphological and rDNA sequence (partial lsu and its) approach. *J. Phycol.* 46, 1239–1251. doi: 10.1111/j.1529-8817.2010.00918.x

Miranda, L. N., Zhuang, Y., Zhang, H., and Lin, S. (2012). Phylogenetic analysis guided by intragenomic SSU rDNA polymorphism refines classification of "*Alexandrium tamarense*" species complex. *Harmful Algae* 16, 35–48. doi: 10.1016/j.hal.2012.01.002

Montresor, M., John, U., Beran, A., and Medlin, L. K. (2004). *Alexandrium tamutum* sp. *nov. (Dinophyceae):* a new nontoxic species in the genus Alexandrium. *J. Phycol.* 40, 398–341. doi: 10.1111/j.1529-8817.2004.03060.x

Nagai, S., Lian, C. L., Yamaguchi, S., Hamaguchi, M., Matsuyama, Y., Itakura, S., et al. (2007). Microsatellite markers reveal population genetic structure of the toxic dinoflagellate *Alexandrium tamarense* (Dinophyceae) in Japanese coastal waters. *J. Phycol.* 43, 43–54. doi: 10.1111/j.1529-8817.2006.00304.x

Nagai, S., Yoshida, G., and Tarutani, K. (2011). "Changes of species composition and distribution of algae in coastal waters of western Japan," in *Global Warming Impacts - Case Studies on the Economy, Human Health, and on Urban and Natural Environments,* ed S. Casalegno (Rijeka: InTech-Open Access Publisher), 209–235.

Natsuike, M., Saito, R., Fujiwara, A., Matsuno, K., Yamaguchi, A., Shiga, N., et al. (2017). Evidence of increased toxic *Alexandrium tamarense* dinoflagellate blooms in the eastern Bering Sea in the summers of 2004 and 2005. *PLoS ONE* 12:e0188565. doi: 10.1371/journal.pone.0188565

Odebrecht, C., Mendéz, S., and Garcia, V. M. T. (1997). "Oceanographic processes and HAB in the subtropical southwestern Atlantic (28–368 S)," in *Abstracts of the 8th International Conference on Harmful Algae*, eds B. Reguera, J. Blanco, M. L. Fernández, and T. Wyatt (Vigo), 152.

Omachi, C. Y., Tamanaha, M. D. S., and Proença, L. A. O. (2007). Bloom of *Alexandrium fraterculus* in coastal waters off Itajaí, SC, Southern Brazil. *Braz. J. Oceanog.* 55, 57–61. doi: 10.1590/S1679-87592007000100007

Orlova, T. Y., Selina, M. S., Lilly, E. L., Kulis, D. M., and Anderson, D. M. (2007). Morphogenetic and toxin composition variability of *Alexandrium tamarense* (Dinophyceae) from the east coast of Russia. *Phycologia* 46, 534–548. doi: 10.2216/06-17.1

Penna, A., Fraga, S., Masa, M., Giacobbe, M. G., Bravo, I., Vila, M., et al. (2008). Phylogenetic relationships among the Mediterranean *Alexandrium* (Dinophyceae) species based on sequences of 5.8S gene and internal transcribed spacers of the rRNA operon. *Eur. J. Phycol.* 43, 163–178. doi: 10.1080/09670260701783730

Penna, A., Garcés, E., Vila, M., Giacobbe, M. G., Fraga, S., Lugli,è, A., et al. (2005). *Alexandrium catenella* (Dinophyceae), a toxic ribotype expanding in the NW Mediterranean Sea. *Mar. Biol.* 148, 13–23. doi: 10.1007/s00227-005-0067-5

Persich, G. R., and Garcia, V. M. (2003). Ocorrência de cistos de dinoflagelados, com ênfase em espécies potencialmente nocivas, no sedimento próximo à desembocadura da Laguna dos Patos (RS). *Atlântica (Rio Grande)* 25, 123–133.

Persich, G. R., Kulis, D. M., Lilly, E. L., Anderson, D. M., and Garcia, V. M. (2006). Probable origin and toxin profile of *Alexandrium tamarense* (Lebour) Balech from southern Brazil. *Harmful Algae* 5, 36–44. doi: 10.1016/j.hal.2005.04.002

Pfennig, D. W., and Pfennig, K. S. (2010). Character displacement and the origins of diversity. *Am. Nat.* 176(Suppl. 1), S26–44. doi: 10.1086/657056

Pigliucci, M., Murren, C. J., and Schlichting, C. D. (2006). Phenotypic plasticity and evolution by genetic assimilation. *J. Exp. Biol.* 209, 2362–2367. doi: 10.1242/jeb.02070

Prud'homme van Reine, W. F. (2017). Report of the nomenclature committee for algae: 15. *Taxon* 66, 191–192. doi: 10.12705/661.16

Rhodes, L., and Munday, R. (2016). "Harmful algae and their commercial implications," in *Algae Biotechnology*, eds F. Bux and Y. Chisti (Cham: Springer), 301–315. doi: 10.1007/978-3-319-12334-9_15

Ronquist, F., Teslenko, M., Mark, P., Ayres, D. L., Darling, A., Höhna, S., et al. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 61, 539–542. doi: 10.1093/sysbio/sys029

Ruiz-Sebastián, C., Etheridge, S. M., Cook, P. A., Oryan, C., and Pitcher, G. C. (2005). Phylogenetic analysis of toxic Alexandrium (Dinophyceae) isolates from South Africa: implications for the global phylogeography of the *Alexandrium tamarense* species complex. *Phycologia* 44, 49–60. doi: 10.2216/0031-8884(2005)44[49:PAOTAD]2.0.CO;2

Sato, S., Nishimura, T., Uehara, K., Sakanari, H., Tawong, W., et al. (2011). Phylogeography of *Ostreopsis* along West Pacific coast, with special reference to a novel clade from Japan. *PLoS ONE* 6:e27983. doi: 10.1371/journal.pone.0027983

Scholin, C. A., Herzog, M., Sogin, M., and Anderson, D. M. (1994). Identification of group- and strain-specific genetic markers from globally distributed *Alexandrium* (Dinophyceae). II. Sequence analysis of fragments of the LSU rRNA gene. *J. Phycol.* 30, 999–1011. doi: 10.1111/j.0022-3646.1994.00999.x

Selina, M. S., and Morozova, T. V. (2005). First records of dinoflagellates *Alexandrium margalefi* Balech, 1994 and *A. tamutum* Montresor, Beran et John, 2004 in the seas of the Russian Far East. *Russ. J. Mar. Biol.* 31, 187–191. doi: 10.1007/s11179-005-0066-x

Sierra-Beltrán, A. P., Cruz-Villacorta, A., Núñez-Vázquez, Del Villar, L. M., Cerecero, J. J., and Ochoa, J. L. (1998). An overview of the marine food-poisoning in Mexico. *Toxicon* 36, 1493–1502. doi: 10.1016/S0041-0101(98)00139-1

Silva, N. J., Tang, K. W., and Lopes, R. M. (2013). Effects of microalgal exudates and intact cells on subtropical marine zooplankton. *J. Plank. Res.* 35, 855–865. doi: 10.1093/plankt/fbt026

Sunday, J. M., Calosi, P., Dupont, S., Munday, P. L., Stillman, J. H., and Reusch, T. B. H. (2014). Evolution in an acidifying ocean. *Trends Ecol. Evol.* 29, 117–125. doi: 10.1016/j.tree.2013.11.001

Swan, S. C., and Davidson, K. (2014). "*Alexandrium* and risk management within the Scottish phytoplankton monitoring programme," in: *Marine and Freshwater Harmful Algae 2014. Proceedings of the 16th International Conference on Harmful. Algae*, eds A. Lincoln MacKenzie [Cawthron Institute, Nelson, New Zealand and the International Society for the Study of Harmful Algae (ISSHA)].

Tillmann, U., Krock, B., Alpermann, T. J., and Cembella, A. (2016). Bioactive compounds of marine dinoflagellate isolates from western Greenland and their phylogenetic association within the genus *Alexandrium*. *Harmful Algae* 51, 67–80. doi: 10.1016/j.hal.2015.11.004

Touzet, N., Franco, J. M., and Raine, R. (2008). Morphogenetic diversity and biotoxin composition of *Alexandrium (*Dinophyceae) in Irish coastal waters. *Harmful Algae* 7, 782–797. doi: 10.1016/j.hal.2008.04.001

Wang, D.-Z. (2008). Neurotoxins from marine dinoflagellates: a brief review. *Mar. Drugs* 6, 349–371. doi: 10.3390/md6020349

Wang, D.-Z., Lin, L., Gu, H.-F., Chan, L. L., and Hong, H.-S. (2008). Comparative studies on morphology, ITS sequence and protein profile of *Alexandrium tamarense* and *A. catenella* isolated from the China Sea. *Harmful Algae* 7, 106–113. doi: 10.1016/j.hal.2007.06.002

Wang, L., Zhuang, Y., Zhang, H., Lin, X., and Lin, S. (2014). DNA barcoding species in *Alexandrium tamarense* complex using ITS and proposing designation of five species. *Harmful Algae* 31, 100–113. doi: 10.1016/j.hal.2013.10.013

Yoshida, M. (2002). *Alexandrium* spp. (Dinophyceae) in the western North Pacific. *Fish Sci.* 68, 511–514.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Menezes, Branco, Miotto and Alves-de-Souza. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Marine Science

ORIGINAL RESEARCH
published: 04 December 2018
doi: 10.3389/fmars.2018.00454

Mass Mortality of Cultivated Northern Bluefin Tuna *Thunnus thynnus orientalis* Associated With *Chattonella* Species in Baja California, Mexico

Ernesto García-Mendoza[1], Jorge Cáceres-Martínez[2], David Rivas[1], Michelle Fimbres-Martinez[1], Yaireb Sánchez-Bravo[1], Rebeca Vásquez-Yeomans[3] and Jennifer Medina-Elizalde[4]*

[1] Departamento de Oceanografía Biológica, Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, Mexico, [2] Departamento de Acuicultura, Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, Mexico, [3] Instituto de Sanidad Acuícola, A.C. (ISA), Ensenada, Mexico, [4] Departamento de Biotecnología Marina, Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, Mexico

OPEN ACCESS

Edited by:
Marius Nils Müller,
Federal University of Pernambuco, Brazil

Reviewed by:
Raphael M. Kudela,
University of California, Santa Cruz, United States
Chui Pin Leaw,
University of Malaya, Malaysia
Kazuhiro Aoki,
Japan Fisheries Research and Education Agency (FRA), Japan

***Correspondence:**
Ernesto García-Mendoza
ergarcia@cicese.mx

Specialty section:
This article was submitted to Marine Biogeochemistry, a section of the journal Frontiers in Marine Science

Received: 05 August 2018
Accepted: 13 November 2018
Published: 04 December 2018

Citation:
García-Mendoza E, Cáceres-Martínez J, Rivas D, Fimbres-Martinez M, Sánchez-Bravo Y, Vásquez-Yeomans R and Medina-Elizalde J (2018) Mass Mortality of Cultivated Northern Bluefin Tuna Thunnus thynnus orientalis Associated With Chattonella Species in Baja California, Mexico. Front. Mar. Sci. 5:454. doi: 10.3389/fmars.2018.00454

In 2016 a mass mortality period (MMP) of cage cultured tuna occurred in the northwest coast of Baja California, Mexico. Nine die-offs occurred from May 31st to August 2nd in Todos Santos Bay, Salsipuedes Bay and Coronado Islands. The organisms were disoriented, gasping, swimming erratically, and died hours after these signs were detected. Necropsies and histopathological analyses were performed on dead organisms. Abundant mucus and congestion was observed in the gills. Histopathological analysis of the gills showed hyperplasia, fusion of gill filaments and lamellae, telangiectasia, edemas, increased numbers of mucus cells, and in some cases severe hemorrhage. Water samples were analyzed and a sampling campaign was implemented in some cultivation areas to evaluate the presence of ichthyotoxic microalgae. *Chattonella* spp. (mainly *C.* cf. *marina*) were detected in the water column during the MMP. At the end of May abundances of 5×10^3 cells L^{-1} were detected in sea surface samples and *Chattonella* spp. represented ∼20% of the microphytoplankton community. Abundance of these species at surface increased to 33×10^3 cells L^{-1} in June and represented 85% of the phytoplankton community. No other environmental stressful variables were detected during the MMP. The presence of *Chattonella* spp. in the water column explains the dead of the tuna since behavior, necropsies, and histopathological analyses of the gills indicate a severe reaction to an environmental noxa that could be related to the characteristic toxic effect of these species. Before the MMP, ichthyotoxic species have not been reported in the phytoplankton community of the region. Accumulation of *Chattonella* spp. was probably associated with abnormally high temperatures present during the two previous years before the MMP. Surface temperature anomalies of 3°C were registered during 2015. Mesoscale oceanographic and atmospheric phenomena brought the environmental conditions for a change in the

phytoplankton community in the region. Phytoplankton biomass was low and associated with a decrease in the abundance of diatoms and dinoflagellates. The absence of diatoms together with upwelling events followed by stratification before the MMP probably favored the accumulation of *Chattonella* spp. that affected importantly tuna ranching activities in Northwest Baja California.

Keywords: ichthyotoxic species, *Chattonella marina*, environmental noxa, gill damage, El Niño

INTRODUCTION

Harmful algal blooms (HABs) can affect economically important coastal activities. Among these, marine aquaculture is highly susceptible to HABs. Phycotoxin accumulation and mass mortalities are the two major noxious aspects of HABs on cultivated organisms. From an economic perspective, massive mortalities of fish is the main negative effect of HABs to marine aquaculture (Lewitus et al., 2012; Hallegraeff et al., 2017). Mortalities are associated with different causes but mainly with the presence of marine fish-killing microalgae species. For example, in 2016 more than 40,000 t of cultivated salmon in Chile were affected by the presence of the microalga *Pseudochattonella* cf. *verruculosa* (Clement et al., 2016; León-Muñoz et al., 2018). This is the most extraordinary mass mortality event of cultivated fish ever recorded, referred as the "Godzilla Red Tide" that caused an estimated loss of over 800M USD (Hallegraeff et al., 2017).

Ichthyotoxic microalgae belong to different taxonomic groups. Dinoflagellates, cyanobacteria, raphydophytes, chrysophytes, dictyochophytes (Silicoflagellates) have been implicated in wild and cultivate massive fish mortalities (Kim et al., 2007; Aoki et al., 2012; Imai and Yamaguchi, 2012; Kudela and Gobler, 2012). The rhaphydophytes is one of the most important group of ichthyotoxic microalgae that has caused significant economic losses worldwide. Mortalities have been associated mainly with blooms of *Chattonella* and *Heterosigma akashiwo*. This latter species has affected fish aquaculture principally in northern latitudes, while *Chattonella* species are distributed in tropical waters (Imai and Yamaguchi, 2012). In 1980, ∼1,500 t of salmon cultivated in British Colombia were lost due to a *H. akashiwo* bloom (Bruslé, 1995; Lewitus et al., 2012; Esenkulova et al., 2014). The estimated loss was 35M USD (Rensel and Whyte, 2004; Hallegraeff et al., 2017). In 1990, *H. akashiwo* was again associated with another mass mortality of cultivated salmon in the same region (Black et al., 1991). *Chattonella marina* had been implicated in more fish mass mortalities than other raphydophytes species. Particularly, blooms of this microalga have affected importantly yellowtail finfish (*Seriola quinqueradiata*) aquaculture in Japan and Korea. The first registered mass mortality occurred in 1973 in the Japanese Seto Inland Sea (Imai et al., 1991). The estimated loss was 90M USD (Imai and Yamaguchi, 2012). In the 1970s and mid1980s, *C. marina* blooms caused other mass mortalities of yellowtail finfish (Imai et al., 2006). This species was also associated with a mass mortality of captive southern bluefin tuna *Thunnus maccoyii* in Australia (Munday and Hallegraeff, 1998). In April 1996, ∼1700 t of captive tuna died in Boston Bay, South Australia (Munday and Hallegraeff, 1998). The association of the fish mortality with the presence of *Chattonella* was unclear since the microalga was present at much lower abundances (maximum 66×10^3 cells L^{-1}) than the ones reported when yellowtail finfish mortalities occur. Mortalities of this species occurs when cell abundances are in the order of millions per liter (Imai and Yamaguchi, 2012). However, microalgal toxicosis due to *C. marina* was the most plausible explanation for the tuna die-off (Munday and Hallegraeff, 1998). This is the only report of a negative impact to the tuna ranching industry by an ichthyotoxic microalga.

Tuna cultivation of northern bluefin tuna (*Thunnus thynnus orientalis*) is an important aquaculture activity in Mexico. Tuna ranching was established in 2002 in northern Baja California and three companies operate currently close to Todos Santos bay. Cultivated tuna represents more than 90% of the aquaculture production of Baja California and it is the second largest (behind shrimp production) aquaculture activity in terms of revenue in Mexico. Annual production of the last 5 years fluctuated around 6,000 t per year (*Boletines Estadísticos de la producción pesquera y acuícola de Baja California*; www.sepescabc.gob.mx). In summer of 2016, this industry experienced a period of mass mortalities of organisms maintained in different areas in northern Baja California. First mass mortalities were registered in May 31 to June 2 (**Figure 1**). Eighty percent of the biomass of some culture pens located in Punta Banda was affected (Personal communication; Baja Aquafarms Co.). In June 14, a second outbreak occurred in Salsipuedes Bay followed by another mass mortality in Punta Banda after 2 days (**Figures 1**, **2** for the location of the cultivation areas). Some cultivation pens were relocated from Todos Santos Bay to Coronado Islands as a response to the registered events. However, mortalities also occurred in this location at the end of July. In total, nine die-offs were registered at different locations from May 30 to August 4 (**Figure 1**) hereafter, referred as the mass mortality period (MMP). This phenomenon has never been recorded before in the region. We implemented a sampling campaign during the MMP to identify the causes of the tuna mass mortalities. Water samples and hydrographic variables were analyzed. In addition, analyses on affected organisms were conducted. Here, we report that the tuna disease causative agent was raphydophyte species of the genus *Chattonella*, mainly *C. marina.* The presence of these species was probably associated with abnormal environmental conditions present in the region before the MMP. In addition, we describe the behavior and lesions of affected organisms caused by these microalgae.

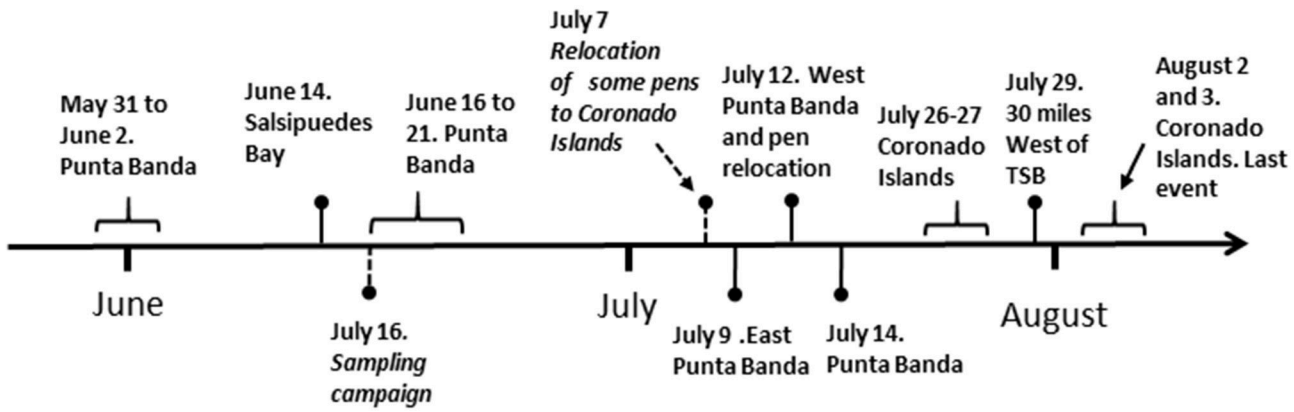


FIGURE 1 | Time line of the events during the period of occurrence of mass mortalities of northern bluefin tuna *Thunnus thynnus orientalis* cultivated in the northwest coast of Baja California, Mexico.

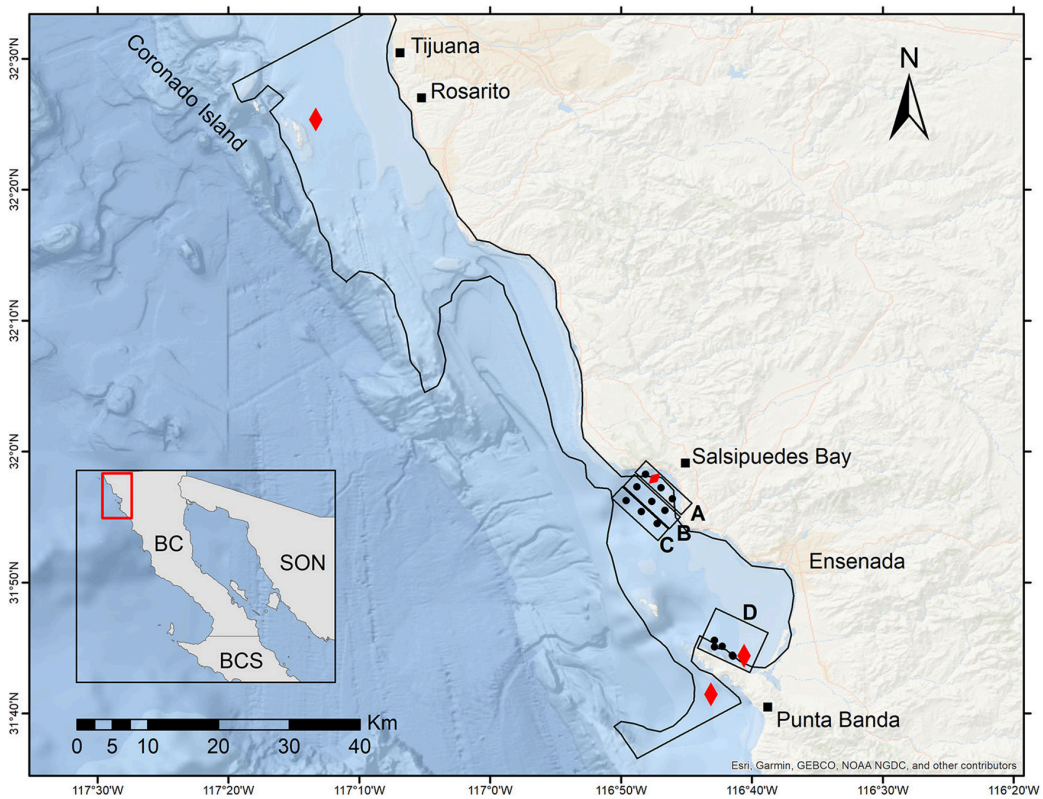


FIGURE 2 | Location of tuna production areas (red symbols) in the northwest coast of Baja California, Mexico. The round closed symbols represent the stations covered during the sampling campaign implemented the 16 of June during the mass mortality period. The coastal (line **A**) and offshore (lines **B** and **C**) stations are indicated in the map. In addition, stations in Punta Banda area are indicated **(D)**. The black line delimits the integration area *A* used in the calculation of *VTN*.

MATERIALS AND METHODS

Description of the Study Area and Location of the Cultivation Zones

The study area is located in Todos Santos Bay (TSB) region on the Baja California Peninsula (31° 52′ N, 116° 37′ W). The bay has two mouths, which communicate with the Pacific Ocean. Two islands, called Todos Santos, divide the two communication mouths of the bay. Seventy five percent of the area presents depths <50 m and 25% is part of a narrow submarine canyon located between Punta Banda peninsula and Todos Santos Islands (**Figure 2**). TBS is an area influenced by the California Current (CC) that transports water southward. Additionally, wind forcing causes coastal upwelling with a marked seasonal cycle (Durazo, 2009). The concessions for cage-cultivation of tuna are located mainly in Todos Santos Bay region (Punta Banda and Salsipuedes Bay). A systematic sampling was implemented in these locations (**Figure 2**).

Sample Campaign (June 16) and Data Collection

The sampling campaign was conducted aboard the research boat Rigel of the Center for Scientific Research and Higher Education at Ensenada (CICESE). Water samples were collected at 16 stations in TSB region. At each station, temperature, salinity, and pressure were measured with a continuous temperature, salinity depth profiler (Sontek, CastAway CTD). The water samples were collected at different depths with a 5-L Niskin bottle.

Phytoplankton Community Evaluation and Pigment Analysis

Of the water collected with the Niskin bottles, a 50 mL aliquot was placed in amber bottles (Nalgene type). The samples were fixed with a solution of paraformaldehyde, glutaraldehyde 1% with HEPES buffer (pH 7.4) and sucrose, as described by Katano et al. (2009). This solution preserved microalgae species with ichthyotoxic potential. The phytoplankton community was analyzed using the Utermöhl method (Sournia, 1978). Ten, 25, or 50 mL were sedimented and the complete area of the sedimentation chamber was analyzed with an inverted light microscope (Leica DM3000 model). Diatoms, dinoflagellates, raphidophytes, and other phytoplankton groups were identified up to genus level and in some cases to species level according to Tomas (1997) and Omura et al. (2012). Abundance is reported in cells L^{-1}. In addition, the presence of *Chattonella* species is expressed as relative abundances in relation of the total abundance of the microphytoplankton community.

One liter of water was vacuum filtered to determine the concentration of phytoplankton pigments. The water was passed through GF/F (Whatman) glass fiber filters. The analysis of the pigments was performed by high-performance liquid chromatography (HPLC). The method used was the one described by Van-Heukelem and Thomas (2001) and modified by Almazán-Becerril and García-Mendoza (2017).

Analyses on Affected Organisms

Histological Procedures

During the MMP, 14 tunas (136.6 ± 19.5 cm of total length) were analyzed. Moribund animals were captured directly from the cage nets and sacrificed *in situ*. Pieces of gills were excised and fixed in 10% neutral buffered formalin, placed in histological cassettes and send to the laboratory. The tissues were decalcified with tetrasodium ethylenediaminotetracetate (EDTA) for a period of 3 to 10 days. The gills were dehydrated in an ethyl alcohol series of ascending concentrations, embedded in paraffin and sectioned at 5 mm. The tissue sections were stained with haematoxilin-eosin (HE), 3 sections of each tissue from each fish were examined by light microscope. The presence of histological alterations was recorded and photographed.

Analysis of Environmental Variables

Regional-Environmental Indicators

Long-term series of monthly anomalies of several physical and biological fields were constructed in order to describe the interannual variability of the environmental conditions close to the study area. The period for these series was from the year 2003 through year 2017, coincident with the availability of the satellite imagery used in this analysis. For all the variables, which are described below, those data points located over the continental shelf (shoreward of the 200-m isobath) and between Punta Banda Peninsula and Coronado Islands (see **Figure 2**) were averaged (or integrated) for each month in the series. A monthly-mean climatology was then calculated and subtracted to the monthly composites to obtain the anomalies for the series.

Satellite Imagery

Monthly composites of satellite-derived products were used in the long-term anomaly series. These products included the 4 km-resolution version of the 4 μ night-time sea surface temperature (SST) and default algorithm (OCI) based chlorophyll-*a* (CHL), taken from the Moderate Resolution Imaging Spectroradiometer (MODIS)—Aqua product. These data were obtained from the National Aeronautics and Space Administration (NASA) OceanColor Web: http://oceancolor.gsfc.nasa.gov.

Coastal Upwelling Index

To analyze the variability of the regional upwelling regime, a monthly coastal upwelling index (CUI) was calculated using the wind stress obtained from the 3-h 25 km-resolution wind vector at 10-m height from the North American Regional Reanalysis (NARR; Mesinger et al., 2006) and using parameterizations proposed by Smith (1988). This calculation was done according to the definition used by Bakun (1973) and Schwing et al. (1996), assuming a coast oriented 28° Clockwise from the north.

Numerical Oceanic Model

Numerical-model outputs were also included in the series to obtain information of the conditions of the water column during the anomalies of the surface fields described above.

The numerical model used in this analysis was the regional physical-biogeochemical coupled model used in Cruz-Rico and Rivas (2018) but with some differences (described below). This biogeochemical model is a nutrients-phytoplankton-zooplankton-detritus (NPZD) model based on nitrogen (Powell et al., 2006). The total nitrogen at any point is partitioned between dissolved nitrogen (N), phototrophic phytoplankton (P), the herbivorous zooplankton (Z), and particulate nitrogen (D: Detritus). Major biological processes (i.e., photosynthetic growth and uptake of nitrogen by phytoplankton, grazing on phytoplankton by zooplankton, mortality of both phytoplankton and zooplankton, and sinking and remineralization of detritus), and physical processes (i.e., advection and mixing) affecting the biological components (N, P, Z, D) are included in the biochemical model which runs together with the physical model as one (Powell et al., 2006).

The differences between our model approach and that used by Cruz-Rico and Rivas (2018) are the boundary data and surface forcing. In our approach, monthly data from the Global Ocean Data Assimilation System (GODAS; e.g., Huang et al., 2008; Ravichandran et al., 2013) were used in the model's lateral open boundaries, and daily wind stress calculated from the NARR's wind and monthly heat fluxes from the GODAS were used in the model's free surface. The simulation period was also 2003–2017.

An indicator of the nutrient availability for phytoplankton growth was diagnosed from the numerical-model outputs and the vertical transport of nitrogen through the 50-m depth. This transport was calculated as

$$VTN = \int_A wN \, dA, \tag{1}$$

where w is the vertical velocity, N is the dissolved nitrogen (nitrate), and A is the area of the horizontal plane limited offshore by the 200-m isobath and extending from Todos Santos Bay to Coronado Islands located at 50-m depth; the total area A is 1.546×10^3 km^2.

On the other hand, the Brunt-Väisälä frequency squared (BVF^2) was calculated as an indicator of the stratification of the water column. The maximum of BVF^2 ($maxBVF^2$) and its depth were used to characterize the water-column conditions around the study area.

Teleconnection Index

A climatic index was also compared to the oceanographic variables around the study area in order to explore a possible relation between such variables and the large-scale climatic patterns. The Southern Oscillation Index (SOI) was used as an indicator of the El Niño activity around the study area. As reported in previous papers (e.g., Cruz-Rico and Rivas, 2018), the El Niño phenomenon modulates much of the dynamic over Baja California's shelf. Indeed, in particular for the warm anomaly occurred in the period 2013–2016, many of the effects are attributed to an intense El Niño event (e.g., McClatchie et al., 2016).

RESULTS

Affectation of Organisms, Necropsy and Gill Histopathological Results

The first signs of distress of cultivated tuna were observed in May 31 (**Figure 1**). The organisms were disoriented, swimming slowly, and erratically, not moving in circles as in normal conditions. Some fishes were gasping, the opercula and mouth were open and the most affected organisms crashed against the nets of the pen (**Video S1; Supplementary Material**). The organisms died and sank to the bottom of the pens ∼4 h after these signs were noticed.

Necropsies were performed in different organisms and organs were analyzed macroscopically and histopathologically. Liver and kidneys did not present signs of damage that represented a malfunction of the organs. In contrast, gills of the evaluated organisms were heavily affected. Excessive mucus and excretion of blood was observed in the gills of an organism analyzed in June 15. The observation of mucus under the microscope revealed the presence of microalgae species (**Figure 1**, **Supplementary Material**). Some cells of *Tripos furca* were visible and round or oval cells were also observed that probable were *Chattonella* spp., see below.

Histopathological analysis revealed the presence of diffuse congestion by erythrocytes of branchial lamellae (**Figures 3A,B**). Multifocal telangiectasia, which is the result of rupture of pillar cells in the distal part of the branchial lamellae was also observed and in some areas rupture of lamellae epithelia was evident releasing erythrocytes (**Figures 3C,D**). In some cases, multifocal telangiectasia occupied the entire branchial lamellae showing the magnitude of damage (**Figures 3E,F**). Severe inflammation and hyperplasia of both, gill filaments, and lamellae, including fusion and shortening of lamellae were common recorded (**Figures 4A,B**). In several cases, the total loose of gill lamellae with severe inflammation, hyperplasia, and necrosis were noted (**Figures 4C,D**). Additionally, detachment of lamellae epithelium, consistent with diffuse edema, and vacuolization were observed (**Figures 4E,F**). Hemorrhagic zones were observed over the gills filaments and lamellae (**Figures 5A,B**).

Identification of the Causative Agent

Analyses of water samples from Punta Banda and Salsipuedes Bay started after the report of the first die-off. Samples were collected at different days during the mass mortality period (MMP, **Figure 1**) and to the end of August in 2016. After this month, analysis of samples continued on weekly basis as part of the continuous monitoring programs of Baja Aquafarms Co. and CICESE. The ichthyotoxic microalgae *Chattonella* spp. were present in the tuna cultivation areas during the MMP. The species was identified as *C. marina* according to cell morphology of the organism observed in water samples without any fixative (**Figures 6A,B**). The cells presented a tear (oblong to ovoid) shape morphology and were 35 to 60 μm long. Several chloroplasts arranged close to the cell wall were evident (**Figures 6A,B**). Cells with different morphologies were also present in the samples (**Figures 6C,D**) but the most conspicuous and abundant organism was the

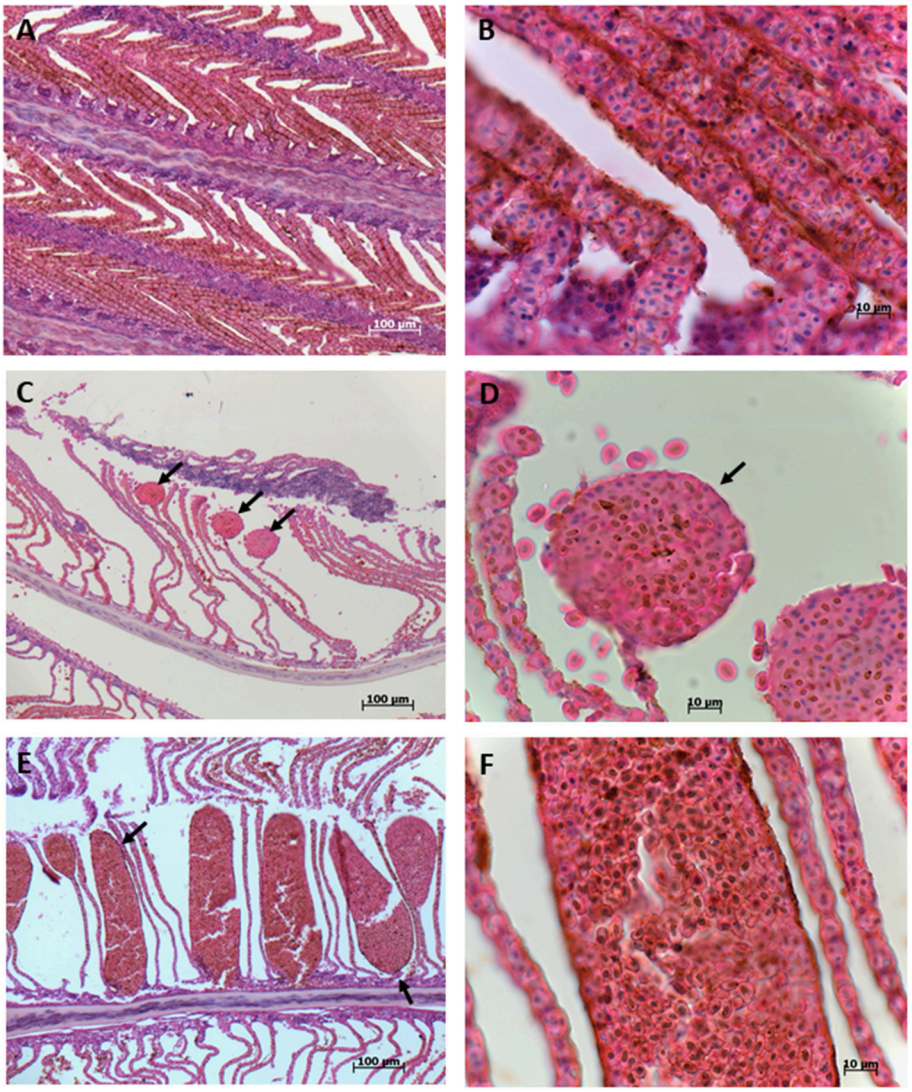


FIGURE 3 | Gills of blue tuna (*Thunnus thynnus orientalis*), exposed to a harmful algal bloom of *Chattonella marina*. **(A)** The presence of diffuse congestion by erythrocytes of the branchial lamellae is observed. **(B)** Detail of erythrocyte accumulation (congestion) of the branchial lamellae. **(C)** Multifocal telangiectasia (arrows), resulted of rupture of pillar cells, in the distal part of branchial lamellae (aneurysm). **(D)** Detail of the top of branchial lamellae telangiectasia (arrow) where release of some erythrocytes is observed, evidencing rupture of lamellae epithelia. **(E)** Large multifocal telangiectasia of entire branchial lamellae plethoric of erythrocytes. **(F)** Close up of erythrocytes accumulated in branchial lamellae, note hemosiderin granules.

one identified as *C. marina*. Raphydophytes are susceptible to some preservatives and cell morphology was affected by the addition of lugol-acetate to the samples (**Figure 6E**). Several fixatives were tested during the monitoring period. Using paraformaldehyde, 1% glutaraldehyde, HEPES and sucrose (Katano et al., 2009) proved to be adequate to preserve the samples with minor alterations of *Chattonella* cells (**Figure 6F**). Although it was possible to identify the organisms as *Chattonella*, the differentiation between morphotypes was not possible in preserved samples. Identification of *Chattonella* species using morphological characteristics is sometimes ambiguous. Therefore, several isolates were established from cells collected during the MMP. Cultures of two strains were established. The strains were identified with the D1/D2 large DNA subnunit sequences as *Chattonella marina var. ovata* (**Figure 6G**) and *C. minima* (**Figure 6H**) (Ahumada-Fierro, 2017). Therefore, different species (or varieties) of *Chattonella* were present during the die-off period and the abundance is presented as *Chattonella* spp.

The abundance of phytoplankton and *Chattonella* spp. in samples collected in Punta Banda and Salsipuedes from the end of May to December 2017 is presented in **Figure 7**. *Chattonella* spp. abundance at the end of May was $\sim 5 \times 10^3$ cells L^{-1} and reached a maximum of 30×10^3 cells L^{-1} in the second week of June (**Figure 7B**). *Chattonella* spp. abundance was higher than 10×10^3 cells L^{-1} from June to the beginning

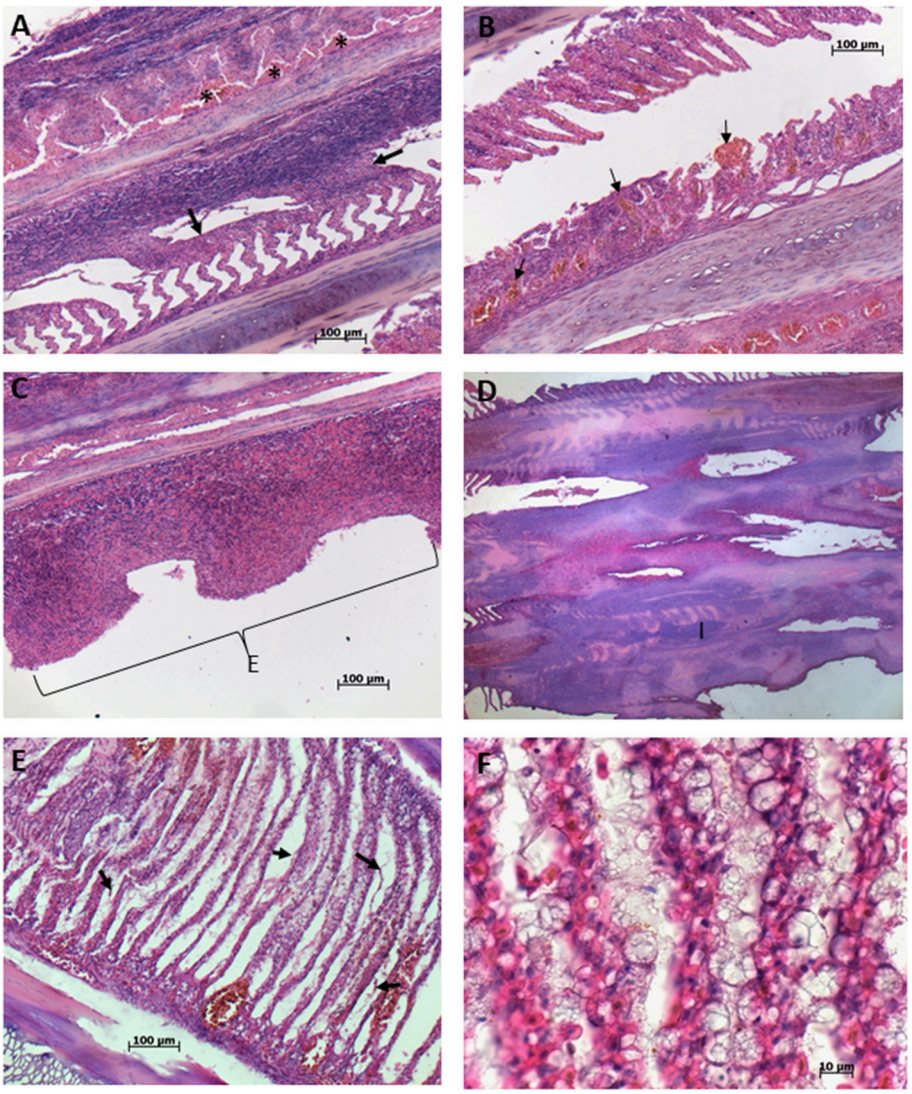


FIGURE 4 | Gills of blue tuna (*Thunnus thynnus orientalis*), exposed to a harmful algal bloom of *Chattonella marina*. **(A)** Severe inflammation and hyperplasia of gill filaments and lamellae, showing fusion of lamellae (arrows), note accumulation of erythrocytes (asterisks) in the middle area of filament. **(B)** Fusion and shortening of gill lamellae with multifocal telangiectasia (arrows). **(C)** Total loose of gill lamellae with severe inflammation and necrosis of the gill filament. **(D)** Severe inflammation, hyperplasia, fusion, and necrosis of filament with almost the totality loose of gill lamellae. **(E)** Detachment of lamellae epithelium (arrow) consistent with diffuse edema and vacuolization. **(F)** Close up of gill lamellae severely vacuolated.

of August, particularly in surface samples (**Figure 7B**). Cell abundance decreased after August and only an abundance above 10×10^3 cells L^{-1} was detected in one surface sample after this month. Low phytoplankton abundances (below 50×10^3 cells L^{-1}) were registered during the period of the appearance of *Chattonella* spp. (**Figure 7A**). Therefore, these species were highly represented in the phytoplankton community. In some samples, relative abundances of *Chattonella* spp. were higher than 60% (maximum relative abundance of 83% by the middle of June) of the microphytoplankton community (**Figure 7C**). These species were not detected or were present at abundances lower than 1×10^3 cells L^{-1} after August. Phytoplankton abundance increased after this month (**Figure 7A**).

Environmental Conditions Associated With the Presence of Chattonella

To characterize the spatial distribution of ichthyotoxic species and environmental variables close to the tuna cultivation areas, a sampling campaign was implemented after the second mass mortality episode that occurred in Salsipuedes bay (**Figure 1**). In June 16, phytoplankton abundance was evaluated in nine sampling stations in this area (**Figure 2**, lines marked as A, B, and C) and in five stations in Punta Banda (**Figure 2**, area D). We found high abundances of *Chattonella* spp. in the surface and close to the coast in Salsipuedes bay. Abundances of ∼30 $\times 10^3$ cells L^{-1} were detected at surface in three stations located between 0.77 and 1.79 miles from the coast (**Figure 8A**).

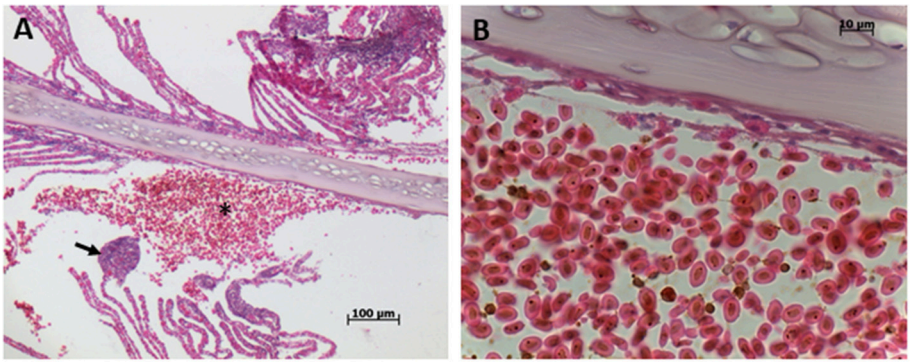


FIGURE 5 | Gills of blue tuna (*Thunnus thynnus orientalis*), exposed to a harmful algal bloom of *Chattonella marina*. **(A)** General view of gill filament with hemorrhagic zone (asterisk) where all lamellae are loosed, note telangiectasia in the distal area of gill lamellae (arrow) of the contiguous filament. **(B)** Detail of hemorrhagic zone plenty of erythrocytes with hemosiderin granules, note necrosis of the basal epithelia close to the cartilage of the filament.

Chattonella abundance decreased to ∼25 × 10^3 cells L^{-1} at 10 m and decreased significantly at 20 m sampling depth in these stations (**Figure 8A**). The abundance of this species at surface decreased to approximately to 10 × 10^3 cells L^{-1} and 5 × 10^3 cells L^{-1} in offshore stations (**Figures 8B,C**). In Punta Banda (**Figure 8D**) *Chattonella* spp abundance was lower than in Salsipuedes Bay. These species were detected from surface to ∼15 m depth (**Figure 8D**).

Phytoplankton pigments were also determined in samples collected during the campaign. CHL, fucoxanthin, violaxanthin, and chlorophyll C2 were highly represented in samples dominated by *Chattonella* spp. (**Figure 2A**, **Supplementary Material**). Pigment concentration was high relative to phytoplankton abundance registered in water samples. CHL concentration was ∼3 µg L^{-1} in samples collected at surface in coastal stations. In these stations, microphytoplankton abundance was lower than 40 × 10^3 cells L^{-1} and *Chattonella* spp. represented ∼80% of this abundance. Therefore, accumulation of CHL was associated with the presence of *Chattonella* spp. in the water column (**Figure 2B**, **Supplementary Material**).

There was not a clear relation between the distribution of *Chattonella* spp. and the thermal structure of the water column during the sampling campaign. Surface temperature was similar (mean of 17.8°C) in all Salsipuedes stations (**Figures 8A–C**). Also, a clear thermal stratification of the water column was not evident between coastal and offshore stations. There was no evident mixed layer and the temperature decreased monotonically with the depth in most of Salsipuedes stations (**Figures 8A–C**). In contrast, a well-defined mixed layer was evident in some stations of Punta Banda (inside TSB). The thermocline was evident at ∼8 m depth in some stations (**Figure 8D**). Also, surface temperature in Punta Banda was higher than in Salsipuedes. The maximum surface temperature was 20.3°C in this area (**Figure 8D**). Under these conditions, *Chattonella* abundances were ∼10 × 10^3 cells L^{-1} from surface to the bottom of the thermocline in Punta Banda (**Figure 8D**).

Chattonella species thrive in warm temperatures and blooms of this species have been reported when water temperature is above 20°C (Edvardsen and Imai, 2006; Imai and Yamaguchi, 2012). The presence of these species and its relation with the thermal conditions was not clear during the sampling campaign. Therefore, we analyzed the variation of the temperature measured continuously *in-situ* at various depths in Punta Banda since 2012. Two short upwelling pulses occurred at the beginning of the MMP (**Figure 9**). Thereafter, surface temperatures increased and ∼23°C occurred in the Punta Banda region during the MMP. In addition, stratification was evident during this period and the 18°C isotherm was detected between 8 and 10 m depth. Only in few occasions, this temperature were registered below 10 m depth in the rest of the year (**Figure 9**). Most notably, temperatures above 18°C were registered from surface to the bottom of the water column in the two previous years of the MMP. Temperatures in the bottom of the water column above 18°C prevailed for ∼5 months, from August to December in 2015 (**Figure 9**). This condition was not registered in other years.

In-situ thermal conditions indicate that abnormally warm years occurred in the region before 2016. The interannual variability of the environmental conditions in the region was analyzed to evaluate the anomaly of the conditions related to the MMP. This period (May-August 2016) was characterized by moderate La Niña conditions. Early 2016 and during the MMP the waters were anomalously warm (∼1–2°C) but colder than in the previous months when the anomalies were higher than 2°C with a maximum exceeding 3°C in October 2015 (**Figure 10B**). During this year and in 2014 intense El Niño conditions were evident (**Figure 10A**). Associated with this conditions, almost 3 years of excessively low levels of CHL were registered in the region. Right after one of its lowest values in April 2016, the CHL levels became nearly normal (**Figure 10C**). In the months prior to the MMP the upwelling activity was intense, especially from December 2015 to March 2016, after that the upwelling was nearly normal with slightly intense short periods (**Figure 10D**). The strong

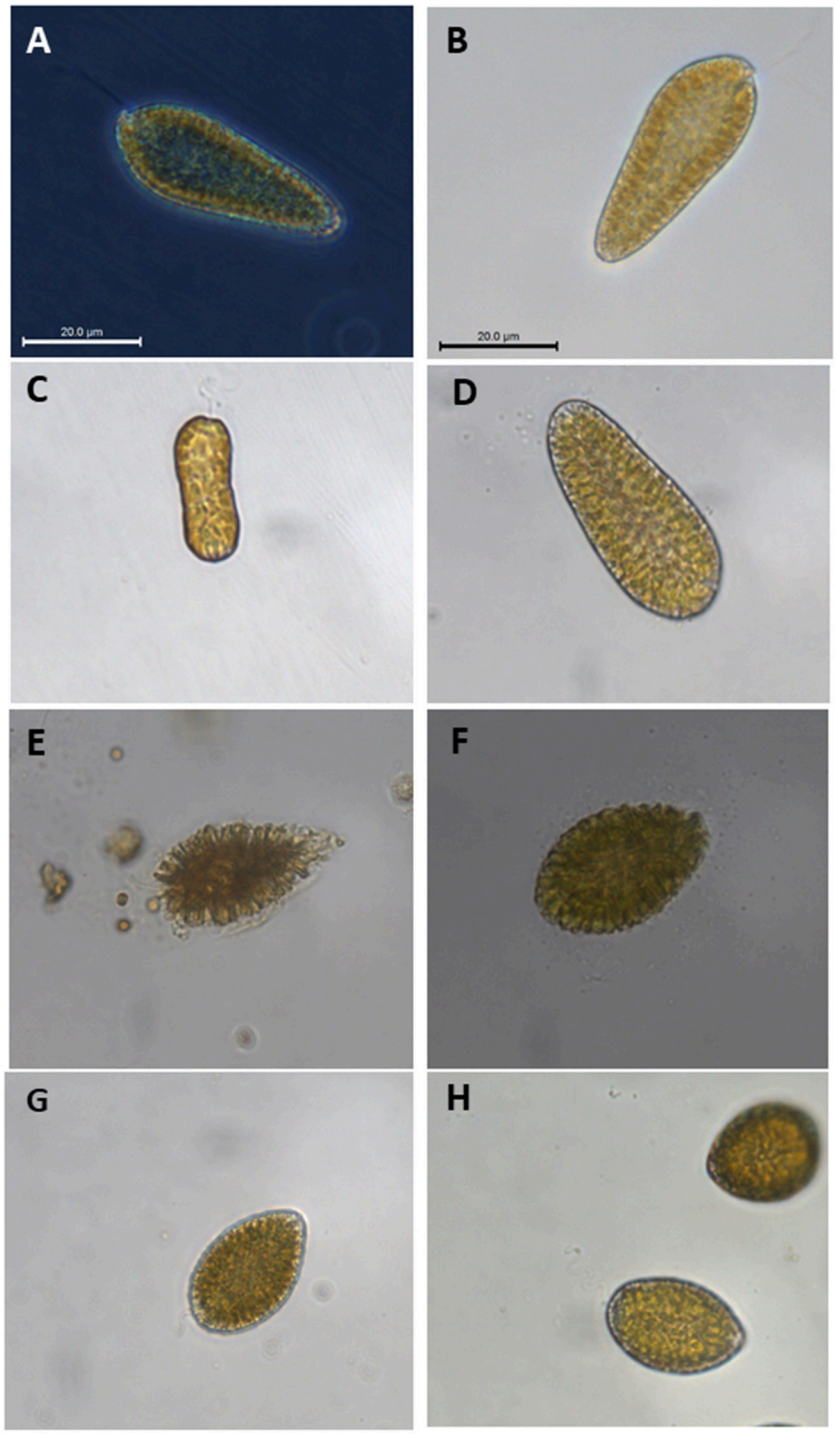


FIGURE 6 | *Chattonella* spp. in water samples collected during the mass mortality period of northern bluefin tuna *Thunnus thynnus orientalis* cultivated in the northwest coast of Baja California, Mexico. Panels **(A–D)** shows cell observed in water samples without any fixative. Addition of Lugol-acetate affected significantly the morphology of the cells **(E)**. Less affectation of *Chattonella* cells was observed **(F)** with paraformaldehyde, 1% glutaraldehyde, HEPES, and sucrose (Katano et al., 2009). Cultivated strains isolated during the (MMP) are also presented: *Chattonella marina var. ovata* **(G)** and *C. minima* **(H)**.

positive upwelling anomaly was related to an intense vertical nutrient supply estimated by numerical-model outputs in the first months of 2016, especially in March, reaching lower values (but still over the normal) in the MMP (**Figure 10E**). From late 2015 (November and December) through the MMP the stratification became stronger, except in July-August 2016, with a thermocline (i.e., depth of the $maxBVF^2$) becoming shallower and then nearly normal (**Figure 10F**). After the MMP, SST, and

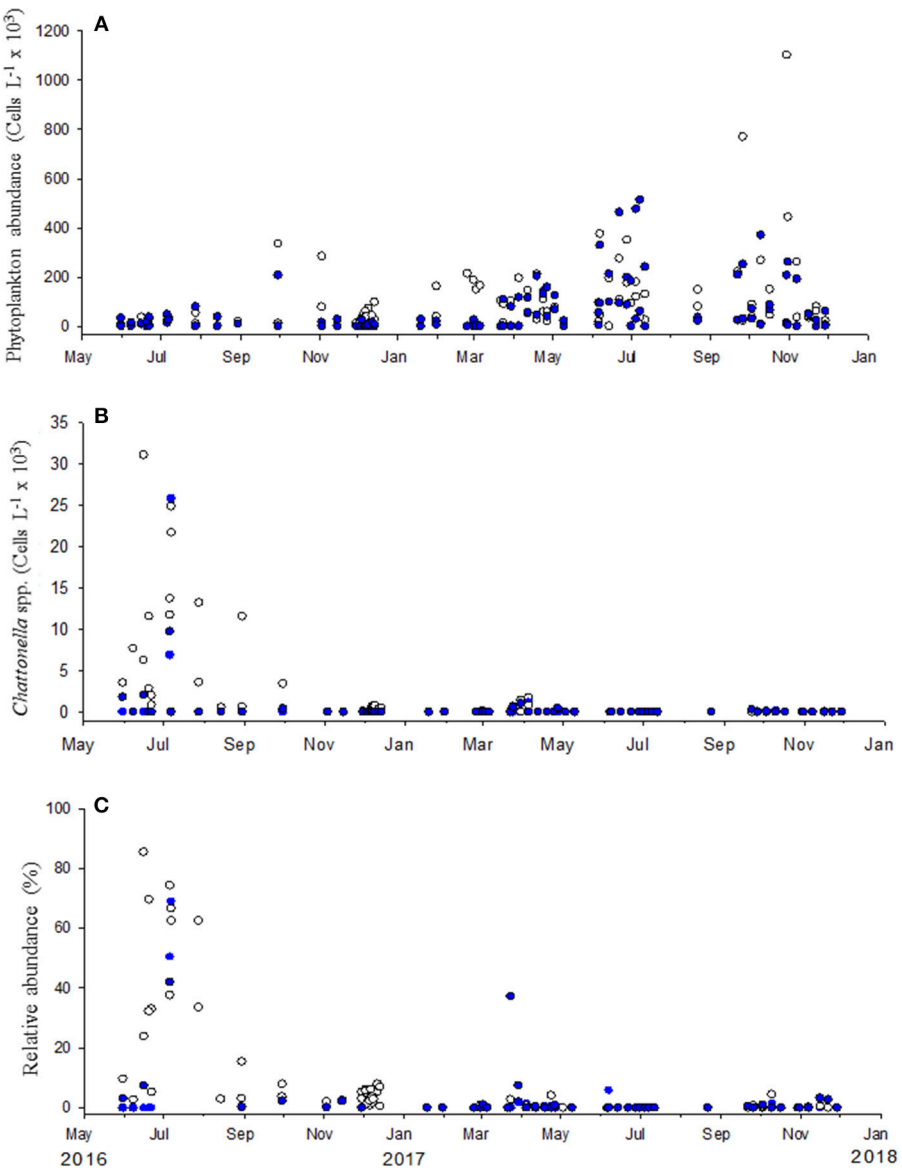


FIGURE 7 | Phytoplankton **(A)** and *Chattonella* spp. **(B)** abundance in samples collected in Punta Banda and Salsipuedes from the end of May to December 2017. The relative abundance of these species is presented in **(C)** Abundances in surface samples are represented by empty symbols and blue circles are abundances detected at 10 m depth.

SOI, CHL concentration were nearly normal, positive or close to the climatological mean and the SOI was nearly normal (**Figure 10**).

DISCUSSION

Here, we document for the first time mass mortalities of cage-reared tuna caused by raphytophytes species of the genus *Chattonella* in Northwest Baja California, Mexico. No other environmental stressful variable was detected during the mass mortality period (MMP). The presence of *Chattonella* species, but most probably *C. marina,* in the water column explains the death of the tuna since behavior of the organisms, and histopathological analyses of the gills indicate a severe reaction to an environmental noxa that could be related to the characteristic toxic effect of these ichthyotoxic raphydophytes.

The gills functions include not only respiration but also osmoregulation and excretion. Since the gills are in contact with the environment, they are particularly sensitive to presence of noxas in the water. The significant morphological changes of the gill tissue of tunas exposed to *C. marina* are characteristic of an exposure of fishes to an acute-type noxa or contaminants (Mallatt, 1985; Godoy, 2016). Changes such as hypertrophy,

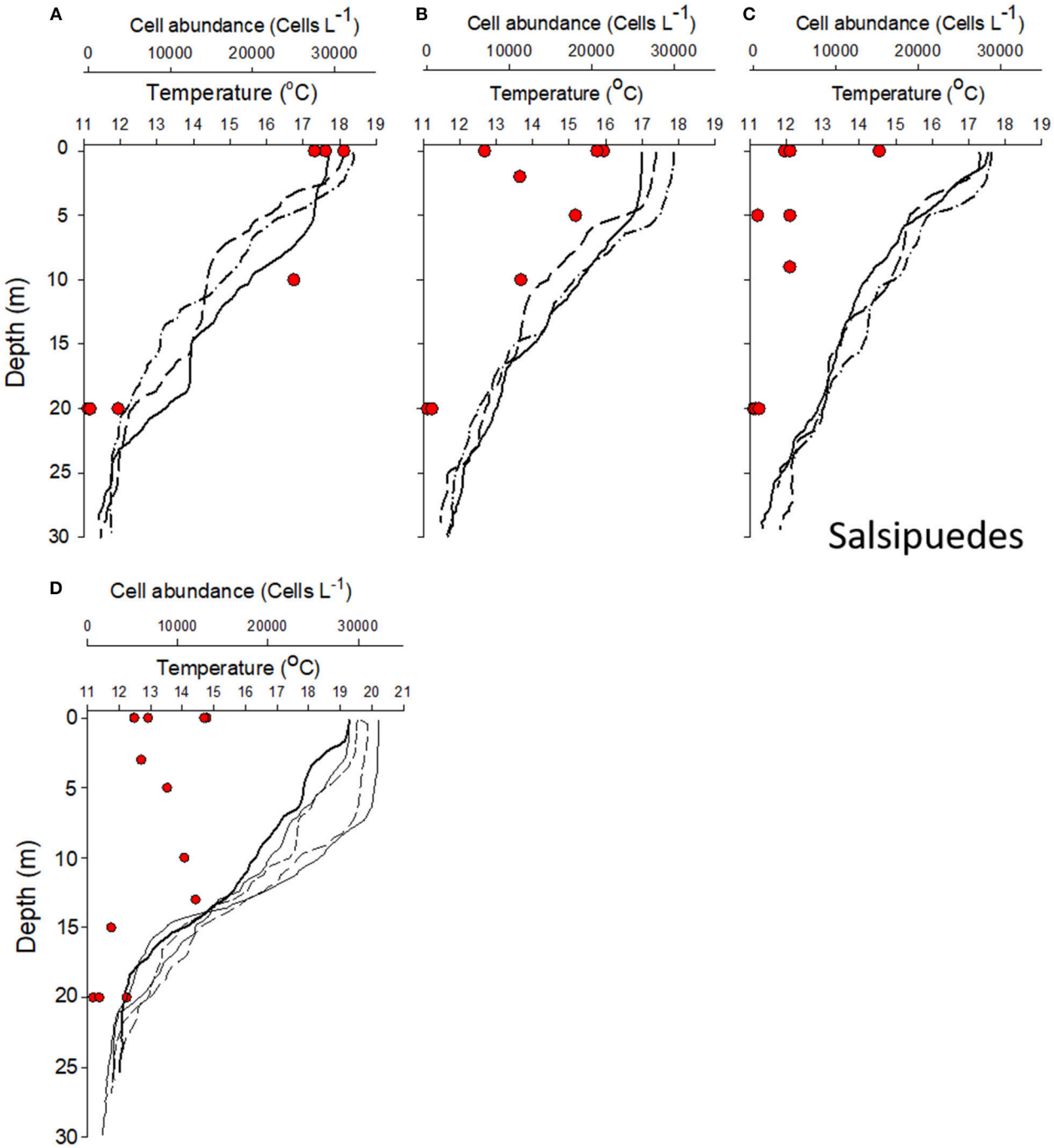


FIGURE 8 | *Chattonella* spp. abundance (in cells L^{-1}, red circles) and temperature distribution (lines) in the water column in tuna cultivation areas after a mass mortality episode that occurred in Salsipuedes Bay. **(A)** presents the abundance and temperature in three stations located close to the coast (see **Figure 2** for the distribution of the stations). **(B,C)** shows the results of samples collected in offshore stations. **(D)** shows the results of registered in Punta Banda area (see Map in **Figure 2** for the localization of sampling stations).

hyperplasia, epithelial lifting, partial fusion of some lamellae of gill epithelium constitute adaptive changes that can be reversed if the external noxa is eliminated. In contrast, other changes are more severe and if are extended, impairment of the normal functioning of the tissue and irreparable damage occurs, even with elimination of the noxa in the water. These changes correspond to lamellar telangiectasis (aneurysm), rupture of epithelial cells with hemorrhages and necrosis

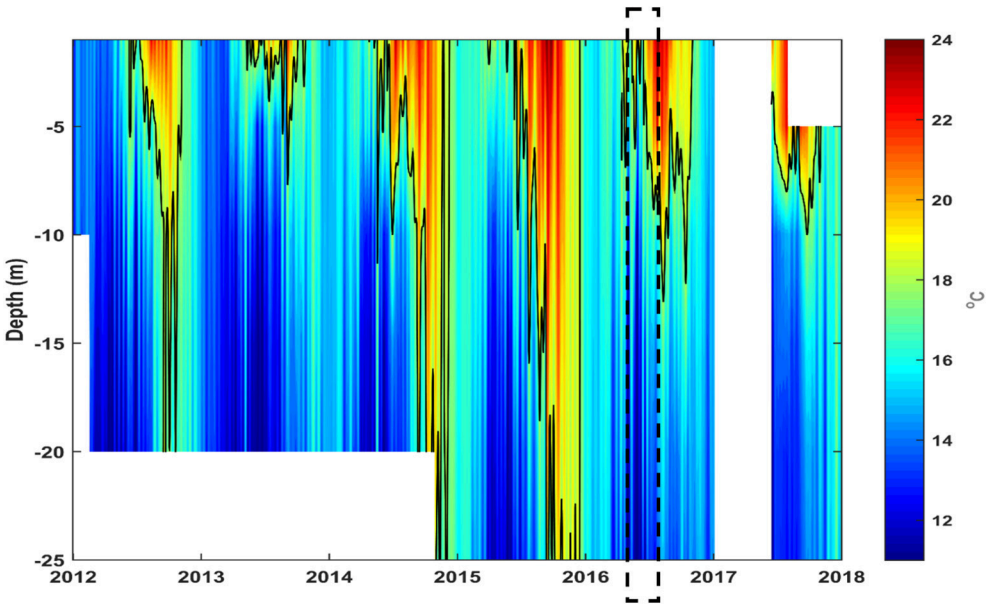


FIGURE 9 | Water temperature measured continuously *in-situ* since 2012 at various depths in Punta Banda cultivation area. The mass mortality period is highlight. The data was obtained with a set of temperature data loggers (HOBO U22-001) placed at different depths in the water column.

(Fernandez and Mazon, 2003; Camargo and Martinez, 2007; Godoy, 2016). These alterations were documented in all gill samples analyzed from organisms that died in different dates during the MMP. Similar alterations in the gills of fishes exposed to *Chattonella* spp. have been observed in yellow tail, *Seriola quinqueradiata* in Japan (Ishimatsu et al., 1996); Tilapia, *Oreochromis mossambicus*, from the Salton Sea, California (Tiffany et al., 2001), and the Atlantic salmon, *Salmon salar* in Chile (Godoy, 2016). Lethal effects associated with *Chattonella* spp. has been recorded in *S. quinqueradiata* in Japan, southern bluefin tuna (*Thunnus maccoyi*) in Australia, and salmon (*S. salar*) in Norway and Chile (Imai and Itoh, 1987; Munday and Hallegraeff, 1998; Godoy, 2016).

The precise affectation mechanisms of the gills by *Chattonella* is not clear. It was proposed (Ishimatsu et al., 1996) that oxygen radicals released from *Chattonella*, stimulate mucus cells in the gills, and the secreted mucus, possibly plus *Chattonella* cells trapped within the mucus, impedes the gas exchange capacity of the gills by shunting respiratory water current away from the lamellae. Khan et al. (1996) proposed that brevetoxins produced by *C. marina* in conjunction with superoxide radicals (ROS) cause gill epithelium to become swollen with massive mucous production resulting in fish suffocation. However, production of brevetoxins by *Chattonella* has been disputed since these phycotoxins have not been detected by LC-MS/MS analysis (Hallegraeff et al., 2017). What is clear is that ROS play an important role in the affectation of the gills since raphydophytes produce high amounts of these compounds and together with polyunsaturated fatty acids (PUFAs) produce other toxic compounds through lipid peroxidation by increasing superoxide dismutase activity and damage gill cell membranes (Dorantes-Aranda et al., 2015). Therefore, the toxicity of PUFAs increased synergistically in the presence of ROS (Marshall et al., 2003).

It well recognized that intense blooms of raphydophytes have been responsible for several mass mortalities of cultivated and wild fish and represent a threat to marine fish aquaculture (Imai and Yamaguchi, 2012). However, two main questions arise from the analysis of the extraordinary event reported in this work: (1) Why a relatively low abundance of *Chattonella* species has such a devastating effect on tuna? and (2) What caused the accumulation of *Chattonella* spp. since these species have not been reported before in the region?

A high toxic potential of *Chattonella* species during the bloom or (together with) a high susceptibility of affected organisms are possible answers for the first question. After the first report of the effect of *Chattonella* to cultured tuna in southern Australia, investigations on strains isolated from this region have been performed. These strains showed higher growth rates than Japanese strains at high irradiances (Marshall and Hallegraeff, 1999). Maximum growth rate of the Southern Australian strain was registered at 400 μmol quanta m^{-2} s^{-1} and they have been recognized as a high-light adapted microalga strains (Marshall and Hallegraeff, 1999; Hallegraeff et al., 2017). In addition, the *C. marina* Australian strain probed to be more toxic to fish gill cells due to an elevated production of the eicosapentaenoic fatty acid and superoxide anions (Dorantes-Aranda et al., 2013). The mortality of cage-reared tuna in the Todos Santos bay region can be related also to the presence of highly toxic *Chattonella* strains. Abundances were in the same order of magnitude during the MMP and the outbreak that occurred in Australia. We registered a maximum cell abundance of 33×10^3 cells L^{-1}. However,

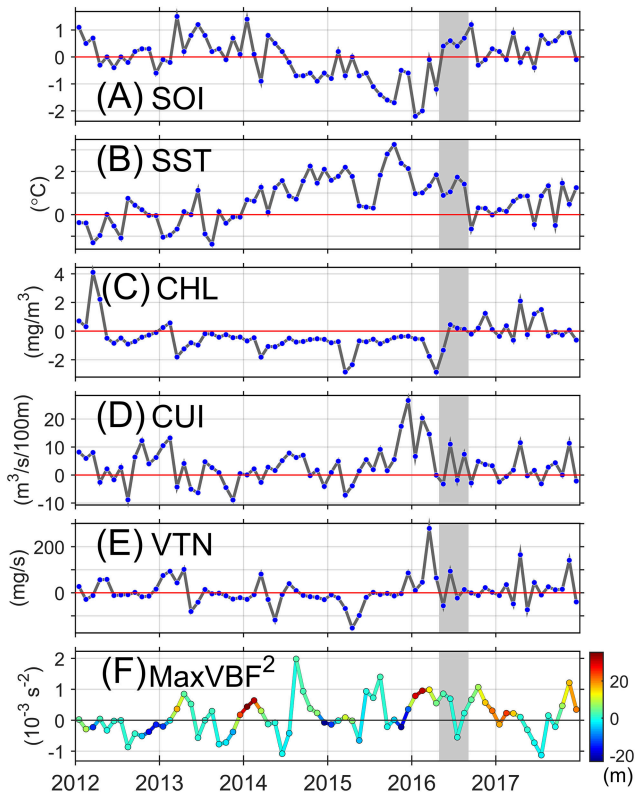


FIGURE 10 | (A) Monthly values of the southern oscillation index (SOI), and monthly anomalies (with respect to the 2003–2017 climatology) of **(B)**, surface temperature (SST); **(C)**, chlorophyll-*a* concentration (CHL); **(D)**, coastal upwelling index (CUI); **(E)**, vertical transport of nutrients (*VTN*), and **(F)**, magnitude and depth (line's color) of the maximum Brunt-Väisälä frequency ($maxBVF^2$) for the period 2012–2017. The shaded area indicates the period mass morality period. Positive values of the SOI correspond to La Niña, whereas negative values correspond to El Niño. Positive values in the *VTN* correspond to an upward nitrogen supply.

a tuna ranching company reported (abundance was evaluated with Sedgwick-rafter chambers) a maximum abundance of 90 × 10^3 cells L^{-1}. In comparison, cells abundances in the Australian outbreak were between 1 × 10^3 cells L^{-1} and a maximum of 66 × 10^3 cells L^{-1} (Munday and Hallegraeff, 1998). Toxic potential of the species from our region has to be investigated but it is clear from the only two reported mass mortalities of cultivated tuna that the lethal abundance for tuna is one order of magnitude lower than the one considered for other economically important fishes. Affectations to the yellow tail *S. quinqueradiata* are expected when *Chattonella* cell abundances are higher than 500 × 10^3 cells L^{-1}.

Tuna susceptibility to ichthyotoxic species has not been investigated. Behavior and affectation of the gills of the organisms during the outbreak of Australia were similar to our observations. Therefore, it seems that tuna is particularly susceptible to ichthyotoxic microalgae. Shen et al. (2011) proposed that susceptibility of marine fish to *C. marina* appears to be inversely related to their tolerance to hypoxia. However, *T. maccoyii* is highly tolerant to conditions of low dissolved oxygen in the environment. The maintenance metabolism, routine swimming, and specific dynamic action of these species are not affected in moderate to severe hypoxia (Fitzgibbon et al., 2010). Probably, oxygen uptake adaptations that support the high metabolic demand of tuna such as a large gill surface area and thin gill epithelium (Korsmeyer and Dewar, 2001) make these organisms extremely susceptible to the ROS and PUFAs produced by ichthyotoxic species. This has to be corroborated but a probably high susceptibility of tuna is the most plausible explanation for the devastating effect of the noxious *C. marina* on these organisms. Striped bass is also cultivated in the region and there were no reports of affection to this species during the MMP.

What caused the accumulation of *Chattonella* spp. since these species have not been reported before? It is important to try to understand how the *Chattonella* spp. HAB developed in the region. We have been monitoring the phytoplankton community intermittently for more than 15 years at different sampling points in TSB and semicontinuously since 2010 in Rincón de Ballenas where bivalve mollusks are cultivated and it is located 2 miles south from Punta Banda (**Figure 2**). There are also other phytoplankton monitoring programs in the region and *Chattonella* spp. have not been reported before. After the MMP the presence of ichthyotoxic species were monitored and *Chattonella* spp were registered in several samples. Abundances lower than 1000 cells L^{-1} were registered during some periods of the year and relative contribution to total abundance was generally not higher than 5% (see **Figure 7**). This indicates that *Chattonella* spp. are common species in the phytoplankton community of the region and are present at low abundances. Probably, these species were not detected before the MMP since the monitoring programs were not focused on the detection of ichthyotoxic species and samples were preserved with solutions that affect fragile phytoplankton cells. If they were present, probably they represented the seeding population for the HAB that affected the cultivated tuna. Alternatively, *Chattonella* spp. were transported into the region from other areas or cysts in the sediment were probably the source of cells for the initiation of the HAB during the MMP. Cysts were detected in sediments after the 2016 HAB (data not shown).

The source of the seeding population that originated the HAB during the MMP remains elusive. We cannot prove that *Chattonella* spp. or cysts were present before the MMP. However, it is important to try to identify the conditions that favored the accumulation of *Chattonella*. This was an extraordinary event not documented before in the region. Extraordinary environmental conditions were also present in the region before the MMP. Abnormally warm conditions were present the two previous years before 2016 and temperatures above 18°C were detected in the entire water column close to Punta Banda. In addition, there were two upwelling periods before the rise of the temperature and stratification in the water column during the MMP. The mesoscale interannual variability analysis demonstrated that these abnormal conditions started 3 years before 2016. In most of these 3 years before the MMP the El Niño conditions dominated in the study area. Notably, upwelling conditions were close to the climatological mean but high SST and low levels

of CHL were present on these years. By the end of the year 2015 an upwelling intensification occurred, associated with an atmospheric low-pressure anomaly over the northeastern Pacific and an intensification of the North Pacific High Pressure System (**Figure 3**, **Supplementary Material**). This condition affected the northwestern Baja California coast through the first months of 2016, which caused a weakening of the SST anomaly, although it did not reach its normal values and the CHL showed no increase. According to the numerical model results the enhanced upwelling caused a stronger nutrient supply into the surface waters over the shelf combined with an enhanced stratification. Thus, the environmental conditions during the MMP turned into La Niña colder conditions with a relatively intense upwelling, accompanied with high levels of nutrients according to the model, and a relatively strong stratification, which caused an increment of the CHL but not phytoplankton accumulation. Notably, during the MMP microphytoplankton counts were extremely low but concentrations higher than 3 $\mu g\ L^{-1}$ of CHL were detected in some samples. The high chlorophyll concentration was associated with the presence of *Chattonella*. This species presents high CHL cellular concentrations, up to 250 ng CHL $cell^{-1}$ had been reported for *C. marina* maintained under culture conditions (Marshall and Newman, 2002).

We propose that extraordinary environmental conditions before the MMP permitted *Chattonella* spp. to thrive in the region. These conditions caused a change in the phytoplankton community normally observed in the region. The abundance of diatoms and dinoflagellates decreased significantly. Specifically, diatom abundance was unusually low in the previous years before 2016 (data not shown) and particularly during the MMP (**Figure 4**, **Supplementary Material**). Blooms of *Chattonella* occur when diatoms are scarce (Imai, 1990; Onitsuka et al., 2011; Aoki et al., 2015). The change in the phytoplankton community during the abnormal period and conditions (upwelling events followed by stratification periods) before the MMP were the probable causes for the accumulation of *Chattonella* spp. according to the conceptual model for the formation of blooms of these species.

The ecological interaction between *Chattonella* and diatoms is explained by the "Diatom resting hypothesis" proposed by Imai and Yamaguchi (2012). According to this hypothesis, blooms of *Chattonella* spp. are formed when diatoms are scare in surface waters and there is an input of nutrients associated with mixing processes after a period of strong stratification. Chattonellas show lower growth rate than diatoms (Imai and Yamaguchi, 2012). However, Chattonellas can dominate the phytoplankton community when diatoms population is in a resting stage in the sediments or they are physiologically inactive in the water column. Some diatoms species form resting stages and sink when there is a strong stratification accompanied by depletion of surface nutrients. Formation of diatom resting stages are essential for the increase of *Chattonella* abundance when the stratification breaks and nutrient are pumped into surface waters (Imai and Yamaguchi, 2012).

The increase in *Chattonella* spp. abundances during the MMP fits to this conceptual model for HABs formation in Seto Inland Sea in Japan developed after the observation of recurrent events in this region (Imai and Yamaguchi, 2012). Probably in the BTS region, the decrease of diatom abundance during the long period of abnormally warm conditions and the upwelling events that occurred at the end of 2015 and before the MMP brought the conditions for the increase of *Chattonella* abundance. After the MMP, "normal conditions" returned in 2016 and 2017 and phytoplankton abundance increased accompanied with the representation of diatoms in the phytoplankton community. We related a HAB not registered before to extraordinary environmental conditions that were present in the region. These two phenomena must occur recurrently to validate the concept that they were related. Logically, these will not be favorable for the mariculture industry of the region.

Impact to the Industry and Management of the Problem

The MMP affected importantly tuna-ranching activities in Northwest Baja California. This event is one of the largest economical negative impact for marine aquaculture industry related to a microalgae bloom in Mexico. An estimated value of the loses in one of the companies of the region was 42 million dollars according to insurance records (http://www.abacoadjusters.com/referencia/mortandad-de-atun-2/). Ecological information of some noxious species has been gathered from other regions with a long affectation history. This information is essential to understand the development of extraordinary blooms in our region. It is important to continue the monitoring of the phytoplankton community together with the characterization of environmental conditions to understand the bloom dynamics of *Chattonella* or other noxious microalga. Risk indexes for the presence of the noxious species including environmental and ecological variables should be developed to implement management plans to mitigate the effect of the species to the industry especially, if extraordinary events will become normal conditions.

AUTHOR CONTRIBUTIONS

EG-M, analyzed and interpreted the data, reviewed the data for accuracy and integrity, wrote, and edited this manuscript. JC-M and RV-Y processed and analyzed histopathological samples, interpreted the results, wrote, and edited the manuscript. DR analyzed and interpreted mesoscale interannual data of the region, wrote, and edited the manuscript. MF-M analyzed samples, interpreted the data, elaborated figures, and participate in the edition of the manuscript. YS-B, analyzed phytoplankton samples and interpreted the data. JM-E participated in editing the manuscript.

FUNDING

CONACyT scholarship to MF-M. FORDECYT—CONACyT project number 260040-2015; Red Temática sobre Florecimientos Algales Nocivos (RedFAN) CONACyT 2015-2017 projects. DR was funded by CICESE through internal Project 625118.

ACKNOWLEDGMENTS

We thank Baja Aquafarms S.A. de C.V for the help, support, and collaboration with CICESE, especially with FICOTOX laboratory. Also, we thank the company for sharing important information of the event. Particularly, we greatly appreciate the help and support of Javier Vivanco-Ocampo and Andres Ortinez-Escorza.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2018.00454/full#supplementary-material

Video S1 | The video was recorded during a mass mortality event of organisms maintained in Punta Banda. Organisms were disoriented, swimming slowly and erratically, not moving in circles as in normal conditions. The video shows a severely affected tuna crashing against the nets of the pen (video courtesy of Baja Aquafarms Co.).

Figure S1 | Mucus present in the gills of a dead northern bluefin tuna *Thunnus thynnus orientalis* analyzed in June 15 in FICOTOX laboratory of CICESE. Cells of *Tripos furca* and round or oval cells were also observed.

Figure S2 | A chromatograph of a typical pigment profile of samples dominated by *Chattonella* spp. **(A)** station E1, 33,000 cells L^{-1}. Chlorophyll a concentration and *Chattonella* spp. abundance relation **(B)**. The data was fitted to a linear regression model (solid line) passing through the origin considering that when *Chattonella* is not present the chlorophyll concentration is associated to other phytoplankton groups.

Figure S3 | Geopotential height anomaly (with respect to the 1979–1995 climatology) at 500 hPa for December 2015. Plot taken from https://www.esrl.noaa.gov/psd/data/histdata.

Figure S4 | Diatom abundance in samples collected in Punta Banda and Salsipeudes bay from the end of May to December 2017. Abundances in surface samples are represented by empty symbols and blue circles are abundances detected at 10 m depth.

REFERENCES

Ahumada-Fierro, V. (2017). *Caracterización de Aislados de Chattonella spp. de la Bahía de Todos Santos, y Evaluación de su Efecto Sobre Estadios Tempranos del Ostión Crassostrea gigas*. Unpublished Bachelor's dissertation, Universidad Autónoma de Baja California, Ensenada, Mexico.

Almazán-Becerril, A., and García-Mendoza, E. (2008). Maximum efficiency of charge separation of photosystem II of the phytoplankton community in the Eastern Tropical North Pacific off Mexico: a nutrient stress diagnostic tool? Ciencias Marinas 34, 29–43. doi: 10.7773/cm.v34i1.1151

Aoki, K., Onitsuka, G., Shimizu, M., Kuroda, H., Matsuyama, Y., Kimoto, K., et al. (2012). Factors controlling the spatio-temporal distribution of the 2009 Chattonella antiqua bloom in the Yatsushiro Sea, Japan. *Estuarine Coast. Shelf Sci.* 114, 148–155. doi: 10.1016/j.ecss.2012.08.028

Aoki, K., Onitsuka, G., Shimizu, M., Yamatogi, T., Ishida, N., Kitahara, S., et al. (2015). Chattonella (Raphidophyceae) bloom spatio-temporal variations in Tachibana Bay and the southern area of Ariake Sea, Japan: interregional displacement patterns with Skeletonema (Bacillariophyceae). *Mar. Poll. Bull.* 99, 54–60. doi: 10.1016/j.marpolbul.2015.07.063

Bakun, A. (1973). *Coastal Upwelling Indices, West Coast of North America*. 194671. NOAA Tech. Rep. NMFS SWFSC 671. Seattle, WA: NOAA.

Black, E. A., Whyte, J. N. C., Bagshaw, J. W., and Ginther, N. G. (1991). The effects of *Heterosigma akashiwo* on juvenile *Oncorhynchus tshawytscha* and its implications for fish culture. *J. Appl. Ichthyol.* 7, 168–175. doi: 10.1111/j.1439-0426.1991.tb00523.x

Bruslé, J. (1995). *The Impact of Harmful Algal Blooms on Finfish: Mortality, Pathology and Toxicology*. Brest: REPÈRES OCEAN.

Camargo, M. M. P., and, Martinez, C. B. R. (2007). Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. *Neotrop. Ichthyol.* 5, 327–336. doi: 10.1590/S1679-62252007000300013

Clement, A., Lincoqueo, L., Saldivia, M., Brito, C. G., Muñoz, F., Fernández, C., et al. (2016). Exceptional summer conditions and HABs Pseudochattonella in southern chile create record impacts on salmon farms. *Harm. Algal News* 53, 1–3.

Cruz-Rico, J., and Rivas, D. (2018). Physical and biogeochemical variability in Todos Santos Bay, northwestern Baja California, derived from a numerical NPZD model. *J. Mar. Sys.* 183, 63–75. doi: 10.1016/j.jmarsys.2018.04.001

Dorantes-Aranda, J. J., Nichols, P. D., Waite, T. D., and Hallegraeff, G. M. (2013). Strain variability in fatty acid composition of Chattonella marina (*Raphidophyceae*) and its relation to differing ichthyotoxicity toward rainbow trout gill cells. *J. Phycol.* 49, 427–438. doi: 10.1111/jpy.12053

Dorantes-Aranda, J. J., Seger, A., Mardones, J. I., Nichols, P. D., and Hallegraeff, G. M. (2015). Progress in understanding algal Bloom-mediated fish killing: the role of superoxide radicals, phycotoxins and fatty acid. *PLoS ONE* 10:e133549. doi: 10.1371/journal.pone.0133549

Durazo, R. (2009). Climate and upper ocean variability off Baja California, Mexico: 1997-2008. *Prog. Ocean.* 83, 361–368. doi: 10.1016/j.pocean.2009.07.043

Edvardsen, B., and Imai, I. (2006). "The ecology of harmful flagellates within Prymnesiophyceae and Raphidophyceae," in *Ecology of Harmful Algae,* eds E. Granéli and J. T. Turner (Berlin, Heidelberg: Springer), 67–79.

Esenkulova, S., Haigh, N., Keddy, L. J., and Pearce, C. M. (2014). Germinating cysts of *Heterosigma akashiwo* from marine sediments of British Columbia, Canada. *Harm. Algae News* 48, 4–5.

Fernandez, M. N., and Mazon A. F. (2003). "Environmental pollution and fish gill morphology," in *Fish Adaptations,* eds A. L. Val and B. G. Kapoor (Enfield, CT: Science Publishers), 203–231.

Fitzgibbon, Q. P., Seymour, R. S., Buchanan, J., Musgrove, R., and Carragher, J. (2010). Effects of hypoxia on oxygen consumption, swimming velocity and gut evacuation in southern Bluefin tuna (*Thunnus maccoyii*). *Environ. Biol. Fish* 89, 59–69. doi: 10.1007/s10641-010-9690-1

Godoy, M. (2016). *Patología en Acuicultura, 2016 Histopatología Branquial Asociada a la Exposición a Bloom por Chattonella sp. (Raphidophyceae) en Salmón del Atlántico (Salmo salar).* Available online at: http://goo.gl/e0SdFI

Hallegraeff, G. M., Dorantes-Aranda, J. J., Mardones, J., and Seger, A. (2017). "Review of Progress in our understanding of fish-killing microalgae: implications for management and mitigation," in *Marine and Fresh-Waters Harmful Algae. Procedings of the 17th International Conference on Harmful Algae 2017,* eds L. A. O. Proença and G. M. Hallegraeff, (International Society for the Study of Harmful Algae).

Huang, B., Xue, Y., and Behringer, D. W. (2008). Impacts of argo salinity in NCEP global ocean data assimilation system: the tropical Indian Ocean. *J. Geophys. Res.* 113:C08002. doi: 10.1029/2007JC004388

Imai, I. (1990). Distribution of diatom resting cells in sediments of Harima-Nada and northern Hiroshima Bay, the Seto Inland Sea, Japan. *Bull. Coast. Oceanogr.* 28, 75–84.

Imai, I., Itakura, S., and Itho, K. (1991). Life cycle strategies of the Red Tide causing flagellates Chattonella (*Raphidophyceae*) in the Seto Inland Sea. *Mar. Poll. Bull.* 23, 165–170. doi: 10.1016/0025-326X(91)90668-I

Imai, I., and Itoh, K. (1987). Annual life cycle of Chattonella spp., causative flagellates of noxious red tides in the Inland Sea of Japan. *Mar. Biol.* 94, 287–292. doi: 10.1007/BF00392942

Imai, I., and Yamaguchi, M. (2012). Life cycle, physiology, ecology and red tide occurrences of the fish-killing raphidophyte Chattonella. *Harm. Algae* 14, 46–70. doi: 10.1016/j.hal.2011.10.014

Imai, I., Yamaguchi, M., and Hori, Y. (2006). Eutrophication and occurrences of harmful algal blooms in the Seto Inland Sea, Japan. *Plankt. Benthos. Res.* 1, 71–84. doi: 10.3800/pbr.1.71

Ishimatsu, A., Sameshima, M., Tamura, A., and Oda, T. (1996). Histological analysis of the mechanisms of chattonella-induced hypoxemia in yellowtail. *Fish. Sci.* 62, 50–58. doi: 10.2331/fishsci.62.50

Katano, T., Yoshida, M., Lee, J., Han, M.-S., and Hayami, Y. (2009). Fixation of Chattonella antiqua and C. marina (*Raphidophyceae*) using Hepes-buffered

paraformaldehyde and glutaraldehyde for flow cytometry and light microscopy. *Phycologia* 43, 473–479. doi: 10.2216/08-102.1

Khan, S., Arakawa, O., and Onoue, Y. (1996). A toxicological study of the marine phytoflagellate,Chattonella antiqua (*Raphidophyceae*). *Phycologia* 35, 239–244. doi: 10.2216/i0031-8884-35-3-239.1

Kim, S. Y., Seo, K. S., Lee, C. G., and Lee, Y. (2007). Diurnal modification of a red-tide causing organism Chattonella antiqua (*Raphidophyceae*) from Korea. *Algae* 22, 95–106. doi: 10.4490/ALGAE.2007.22.2.095

Korsmeyer, K. E., and Dewar, H. (2001). Tuna metabolism and energetics. *Fish Physiol.* 19, 35–78. doi: 10.1016/S1546-5098(01)19003-5

Kudela, R. M., and Gobler, C. J. (2012). Harmful dinoflagellates blooms caused by Cochlodinium sp.: global expansion and ecological strategies facilitating bloom formation. *Harm. Algae* 14, 71–86. doi: 10.1016/j.hal.2011.10.015

León-Muñoz, J., Urbina, M. A., Garreaud, R., and Iriarte, J. L. (2018). Hydroclimatic conditions trigger record harmful algal Bloom in western Patagonia (summer 2016). *Sci. Rep.* 8:1330. doi: 10.1038/s41598-018-19461-4

Lewitus, A. J., Horner, R. A., Caron, D. A., García-Mendoza, E., Hickey, B. M., Hunter, M., et al. (2012). Harmful algal blooms along the North American west coast region: history, trends, causes and impacts. *Harm. Algae* 19, 133–159. doi: 10.1016/j.hal.2012.06.009

Mallatt, J. (1985). Fish gill structural changes induced by toxicants and other irritants: a statistical review. *Can. J. Fish Aquatic Sci.* 42, 630–648. doi: 10.1139/f85-083

Marshall, J. A., and Hallegraeff, G. M. (1999). Comparative ecophysiology of the harmful alga Chattonella marina (*Raphidophyceae*) from South Australian and Japanese water. *J. Plankton Res.* 21, 1809–1822. doi: 10.1093/plankt/21.10.1809

Marshall, J. A., and Newman, S. (2002). Differences in photoprotective pigments production between Japanese and Australian strains of Chattonella marina (*Raphidophyceae*). *J. Exp. Mar. Biol. Ecol.* 272, 13–27. doi: 10.1016/S0022-0981(02)00034-5

Marshall, J. A., Nichols, P. D., Hamilton, B., Lewis, R. J., and Hallegraeff, G. M. (2003). Ichthyotoxicity of Chattonella marina (Raphidophyceae) to damselfish *(Acanthochromis polycanthus)*: the synergistic role of reactive oxygen species and free fatty acids. *Harmful Algae* 2, 273–281. doi: 10.1016/S1568-9883(03)00046-5

McClatchie, S., Goericke, R., and Leising, A. W. (2016). *State of the California Current 2015-16: Comparisons with the 1997-98 El Niño*. California Cooperative Oceanic Fisheries Investigations Report, 57, 5–61.

Mesinger, F., DiMego, G., Kalnay, E., Mitchell, K., Shafran, P. C., Ebisuzaki, W., et al. (2006). North American regional reanalysis. *Bull. Am. Meteor. Soc.* 87, 343–360. doi: 10.1175/BAMS-87-3-343

Munday, B. L., and Hallegraeff, G. M. (1998). Mass mortality of captive southern bluefin tuna (*Thunnus maccoyii*) in April/May 1996 in Boston Bay, South Australia: a complex diagnostic problem. *Fish Pathol.* 33, 343–350. doi: 10.3147/jsfp.33.343

Omura, T., Iwataki, M., Borja, V. M., Takayama, H., and Fukuyo, Y. (2012). *Marine Phytoplankton of the Western Pacific*. Tokyo: Kouseisha Kouseikaku.

Onitsuka, G., Aoki, K., Shimizu, M., Matsuyama, Y., Kimoto, K., Matsuo, H., et al. (2011). Short-term dynamics of a Chattonella antiqua bloom in the Yatsushiro Sea, apan, in summer 2010: characteristics of its appearance in the southern area. *Bull. Jpn. Soc. Fish Oceanogr.* 75, 143–153.

Powell, T. M., Lewis, C. V. W., Curchister, E. N., Haidvogel, D. B., Hermann, A. J., and Dobbins, E. L. (2006). Results from a three-dimensional, nested biological-physical model of the California current system and comparisons with statistics from satellite imagery. *J. Geophys. Res.* 111:C07018. doi: 10.1029/2004JC002506

Ravichandran, M., Behringer, D., Sivareddy, S., Girishkumar, M. S., Chacko, N., and y Harikumar, R. (2013). Evaluation of the global ocean data assimilation system at INCOIS: the tropical Indian Ocean. *Ocean Modell.* 69, 123–135. doi: 10.1016/j.ocemod.2013.05.003

Rensel, J. E., and Whyte, J. N. C. (2004). "Finfish mariculture and harmful algal blooms," in *Manual on Harmful Marine Microalgae,* eds G. M. Hallegraeff, D. M. Anderson, and A. D. Cembella (Landais: UNESCO Publishing), 693.

Schwing, F. B., Farrell, M. O., and Steger, J. M. (1996). *Coastal Upwelling Indices West Coast of North America*. NOAA Tech. Rep. NMFS SWFSC 231. Seattle, WA: NOAA.

Shen, M., Xu, J., Chan, A. K. Y., and Au, D.W.T. (2011). Susceptibility of fish to Chattonella marina is determined by its tolerance to hypoxia. *Mar. Pollut. Bull.* 63, 189–194. doi: 10.1016/j.marpolbul.2011.06.001

Smith, S. D. (1988). Coefficients for sea surface wind stress, heat flux, and wind profiles as a function of wind speed and temperature. *J. Geophys. Res.* 93, 15467–15472. doi: 10.1029/JC093iC12p15467

Sournia, A. (1978). *Phytoplankton Manual. Monographs on Oceanographic Methodology*. Norwich: UNESCO.

Tiffany, M. A., Barlow, S. B., Matey, V. E., and Hulbert, S. H. (2001). Chattonella marina (*Raphidophyceae*), a pontentially toxic alga in the Salton Sea, California. *Hydrobiologia* 466, 187–194. doi: 10.1023/A:1014503920898

Tomas, C. R. (1997). *Identifying Marine Phytoplankton*. San Diego, CA: Elsevier. 858.

Van-Heukelem, L., and Thomas, S. C. (2001). Computer-assisted high-performance liquid chromatography method development with applications to the isolation and analysis of phytoplankton pigments. *J. Chromatogr. A* 910, 31–49. doi: 10.1016/S0378-4347(00)00603-4

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 García-Mendoza, Cáceres-Martínez, Rivas, Fimbres-Martinez, Sánchez-Bravo, Vásquez-Yeomans and Medina-Elizalde. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Marine Science

ORIGINAL RESEARCH
published: 18 December 2018
doi: 10.3389/fmars.2018.00491

Association of the Toxigenic Dinoflagellate *Alexandrium ostenfeldii* With Spirolide Accumulation in Cultured Mussels (*Mytilus galloprovincialis*) From Northwest Mexico

Patricia Paredes-Banda[1], Ernesto García-Mendoza[2]*, Elizabeth Ponce-Rivas[1], Juan Blanco[3], Antonio Almazán-Becerril[4], Clara Galindo-Sánchez[1] and Allan Cembella[5]*

[1] Departamento de Biotecnología Marina, Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, Mexico, [2] Departamento de Oceanografía Biológica, Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, Mexico, [3] Centro de Investigacións Mariñas, Vilanova de Arousa, Spain, [4] Centro de Investigación Científica de Yucatán, Unidad de Ciencias del Agua, Cancún, Mexico, [5] Alfred-Wegener-Institut, Helmholtz Zentrum für Polar-und Meeresforschung, Helmholtz-Gemeinschaft Deutscher Forschungszentren, Bremerhaven, Germany

OPEN ACCESS

Edited by:
Marius Nils Müller, Federal University of Pernambuco, Brazil

Reviewed by:
Mariângela Menezes, Universidade Federal do Rio de Janeiro, Brazil
Mohamed Laabir, Université de Montpellier, France

***Correspondence:**
Patricia Paredes-Banda
eparedes@cicese.edu.mx
Ernesto García-Mendoza
ergarcia@cicese.mx

Specialty section:
This article was submitted to Marine Biogeochemistry, a section of the journal Frontiers in Marine Science

Received: *01 September 2018*
Accepted: *04 December 2018*
Published: *18 December 2018*

Citation:
Paredes-Banda P, García-Mendoza E, Ponce-Rivas E, Blanco J, Almazán-Becerril A, Galindo-Sánchez C and Cembella A (2018) Association of the Toxigenic Dinoflagellate Alexandrium ostenfeldii With Spirolide Accumulation in Cultured Mussels (Mytilus galloprovincialis) From Northwest Mexico. Front. Mar. Sci. 5:491. doi: 10.3389/fmars.2018.00491

Spirolides are polyether cyclic imines considered as "fast acting toxins." Long-term human health consequences of spirolide ingestion are uncertain, and hence regulatory limits for human consumption have not been established. Nevertheless, monitoring these toxins in shellfish is essential because they can interfere with detection by mouse bioassay of lipophilic regulated toxins. Todos Santos Bay (TSB), in the northwest of the Baja California Peninsula, is an important shellfish cultivation and fish-farming area in Mexico. The toxin analog 13-desmethyl spirolide C has been reported in cultivated mussels (*Mytilus galloprovincialis*) from TSB, but the causative species associated with accumulation of this toxin has not been previously identified. We assessed the occurrence of *Alexandrium ostenfeldii*, the unique known producer of spirolides, by inverted light microscopy and by PCR with species-specific oligonucleotides designed for the ITS and 18S rDNA. We determined the presence and abundance of this species at the surface and at the thermocline from samples collected over two annual sampling periods (2013–2014 and 2016–2017). During the 2013–2014 period, *A. ostenfeldii* was found in 50% of the samples analyzed by light microscopy. The highest cell abundance (about 3.6×10^3 cells L^{-1}) occurred in October 2013. During 2016–2017 the dinoflagellate was present in low cell abundances ($<5 \times 10^2$ cells L^{-1}) and was detected in only 20.9% of the samples. Cells of this species were usually found when sea surface temperature ranged from 17 to 20°C. We also evaluated spirolide accumulated in cultivated mussels from TSB by tandem mass spectrometry (LC-MS/MS). The only spirolide detected was 13-desmethyl spirolide C, found mainly during the 2013–2014 sampling period, with the highest concentration (1.05 μg kg^{-1}) in June 2014. During winter, toxin concentration was at or below the detection limit. During 2016–2017,

spirolides were below the detection limit, coinciding with the absence of the causative species. Cell abundance of *A. ostenfeldii* and spirolide concentration in mussels did not present a clear correlation. This study represents the first record of *A. ostenfeldii* in TSB and provides evidence that this species is the primary origin of spirolides accumulated in mussels.

Keywords: polyether toxins, Mediterranean mussel, 13-desmethyl spirolide-C, LC-MS/MS, marine dinoflagellates, harmful algal blooms

INTRODUCTION

Spirolides are naturally occurring polyether cyclic imine compounds with a spiro-group attached to the tricyclic ethers. These toxins were first isolated from mussel digestive glands and toxicity was determined by intraperitoneal mouse bioassay during chemical investigations of polar bioactive molecules from microalgae and shellfish from Nova Scotia (Canada) (Hu et al., 1995). The intact cyclic imine moiety is the pharmacophore that confers biological activity (Hu et al., 1996, 2001). Many spirolides, including those belonging to the A, B, C, D groups and their respective desmethyl derivatives, act as "fast-acting toxins" (FAT) characterized by rapid onset of neurological symptoms followed by death after intraperitoneal injection into mice (Cembella et al., 1999; Richard et al., 2001). Spirolides are not considered in the sanitary legislation for human consumption of shellfish since a toxic effect in humans has not been proven and no poisoning symptoms associated with these toxins have been confirmed (Otero et al., 2011). A few complaints of gastric distress and tachycardia were recorded after consumption of mussels containing high spirolide concentrations, but these non-specific symptoms could not be definitively ascribed to spirolides (Richard et al., 2001). Cholinergic receptors (muscarinic and nicotinic) are proposed main targets for spirolides, and the toxic effects could be their activity as irreversible antagonists of the muscarinic acetylcholine receptor (Gill et al., 2003) and weak L-type transmembrane calcium channel activators (Sleno et al., 2004).

Spirolides are coextracted in the methanolic extract of the regulated polyether shellfish toxins, such as okadaic acid, dinophysistoxins, azaspiracids, and yessotoxins. The FAT activity of spirolides in methanolic or lipophilic fractions can therefore interfere with and cause artifacts in the intraperitoneal mouse bioassay (Trainer et al., 2013). False toxicity positives associated with spirolides can cause unnecessary shellfish sanitary closures that will negatively affect economic activities for coastal aquaculture and harvest of wild shellfish.

The causative species associated with spirolide accumulation in cultured shellfish from Nova Scotia was identified as the mixotrophic dinoflagellate *Alexandrium ostenfeldii* (Paulsen) Balech and Tangen (Cembella A. et al., 2000). This species is widely distributed in coastal waters around the globe (Cembella, 2018), from the coast of Washington State, United States (Balech, 1995), the northeastern coast of North America (Hargraves and Maranda, 2002), and including north Atlantic and sub-Arctic waters, as well as the Mediterranean Sea and the brackish Baltic Sea (Suikkanen et al., 2013). Interest in the study of *A. ostenfeldii* and associated toxicity has increased due to the reports of coastal blooms of this species in the Peruvian coast (Sánchez et al., 2004), Narragansett Bay and the New River Estuary along the United States east coast (Borkman et al., 2012), and the Baltic Sea coast of Finland (Hakanen et al., 2012). Blooms of this species occurred recently in Oosterschelde, Netherlands, representing a major concern for public health for poisonings associated with paralytic shellfish toxins (PSTs), but these blooms were not related to human intoxication by spirolides (Van de Waal et al., 2015). The toxin regulatory situation is complicated by the fact that certain *A. ostenfeldii* populations can also produce other neurotoxins, such as PSTs, and/or the cyclic imines known as gymnodimines. The toxin composition and content of *A. ostenfeldii* depends upon genotypic strain differences and geographic origin (Suikkanen et al., 2013), and is therefore difficult to predict and assess.

The Pacific coast of North America from Alaska to Baja California is relatively underrepresented in studies of the biogeographical distribution of *A. ostenfeldii* and associated spirolides (Cembella, 2018). Todos Santos Bay (TSB), in the northwest of the Baja California peninsula, is the region with the highest production of cultivated mussels in Mexico (Maeda Martínez, 2008). Sanitary closures have been implemented in the region due to the presence of lipophilic toxins in shellfish harvested from this bay. Sánchez-Bravo (2013) demonstrated a high percentage of non-specificity in the detection of toxicity with the lipophilic mouse bioassay in comparison with measurements of toxin composition determined by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) in mussel samples from TSB that could be attributed to the presence of spirolides among other "fast acting" toxins. Particularly in 2012, 13-desmethyl spirolide C was detected during almost the whole year with no apparent temporal pattern of appearance (García-Mendoza et al., 2014). Unfortunately, the causative species associated with this spirolide accumulation in shellfish was not identified.

The inability to reliably identify the toxigenic species that produces spirolides is often associated with the complexity in the identification of *Alexandrium* taxa (Touzet et al., 2008), especially by morphological criteria alone. *A. ostenfeldii* has been detected and identified by polymerase chain reaction (PCR) probes involving specific primers to amplify the Internal Transcribed Spacer (ITS) of the rDNA gene (Schwarz, 2011). This represents a useful technique to unequivocally evaluate the presence of this species in the phytoplankton community. In the present work, we evaluated the temporal distribution of *A. ostenfeldii* in TSB as causative agent for the accumulation of spirolides

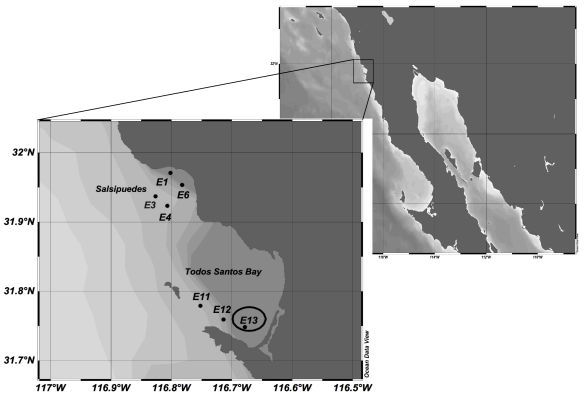


FIGURE 1 | Sampling stations located in Todos Santos Bay (TSB) region. Station 13 enclosed in the circle is inside the mussel cultivation area.

in cultivated bivalve molluscs from the bay. The presence and temporal abundance of this species in the phytoplankton community was evaluated by PCR and by morphological examination of cells from water samples by light microscopy, and combined with analysis of accumulated spirolides in cultivated mussels (*Mytilus galloprovincialis*). The combination of these alternative techniques allowed the detection and confirmation of *A. ostenfeldii* in TSB and provided the first report of this species in the northwest Pacific of Mexico.

MATERIALS AND METHODS

Field Sampling

Todos Santos Bay, located on the northwestern coast of the Baja California peninsula, Mexico, is subject to a regular monitoring program for marine biotoxins in shellfish and associated toxigenic plankton by the FICOTOX laboratory of Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California (CICESE). From July 2013 to June 2014 water and mussel samples were collected periodically from the mussel cultivation area in TSB (St 13; **Figure 1**). Water samples for taxonomic analysis and enumeration were collected at the surface and at 10 m depth with a 2 L Niskin bottle. In addition, plankton samples were analyzed from vertical net tows with 20 μm mesh plankton net conducted through the upper 20 m of the water column. In 2016, the regular monitoring program of FICOTOX was extended to include evaluation of the phytoplankton community where caged tuna aquaculture is located in the region. From 2016, plankton samples were analyzed from Salsipuedes Bay, where some tuna enclosures are located (St 1, 3, 4 and 6; **Figure 1**). Sampling at St 13 continued throughout this period and two more stations (St 11 and 12; **Figure 1**) in the southern sector of TSB were also monitored on a monthly basis from August 2016 to August 2017. Water samples were collected with a 2 L Niskin bottle at the surface and at the thermocline. The vertical temperature profile of the water column was measured with a conductivity, temperature and pressure profiler (CTD, CastAway, YSI, Yellow Springs, OH, United States). The depth of the thermocline was determined from the temperature profile before the samples were collected.

Microscopic Evaluation of *A. ostenfeldii* Abundance

A 250 mL water subsample from the Niskin bottle was fixed with Lugol's iodine-acetate solution. These samples were analyzed with an inverted microscope (Leica DM13000B, Leica Microsystems, Wetzlar, Germany) according to the Utermöhl method (Lund et al., 1958). A 50 mL sample was sedimented and the complete surface of the sedimentation chamber was analyzed to quantify the cell abundance of *A. ostenfeldii*. For net samples only 10 mL were sedimented and the results were presented as the relative abundance (in %) of *A. ostenfeldii* to total phytoplankton cells collected with the 20 μm plankton net.

The thecal plate arrangement and morphology of cells considered provisionally as *A. ostenfeldii* was evaluated by epifluorescence microscopy. Fixed cells from seawater samples were separated by a capillary, and rinsed twice with deionized water to get rid of salts and residual Lugol's solution. Then, 4 μL of Fluorescent Brightener 28 (Sigma-Aldrich, St. Louis, MO, United States) at a concentration of 1 mg mL^{-1} was added to stain the cellulose thecal plates (Fritz and Triemer, 1985). Plate arrangement was characterized under an Axio Imager A2 microscope system and digitally photographed with an AxioCam ICc 1 camera and Axiovision software (Carl Zeiss AG, Oberkochen, Germany).

Molecular Identification of *A. ostenfeldii*

DNA Extraction

Seawater (500 mL to 1 L) was filtered through 8 μm pore-size polycarbonate filters (Nucleopore, Whatman, Darmstadt, Germany), under gentle vacuum to avoid cell disruption. Filters were stored in a 50 mL centrifuge tubes at −80°C until analysis. The DNA was extracted with a DNeasy Plant Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions but with modification of the volume of the lysis buffer to 600 μL. Homogenization of cells was performed with a mini-Bead Beater 24 (BioSpec Products, Bartlesville, OK, United States) at maximum speed (6.5 m s^{-1}) for 45 s (Wohlrab et al., 2010), with 170 μm^3 of zirconium beads of 0.1 mm diameter to disrupt the cells. The DNA was resuspended in sterile Milli-Q water. The purity and concentration of DNA was measured by UV-spectroscopy with a NanoDrop 2000 (Thermo Scientific, Wilmington, DE, United States) and the integrity of the DNA was verified on a 1.2% agarose gel via electrophoresis.

Polymerase Chain Reaction (PCR) Amplification

The primers AOF4 (5′-TGTCAATGCGTGTGCATTCG-3′) and AOR3 (5′-CATTGCAACCAATGCACATGA-3′) were used for identification of *A. ostenfeldii* during the first monitoring period (2013–2014). These primers produce an amplicon of 99 bp of the ITS1, 5.8S and ITS2 region (Schwarz, 2011). The primers were originally designed specifically to differentiate *A. ostenfeldii* from *A. peruvianum* (4 bp), but *A. peruvianum* is now recognized as a heterotypic synonym of the former species (Kremp et al., 2014). For identification of *A. ostenfeldii* in the 2016–2017 samples the

primers AOST213F (5′-GGGAAGGGTTGTGGTCATTAGTTA-3′) and AOST213R (5′-TTGCCATGTAATTCATTCAGTCATT-3′), which produce an amplicon of 89 bp of the 18S region were used (Elferink et al., 2017).

PCR reactions (30 μL) were performed in a Veriti thermal cycler (Applied Biosystems, United States) using 100 ng of DNA. For ITS amplifications, the master mix was prepared with the following concentrations: 0.4 mM of each dNTP, 0.12 μM of each primer, 4 mM of $MgCl_2$, 1 X GoTaq® Flexi buffer (Promega, Madison, WI, United States), and 1.25 U of GoTaq® DNA polymerase. The PCR was carried out at 94°C for 4 min, followed by 40 cycles of 94°C for 30 s, 60°C for 45 s, 72°C for 1 min, and a final extension step of 72°C for 7 min.

For the 18S rDNA amplifications the master mix contained the following concentrations: 0.2 mM of each dNTP, 0.30 μM of each primer, 3 mM of $MgCl_2$, 1 X GoTaq® Flexi buffer (Promega, Madison, WI, United States), and 0.6 U of GoTaq® DNA polymerase. The amplification was carried out under the following conditions: 94°C for 3 min, followed by 30 cycles of 94°C for 30 s, 60°C for 45 s, 72°C for 1 min and a final extension step of 72°C for 7 min. The amplicons were analyzed by electrophoresis on 2% agarose gel.

The specificity of the reaction was confirmed with a DNA template from a monoclonal reference culture of *A. ostenfeldii*. Strain CCMP 1773 was acquired from the Provasoli Guillard Center for Marine Microalgae (CCMP, Boothbay Harbor, ME, United States) and cultivated under optimal growth conditions at 15°C and photon flux density of 100 μmol quanta m^2 s^{-1}. The DNA of this strain was used as a positive control. For the negative control the DNA was replaced with ultrapure water in the PCR reaction.

Sequencing

PCR products of ITS and 18S amplifications were sent for sequence analysis to SeqXcel, San Diego, CA, United States. The analysis of the sequences was carried out using the BLAST tool on the NCBI website and the sequence alignments were performed using the CLUSTAL X software (Thompson et al., 1997) and the Biological sequence alignment editor and analysis program (Bioedit) (Hall, 1999).

Compositional Analysis and Quantification of Spirolides

Spirolides were determined from particulate plankton by filtration of 300–800 mL seawater from the surface and thermocline through Nucleopore polycarbonate filters (3.0 μm pore-size; Whatman, Darmstadt, Germany) under low vacuum. Filters were stored in 50 mL centrifuge tubes at −70°C until extraction. During extraction, filters were repeatedly rinsed with 1 mL 100% MeOH until complete discoloration of the filters. The methanolic extract was filtered through spin-filters (0.45 μm pore-size; Millipore Ultrafree, Eschborn, Germany) and centrifuged for 30 s at 800 × *g*. The filtrate was transferred to an autosampler vial and 5 μL were injected into the LC-MS/MS system. The protocol for the detection of spirolides was as described by Krock et al. (2008) for lipophilic toxins. Toxin analyses were performed on a liquid chromatograph (Agilent 1100, Waldbronn, Germany) coupled to triple-quadrupole mass spectrometer (ABI-SCIEX-4000 Q Trap, Applied Biosystems, Darmstadt, Germany) equipped with a Turbo V ion source. Separation of the lipophilic toxins was performed by reverse-phase chromatography on a C8 column (50 mm × 2 mm) packed with 3 μm Hypersil BDS 120 Å (Phenomenex, Aschaffenburg, Germany). The mobile phase comprised two eluents, where eluent A was water and eluent B was acetonitrile-water (95:5 v:v), both containing 2.0 mmol L^{-1} ammonium formate and 50 mmol L^{-1} formic acid. The limit of detection for SPX-1 (13-desmethyl spirolide C) was 0.3 ng mL^{-1}, determined with reference to an external standard solution of 10 ng mL^{-1} SPX-1 obtained from the CRMP, IMB, National Research Council, Halifax, NS, Canada.

Spirolides in mussels from the cultivation area at St 13 were determined from whole mussel samples provided by Acuacultura Oceanica Co., Ensenada, BC, Mexico. Evaluation of lipophilic toxins in mussels was performed according to the standardized procedure of the European Union for the determination of marine lipophilic biotoxins in molluscs (European Union Reference Laboratory for Marine Biotoxins [EURLMB], 2015). Complete soft tissues of at least 20 commercial-size mussels were homogenized for the determination of phycotoxins. Toxin extraction was performed with 100% methanol on 2.0 ± 0.01 g of shellfish tissue (European Union Reference Laboratory for Marine Biotoxins [EURLMB], 2015). The detection and quantification of lipophilic phycotoxins was performed with the analytical methodology of liquid chromatography coupled to a tandem mass spectrometer (LC-MS/MS). A triple quadrupole Thermo Scientific TSQ Quantum Access Max LC-MS/MS system (San Jose, CA, United States) was used for analysis of mussel samples collected from July 2013 to June 2014, according to the method of Martín-Morales et al. (2013). After extraction, an aliquot of the methanolic extract was filtered through a 0.22-μm-syringe filter and 5 μL were injected into the LC-MS/MS system. The mass spectrometer was operated in positive-ion mode by multiple reaction monitoring (MRM). The Regueiro et al. (2011) method was employed for chromatographic separation with minor modifications for use of a shorter column. Chromatography was performed in reverse-phase mode with a Gemini-NX C18 (Phenomenex, Torrance, CA, United States) (50 × 2 mm; 3 μm) analytical column. The mobile phase was described by Gerssen et al. (2009) to yield alkaline chromatographic conditions; phase A = ammonium hydroxide 6.7 mM, phase B = acetonitrile in ammonium hydroxide 6.7 mM in proportion 9:1 (v:v). A calibration curve of four concentration levels of a certified solution obtained from CIFGA (Lugo, Spain) was used to quantify 13-desmethyl spirolide C. The limit of detection of the method was 0.04 ng mL^{-1}.

For the mussel samples from 2016 to 2017, toxin analyses were performed by the method of Krock et al. (2008), as described above for analysis of spirolides in plankton samples. An aliquot of methanolic extract of mussel tissue was transferred to a spin-filter (pore-size 0.45 μm; Millipore Ultrafree, Eschborn, Germany) and centrifuged for 30 s at 800 × *g*. The filtered sample was transferred to an autosampler vial and 5 μL were injected into the LC-MS/MS system.

RESULTS

Occurrence of *A. ostenfeldii* in Todos Santos Bay

Light microscopic analysis of the phytoplankton community in 134 water samples collected from August 2016 to August 2017 revealed that *A. ostenfeldii* occurred in 20.9% of the samples. The species was detected in the same number of samples from the surface and the thermocline, indicating that *A. ostenfeldii* was present throughout the mixed layer. Nevertheless, the cell abundance was higher at the surface than at the thermocline. There was no apparent seasonality for the species presence, and the low cell abundances did not allow for definition of a clear pattern. The only discernable trend was the low cell abundance during the winter (November–February), when cell densities were consistently low or below the detection limit (**Figures 2A,B**).

At the surface *A. ostenfeldii* was detected at all stations except St 4, but at low abundance throughout the sampling period (**Figure 2A**). The maximum abundance of 480 cells L^{-1} was registered at St 6 (Salsipuedes) at the end of September 2017 (**Figure 2A**). A relatively high abundance of 400 cells L^{-1} in June 2017 and 240 cells L^{-1} at the beginning of March, 2017 were also recorded from St 11 (Rincón de Ballenas). The species was not observed at the thermocline from St 6 and at the other stations the abundance was low (20–80 cells L^{-1}) or absent (**Figure 2B**). The highest abundance of *A. ostenfeldii* in the thermocline was 353 cells L^{-1} detected at St 11 in June (**Figure 2B**). An abundance of 160 cells L^{-1} was found at the end of September from the same station.

Morphological Characterization of *A. ostenfeldii*

The cells of *A. ostenfeldii* presented a globose appearance with slightly rounded margins (**Figures 3A,G**). The theca is very thin and not easily detected with light microscopy (**Figure 3H**). The specimens from TSB were typically observed as solitary cells varying in size from 25 to 35 μm in diameter, in some water samples both vegetative cells and cysts were found (**Figure 3I**). The cingulum is equatorial and slightly excavated with no presence of cingular lists (**Figures 3B,C**). The hypotheca and epitheca have the same height. The tabulation of the cells is typical of *A. ostenfeldii* showing an insert-type tabulation characterized by the contact of the plate 1' with 1", 6"', 2', 4' and the apical pore complex APC (**Figures 3B,C**). This complex is completely occupied by a big comma-shaped pore (**Figure 3B**). The sixth precingular plate (6") is wider than tall. The 1' plate is elongated and asymmetric, with a prominent ventral pore in the inflection (**Figure 3C**). The sulcal plate series is clearly distinguished. The key plate for describing the sulcus is S.p. This plate contacts with s.d.p, s.sp, 5"', 4"', 2"" and 1"""' (**Figures 3D–F**).

Molecular Identification of *A. ostenfeldii*

Molecular identification of *A. ostenfeldii* was conducted on 57 samples analyzed by PCR from the south of TSB (Rincón de Ballenas, St 13) where mussel farms are located. With the AOF4 and AOR3 primers, the 99 bp ITS fragment was detected in 52.6% of the samples collected from July 2013 to June 2014, confirming the presence of *A. ostenfeldii* in the study area. As expected, given the higher plankton biomass collected among the three alternative samples (at surface, from 10 m depth and vertical net haul) the highest number of positive results (68.4%) were obtained from net haul samples, **Figure 4** shows an example of the PCR results. The expected size amplicon was detected in all three fractions of samples from April 8, 2014 collected at surface, from 10 m and by net haul (**Figure 4**), suggesting that this species was present throughout the mixed layer of the water column. In contrast, the absence of an amplicon in the samples from April 22, 2014 indicates that *A. ostenfeldii* was not present on this date (**Figure 4**).

The samples collected from August 2016 to August 2017 at seven stations and analyzed by PCR with the alternative primers AOST213F and AOST213R also confirmed the presence of *A. ostenfeldii* within the TSB region. The 18S amplification fragment of 89 bp was detected in 23.7% of 156 samples processed. For example, in samples of March 1, 2017, the expected amplification fragment was observed in samples from all stations, except in the surface sample from St 4, St 6 at the thermocline, and from St 13 at both surface and thermocline (**Figure 5**).

A. ostenfeldii was detected in 70.5% of the 213 samples from the two sampling periods analyzed by PCR and light microscopy. The PCR method yielded a higher positive detection (by 26.7%) compared to light microscopy. In contrast, PCR results were negative for only 1.88% of the samples in which *A. ostenfeldii* was registered by light microscopy.

PCR products were sequenced with the different primers for specific dates. For example, the alignments and blast analysis conducted with the 99 bp ITS fragment sequence from the sample of August 8, 2013 (St 13) showed 98% identity with *A. ostenfeldii* sequences found in the NCBI GenBank (JX841278.1).

Spirolide Detection in Plankton and Mussels

The presence of spirolide was confirmed in mussel samples collected at Rincón de Ballenas (St 13), but the analog 13-desmethyl spirolide C was the only analog detected by LC-MS/MS in mussels. The chromatogram presented in **Figure 6** shows the quantitative detection of 13-desmethyl spirolide C in a mussel sample of September, 2014. The retention time (RT) (4.25 min) of the 164 *m/z* ion that coincided with the RT of the 692.501 > 444.30 *m/z* transition used as the confirmatory ion for 13-desmethyl spirolide C. The chromatogram of analogs 13-19 didesmethyl spirolide C and the spirolide B are also presented in **Figure 6**, but these analogs were not detected in mussels; the corresponding signals for these analogs were absent or below the detection limit and/or the retention times did not align with those of the expected transitions.

The cell abundances of *A. ostenfeldii* detected in Rincón de Ballenas area (St 13) at the surface and at 10 m or the thermocline depth were compared with the spirolide accumulation in mussels (**Figure 7**). *A. ostenfeldii* was detected in 50% of the samples

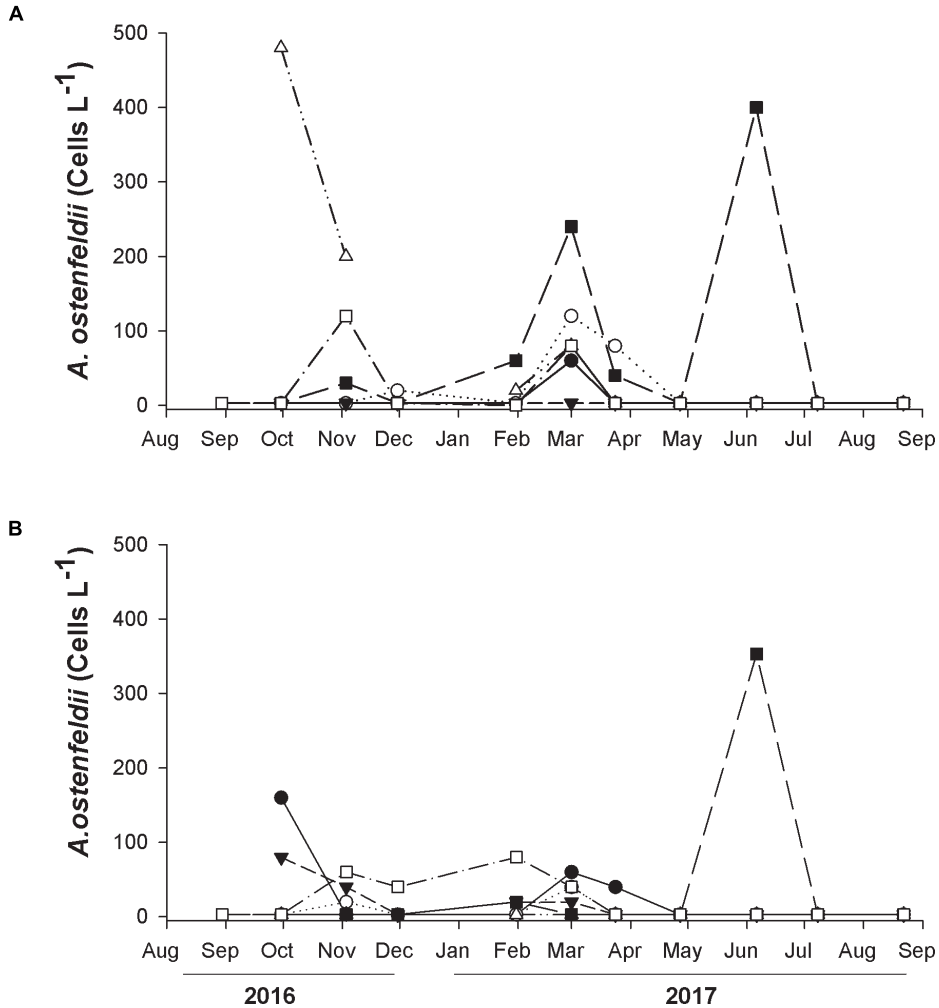


FIGURE 2 | Temporal variation of cell abundance of *A. ostenfeldii* at seven sampling stations located in Todos Santos Bay region: Station 1 (closed circles), Station 3 (open circles), Station 4 (closed triangles), Station 6 (open triangles), Station 11 (closed square), and Station 12 (open square). Samples were collected at surface **(A)** and at the thermocline **(B)** from August 2016 to August 2017.

from 2013 to 2014. The species was heterogeneously distributed from surface to 10 m depth since the detection and abundances coincided in samples from both depths. There was no clear seasonality pattern of occurrence for *A. ostenfeldii* or for the spirolides in mussels. The cell abundance of *A. ostenfeldii* varied throughout the sampling period of 2013–2014 (from 0 to 3.6×10^3 cells L^{-1}). Nevertheless, these variations are not reflected in the concentration of 13-desmethyl spirolide C in mussels, which varied from below the detection limit (0.04 μg kg^{-1}) and remained <1.05 μg kg^{-1} throughout the sampling period (2013–2014) (**Figure 7**). The maximum *A. ostenfeldii* cell abundance was found in October 2013 (3.62×10^3 and 3.59×10^3 cells L^{-1} in surface waters and at 10 m depth, respectively); the concentration of 13-desmethyl spirolide C was 0.54 μg kg^{-1}. From August 2016 to August 2017, *A. ostenfeldii* was absent from all samples, except for a sample obtained at the end of August 2016 (280 cells L^{-1}) at the thermocline. According to the LC-MS/MS results, the concentration of 13-desmethyl spirolide C remained below the detection limit in all of the samples. During the sampling period from 2016 to 2017, no spirolides were detected in the 154 samples of plankton particulate matter.

Certain populations of *A. ostenfeldii* are capable of producing PSP toxins (saxitoxin and derivatives) in addition to or instead of spirolides (Cembella, 2018). As a confirmation, for three samples (from July 8, 2013; August 22, 2013; October 10, 2013) where the cell abundance of *A. ostenfeldii* was high, but the concentration of spirolides in mussels was low, analysis of PSP toxins was carried out on mussels samples. No PSP toxins were detected in these samples.

Water Temperature Associated With the Presence of *A. ostenfeldii*

In an attempt to establish trends of *A. ostenfeldii* cell abundance in relation to water temperature, water temperatures at surface, 5 m and 10 m depth at St 13 are presented together with *A. ostenfeldii*

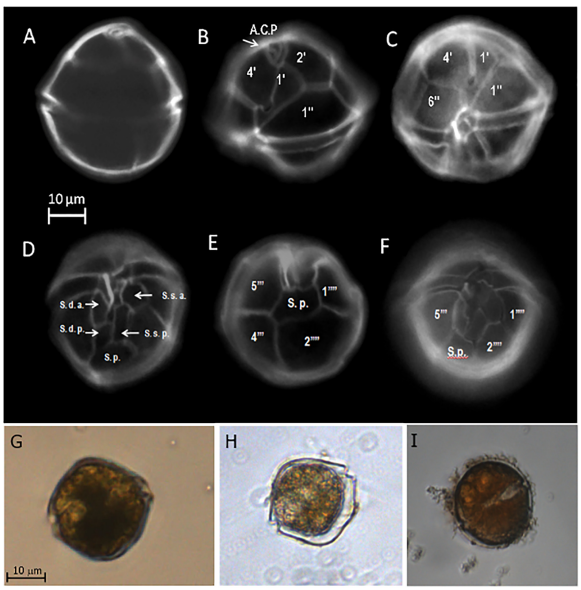


FIGURE 3 | Morphological characteristics of *Alexandrium ostenfeldii* collected in water samples from Todos Santos Bay region. The cells were stained with calcofluor to evaluate the thecal plate arrangement with epifluorescence microscopy. **(A)** Shape of the cells; **(B)** apical pore complex (APC) complex occupied by a prominent comma-shaped pore; **(C)** first precingular (1') plate with a ventral pore; **(D)** ventral view showing the sulcal plates; **(E)** antapical view showing the sulcal posterior plate; **(F)** ventral view; **(G,H)** vegetative cells observed by inverted optical microscopy; **(I)** presence of cysts in the water samples.

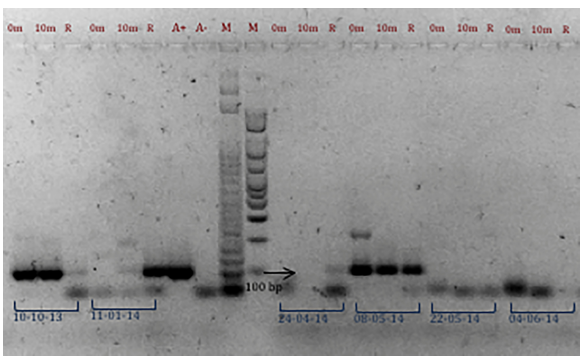


FIGURE 4 | PCR amplification of samples from Station 13 using AOF4 and AOR3 primers. The 99 bp ITS fragment indicates the presences of *A. ostenfeldii*. Results of samples collected at different dates are presented (October 10, 2013; November 1, 2014; April 24, 2014; May 8 and 22, 2014, and June 4, 2014). Samples were collected at 0 and 10 m discrete depth in the water column and by vertical phytoplankton net tows (R). A+: positive PCR control and A–: negative PCR control. M: molecular marker of 50 and 100 bp bioline.

cell abundance during July 2013 to June 2014 and from August 2016 to August 2017 (Rincón de Ballenas) (**Figure 8**). The highest cell abundance of *A. ostenfeldii* was associated with temperatures between 17 and 20°C (**Figure 8**). During 2013–2014, maximum surface temperature of 22.1°C was registered in September 2013.

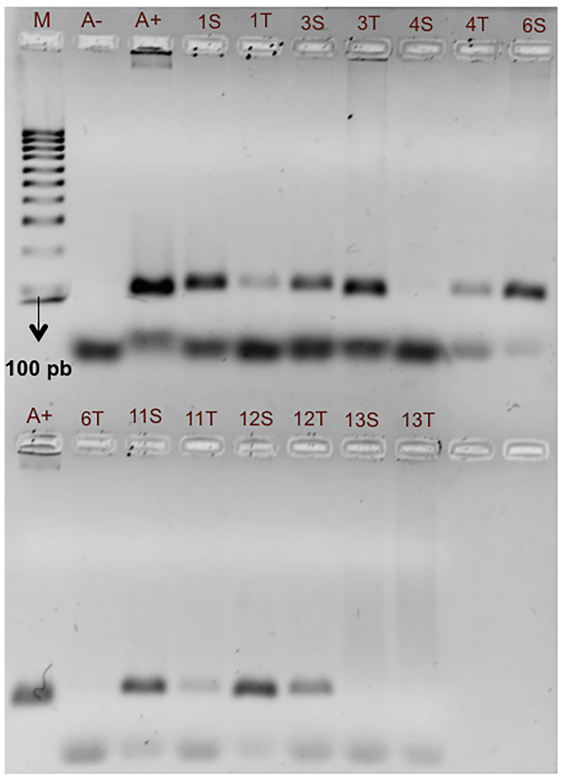


FIGURE 5 | PCR amplification of samples from seven different stations in Todos Santos Bay region using AOST123F and AOST123R primers. The 89 bp fragment indicates the presence of *A. ostenfeldii*. Results of samples collected on March 1, 2017 are presented. The samples were collected at surface (S) and thermocline (T) in the water column. A+: positive PCR control and A–: negative PCR control. M: molecular marker of 100 bp bioline.

The water column was stratified as evidenced by a decrease of 5°C from surface waters at 5 m (17°C) and 8°C at 10 m depth (14°C). Under these conditions, *A. ostenfeldii* was not detected. Abundance of *A. ostenfeldii* was <500 cells L^{-1} during the winter period with lower water temperature (November to April 2013–2014). Strong water stratification was present during the period 2016–2017, with a decrease of 8.5°C from the surface to 10 m depth. During this period, a higher maximum surface temperature of 22.9°C was detected in August 2016 and August 2017. The species was not detected during this period (**Figure 8**). When the water column was homogeneous with respect to temperature and during periods of lower water temperature between November, 2013 and April, 2014 cell abundance was reduced or remained below detection.

DISCUSSION

Dynamics of *A. ostenfeldii* in Todos Santos Bay

The dinoflagellate *A. ostenfeldii* was found in TSB with variable abundances and as a common component of the phytoplankton

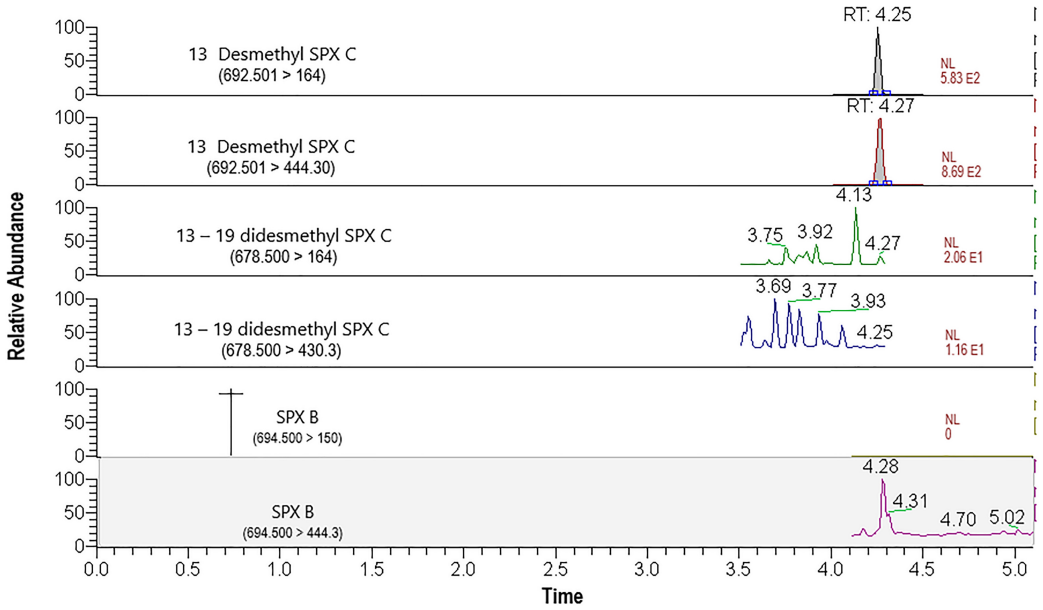


FIGURE 6 | LC-MS/MS chromatograms (in MRM mode) of 13-desmethyl spirolide (SPX) C transitions (692.501 > 164 *m/z* and 692.501 > 444.30 *m/z*). Mass transitions of 13-19-didesmethyl spirolide (SPX) C and spirolide (SPX) B are also shown but were not detected in this sample of Mediterranean mussels (*M. galloprovincialis*) from TSB collected on September 12, 2014.

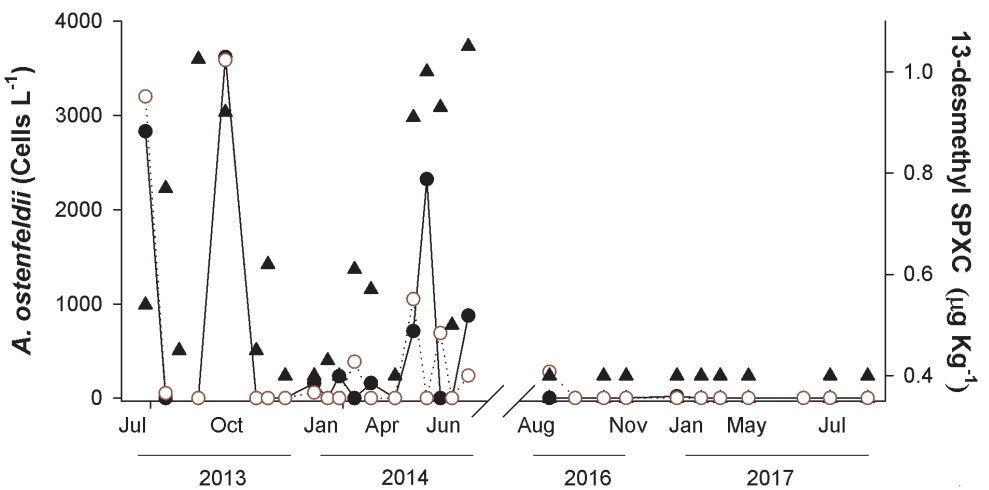


FIGURE 7 | Concentration of 13-desmethyl spirolide (SPX) C (closed triangles) in the Mediterranean mussel *Mytilus galloprovincialis* collected from July 2013 to June 2014 and from August 2016 to August 2017 in Rincón de Ballenas (Todos Santos Bay). The abundance of *A. ostenfeldii* cells at surface (closed circles) and at 10 m or thermocline depth (open circles) is also shown.

community, but this species did not exhibit a clear seasonal occurrence pattern during the sampling period. The maximum cell density (3×10^3 cells L^{-1}) registered at St 13 during late summer to fall 2013 was typical for this species from coastal North Atlantic waters and Scandinavian fjords. In such low water temperature and moderate salinity environments the species tends not to form high biomass blooms, but rather is commonly found as a non-dominant component of the phytoplankton community at low maximal cell abundances ranging typically from 10^2 to 10^3 cells L^{-1} (Balech and Tangen, 1985; Moestrup and Hansen, 1988; Gribble et al., 2005). For example, *A. ostenfeldii* in coastal waters of Nova Scotia tends to form sub-surface aggregations at relatively low cell abundances of less than 4×10^3 cells L^{-1} (Cembella et al., 2001). In Casco Bay, along the Gulf of Maine, United States, a maximum abundance of 244 cells L^{-1} was registered during June 2001 (Gribble et al., 2005). These low abundances contrast with the high bloom concentrations reported from certain locations in northern Europe, typically characterized by a low salinity regime. Maximum cell abundances as high as 2.1×10^5 cells

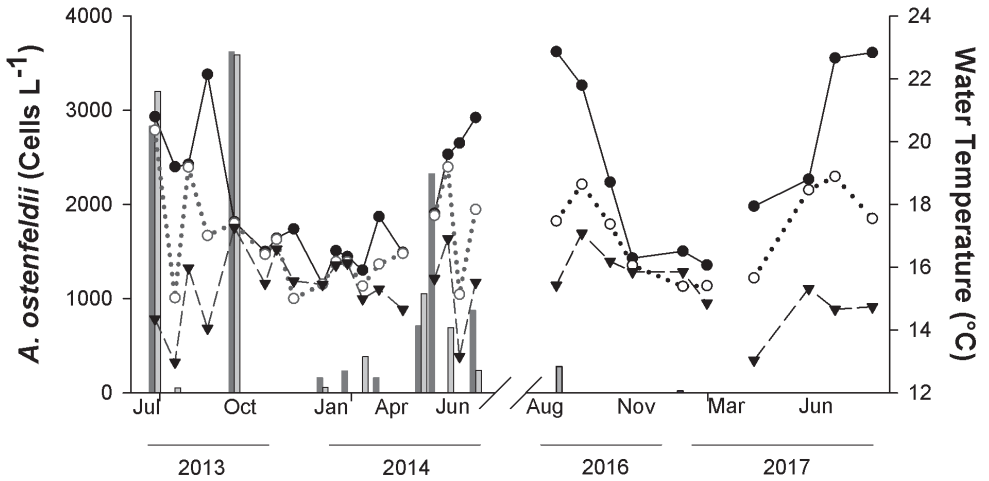


FIGURE 8 | Temperature at surface (closed circles), 5 m (open circles) and 10 m depth (closed triangles) at Station 13 (Rincón de Ballenas) in Todos Santos Bay. The cell abundance of *A. ostenfeldii* in samples at surface (dark gray bar) and 10 m or thermocline (light gray bar) is also presented.

L^{-1} have been recorded from the coast of Finland in the northern Baltic Sea (Hakanen et al., 2012) and in the virtually freshwater Ouwerkerkse Creek, Netherlands, an extreme bloom of *A. ostenfeldii* yielded maximal cell densities of 4.5×10^6 cells L^{-1} (Van de Waal et al., 2015). In any case, the association of extreme blooms of *A. ostenfeldii* with low salinity environments is clearly not applicable to seasonal dynamics of this species in TSB. In comparison with habitats of *A. ostenfeldii* in the North Atlantic, TSB represents an environment characterized by higher seasonal surface water temperatures but only slightly higher salinity, which does not vary dramatically on an annual basis.

Temperature is one of the most important environmental variables that determine the biogeography of phytoplankton species (Butterwick et al., 2005), and which has a direct physiological influence and consequences on metabolic rates and hence growth rate (Anderson, 1998). This suggests a possible regulatory role of temperature in determining the temporal distribution of *A. ostenfeldii* in TSB. In spite of the lack of a definitive seasonal cell abundance pattern, and absence of a clear relationship between the presence of *A. ostenfeldii* and the ambient temperature, the species was apparently not present in the water column during the winter. The cell abundance of *A. ostenfeldii* in TSB decreased from August 2016 to August 2017 to the comparable period in 2013–2014. This indicates that the temporal cell distribution and abundance may be related to prior temperature anomalies. Under this scenario, a low cell abundance of *A. ostenfeldii* could be related to shifts in the phytoplankton community associated with the abnormally warm water conditions in the region, that initiated 3 years before 2016, associated with el Niño events, with temperature anomalies above 3°C (García-Mendoza et al., 2018). These anomalies were accompanied by low phytoplankton biomass. In the California Current System status report these changes are also described for the phytoplankton community, for which the productivity and cell concentrations of diatoms were below average for the southern California coast during 2016 (McClatchie et al., 2016). Jacox et al. (2018) reported that the water temperature anomalies caused changes in the structure and composition of the phytoplankton community, perhaps disadvantageous to *A. ostenfeldii*, while favoring an increase in cell abundances of species better adapted to these conditions. These warm water conditions recorded in TSB in the last few years have caused blooms of other genera such *Cochlodinium* and *Chattonella* that had been previously registered only in low densities in the bay.

Alexandrium ostenfeldii is often considered a "cold-water" species, but "blooms" (or at least maximal cell abundance) consistently occur during the warm period of the annual cycle in the respective environments. In TSB, two peaks of high cell abundance were detected in October, 2013 and May, 2014, when the surface water temperature was 17°C, but also when surface water was around 20°C. This suggests an optimal temperature window of 17–20°C for *A. ostenfeldii* in TSB. By comparison, massive blooms of *A. ostenfeldii* in the northern Baltic Sea occurred at water temperatures close to 20°C (Hakanen et al., 2012). In Narragansett Bay, Gulf of Maine, United States, maximum cell abundances were found within a temperature range from 14.0 to 17.0°C and the species (cited as synonym *A. peruvianum*) was not detected from the water column during autumn when the temperatures were between 13 and 17°C (Borkman et al., 2012). In contrast, in the Beagle Channel, Argentina the species was recorded at lower temperatures (7.5–10°C) during austral spring (Almandoz et al., 2014). The fact that *A. ostenfeldii* is globally distributed (Cembella, 2018) indicates that temperature-adapted variants are common and that comparisons of temperature tolerance windows among geographically disjunct populations are not strictly valid.

Due to the increasing global temperature the surface ocean is anticipated to become more stratified (Stocker et al., 2013). Garneau et al. (2011) recorded the maximum cell abundance for *A. catenella* in summer–autumn along the coast of Oregon and northern California, during the dry season (reduced freshwater runoff) with weak upwelling and low nutrients in surface

waters. Stratification of the water column forming a superficial stable layer may favor the growth of *Alexandrium* over other phytoplankton species during low nutrient conditions in this stratified layer (Horner et al., 1997). Under such circumstances, the influence of the ambient water temperature on the temporal and spatial distribution of *A. ostenfeldii* may also be indirect, via contribution to stratification of the water column. More stratification of the water column should favor growth and persistence of species such as *A. ostenfeldii*, a facultatively mixotrophic dinoflagellate with vertical migration capabilities. This interpretation is consistent with the evidence that cells were absent from the water column at TSB during winter (the 2013–2014 monitoring period) and remained <500 cells L^{-1} during 2016–2017, when the water column was well mixed and homogeneous. Yet a simple association of *A. ostenfeldii* with high stratification is not supported by the data on the apparent lack of cells in the upper water column during August 2016, during a period of high surface water temperature (22.9°C) and strong stratification.

Nutrients are known to influence the dynamics of phytoplankton communities in rivers, estuaries and coastal zones (Anderson et al., 2002). In the same way, inorganic macronutrients could directly influence growth of *A. ostenfeldii* but there were no massive blooms recorded during the sampling periods in TBS. Furthermore, the high mixotrophic potential of this species renders it unlikely that inorganic macronutrient concentrations were directly correlated with or driving cell growth or biomass yield in TSB. This suggestion agrees with both Collos et al. (2007) and Brandenburg et al. (2017) who reported that proliferation of cells of *Alexandrium* species were independent of the inorganic nutrient concentrations because of mixotrophic feeding of the species. During a bloom in the Baltic Sea, the abundance of *A. ostenfeldii* cells was related with warm water, but was not significantly correlated with dissolved inorganic nutrient concentrations, however, nutrient concentration were related to the high concentration of resting cyts in the sediment (Hakanen et al., 2012).

Species Detection and Identification

The presence of *A. ostenfeldii* in TSB was independently confirmed by both light microscopy and positive PCR assays applied to 213 field samples during the two multi-annual periods. There were significant discrepancies between the results of the alternative methods, e.g., the molecular approach yielded 27.6% higher positive detection of the species than microscopy. By comparison, positive registration of toxic dinoflagellate species in the Mediterranean Sea by microscopy did not coincide with detection of the species by PCR. These differences were especially evident with members of the genus *Alexandrium*, and varied among species; the PCR method gave 38% higher detection for *A. catenella*, 36% for *A. minutum* and 20% for undefined *Alexandrium* species (Penna et al., 2007).

These discrepancies are related to differences in cell detection limits for the respective methods. The nominal detection limit of the Utermöhl method is 20 cells L^{-1} in a sedimentation volume of 50 mL when the whole chamber is counted (Edler and Elbrächter, 2010). In comparison, with a reported PCR amplification with 1 pg of genomic DNA for *Alexandrium* (Penna et al., 2007), and an estimated 115 pg per cell nuclear DNA content for *A. ostenfeldii* (Kremp et al., 2009), it is then theoretically possible to detect a single cell of this species in a field sample by PCR technique. Therefore, the possibility of detecting species that are poorly represented in the phytoplankton community, like *A. ostenfeldii,* increased significantly due the use of the PCR technique.

In a few cases (1.88%), *A. ostenfeldii* cells were identified from TSB samples by microscopy but were not detected by PCR. This may be attributable to misidentification of *Alexandrium* taxa due to the difficulty of morphological discrimination among closely related species within this genus (Touzet et al., 2009; Cembella, 2018). In TSB, *A. ostenfeldii* may be present in different life history (vegetative cells, gametes, zygotes) and growth stages of variable morphology. Furthermore, other species of *Alexandrium* genus, such as *A. catenella* (Peña-Manjarrez et al., 2005), may be present in TSB, and could be misidentified as *A. ostenfeldii* in the absence of critical taxonomic analysis.

Accumulation of Spirolides in Mussels

The dinoflagellate *A. ostenfeldii* was likely responsible for the accumulation of spirolides in mussels collected in the TSB region during 2012 (García-Mendoza et al., 2014). Among the diverse structural sub-types and known analogs of spirolides, only 13-desmethyl spirolide C was detected in cultivated mussels from TSB. This indicates that *A. ostenfeldii* populations in the region produce primarily or exclusively this spirolide analog and not other toxins such as PSTs associated with certain *A ostenfeldii* strains from global locations. In a phylogenetic study comparing multiple *A. ostenfeldii* strains from widely biogeographically distributed populations, six groups were defined that cluster genetically and according to toxin profile (Kremp et al., 2014). Group 1 strains produce both 13-desmethyl spirolide C and PSTs (Van Wagoner et al., 2011). In contrast, Group 2 members produce mostly or exclusively 13-desmethyl spirolide C (Franco et al., 2006; Touzet et al., 2008). The strain present in TSB therefore seems to belong to this latter group. Populations of *A. ostenfeldii* Group 2 have been registered mainly from eastern Canada, northeast United States, Spain and Irish coasts (Cembella A. D. et al., 2000; MacKinnon et al., 2004; Percy et al., 2004). However, it is necessary to isolate local strains to characterize their toxin profile and to confirm their phylogenetic relation with other populations of *A. ostenfeldii*. In addition, it is necessary to evaluate the toxin content of vegetative cells and cyts since this might influence the concentrations of spirolides in mussels.

From 2013 to 2014 13-desmethyl spirolide C was detected in 78.9% of the samples analyzed. There was no apparent seasonality in the presence of this toxin, as typified by the time-series data from 2012 when 13-desmethyl spirolide C was detected in most (80%) of the mussel samples analyzed and was present all year long without a clear pattern of appearance (García-Mendoza et al., 2014). In contrast, during the period from 2016 to 2017, 13-desmethyl spirolide C was below the detection limit and this was related with the absence of the causative species.

Concentrations of 13-desmethyl spirolide C in this study were lower than the concentrations recorded in shellfish from other regions. In Catalonia, NW Mediterranean Sea, 2–16 μg kg^{-1} of 13-desmethyl spirolide C was detected in mussels and oysters (García-Altarez et al., 2014). In shellfish samples from the Chinese coast the highest concentration of 13-desmethyl spirolide C was 8.96 μg kg^{-1} (Wu et al., 2015) and in Norwegian blue mussels the concentration of this toxin reached 226 μg kg^{-1} (Rundberger et al., 2011). The concentration of this spirolide previously reported in mussels from TSB was higher during the year 2012 (3 μg kg^{-1}) (García-Mendoza et al., 2014) than recorded in this present study (1.05 μg kg^{-1}).

In July, 2013 among the highest abundance for the species was recorded (2.8 and 3.2 × 10^3 cells L^{-1}), but the concentration of the 13-desmethyl spirolide C was low (0.54 μg kg^{-1}). The toxin cell quota is variable and dependent upon environmental conditions; the physiological state of the cell and the growth phase influence the cellular concentration of the toxin (Boczar et al., 1998; Anderson et al., 2012). This variability could explain the fact that even though one of the highest abundance of *A. ostenfeldii* was recorded and presence of the species was confirmed by PCR, the concentration of 13-desmethyl spirolide C was near the detection limit. Environmental conditions during this sampling period could influence the spirolide production in *A. ostenfeldii* present in the bay, and this could explain why there was not a clear correlation between the abundance of the species and the accumulation of spirolide in mussels.

At the end of August, 2013 and middle of September, 2013 *A. ostenfeldii* was not detected in the water samples, but, nevertheless, one of the highest concentration of 13-desmethyl spirolide C in this study (1.02 μg kg^{-1}) was recorded from mussels. Jensen and Moestrup (1997) have proposed that the wide variation in cell concentrations of this species can also be due to rapid shifts between pelagic and benthic stages in shallow waters. Furthermore, *A. ostenfeldii* has been found to actively produce temporary cysts in cultures (Hakanen and Kremp, unpubl. data) and live field samples, although the trigger mechanisms are not always understood. It has been reported that when there is only low abundance of *A. ostenfeldii* vegetative cells in the water column, the population may have already undergone a life history transition by forming resting cysts deposited to benthic sediments as "seedbeds" for subsequent blooms (MacKenzie et al., 1996). These resting cysts remain toxic and can be ingested by shellfish directly from the sediments or via resuspension events driven by wind and other hydrodynamic mechanisms disrupting the sediments. In such cases, the relatively high concentration of 13-desmethyl spirolide C in the absence of vegetative cells in the water column could be associated with a rapid proliferation of cysts and remobilization from the sediments. A routine operation conducted at shellfish aquaculture farms is the lifting and physical displacement of lines that sink by the weight of the product (oyster modules, mussel socks, etc.), and which thereby causes sediment disruption and resuspension. We cannot therefore discard the possibility that *A. ostenfeldii* cysts were available for the mussels during previous days before sampling the water column and that the mussels accumulated the toxin from such cysts, even when vegetative cells could not be detected.

CONCLUSION

Integration of analytical techniques, i.e., optical microscopy, specific primers for species identification by PCR and LC-MS/MS for toxin determination in plankton and shellfish tissue matrices, allowed the confirmation of *A. ostenfeldii* as the primary and perhaps exclusive source of spirolide in TSB, and is the first record of the species in the northwest Pacific coast of Mexico. The species is a common component of the phytoplankton community but, typically occurs in low cell abundances. The analog 13-desmethyl spirolide C was the only spirolide detected in mussels from TSB, which suggests a very restricted toxin profile is produced by the dinoflagellate. The species was not consistently detected when strong stratification and higher temperatures were recorded in the water column, indicating that these environmental conditions are not favorable for the accumulation of this species in the region.

AUTHOR CONTRIBUTIONS

PP-B determined the abundance of *A. ostenfeldii* cells, extracted the spirolide toxins, conducted the PCR reactions, analyzed and interpreted the data, and wrote the primary draft manuscript. EG-M and EP-R analyzed, interpreted, and reviewed the data for accuracy and integrity, and co-wrote and edited the manuscript. AC did a critical review of the manuscript, and co-authored and edited it. JB performed LC-MS/MS spirolide analysis. AA-B performed the morphological description of the species and CG-S helped with the molecular work.

FUNDING

This work was funded by CONACyT scholarship 280225 – CV 468796; FORDECYT – CONACyT project number 260040-2015; and Red Temática CONACyT (RedFAN) 2015-2017 projects.

ACKNOWLEDGMENTS

We thank Acuacultura Oceanica Co. for providing the cultivated mussels for toxin analysis, and Bernd Krock and Annegret Müller (AWI) for analysis of mussels and filtered plankton samples for spirolides by LC-MS/MS for the period 2016–2017. The participation and financing of Allan Cembella and AWI co-workers was within the PACES II Research Program (Topic II Coast: WP3) of the Alfred-Wegener-Institut, Helmholtz Zentrum für Polar und Meeresforschung under the general theme Earth and Environment, Helmholtz Gemeinschaft, Germany. We also thank CONACYT for financial support of the PhD scholarship 280225 to PP-B, and the FORDECYT project 260040-2015, as well as Red Temática RedFAN 2015-2017 projects for travel funding for the morphological identification in CICY.

REFERENCES

Almandoz, G., Montoya, N., Hernando, M., Benavides, H., Carignan, M., and Ferrario, M. (2014). Toxic strains of the *Alexandrium ostenfeldii* complex in southern South America (Beagle Channel, Argentina). *Harmful Algae* 37, 100–109. doi: 10.1016/j.hal.2014.05.011

Anderson, D., Glibert, P. J., and Burkholder, J. (2002). Harmful algal blooms and Eutrophication: nutrient sources, composition and consequences. *Estuaries* 25, 704–706. doi: 10.1007/BF02804901.

Anderson, D. M., Alpermann, T. J., Cembella, A. D., Collos, Y., Masseret, E., and Montresor, M. (2012). The globally distributed genus alexandrium: multifaceted roles in marine ecosystems and impacts on human health. *Harmful Algae* 14, 10–35. doi: 10.1016/j.hal.2011.10.012

Anderson, D. M. (1998). "Physiology and bloom dynamics of toxic Alexandrium species, with emphasis on life cycle transitions," in *Physiological Ecology of Harmful Algal Blooms* eds D. M. Anderson, A. D. Cembella, and G. M. Hallegraeff (Heidelberg: Springer-Verlag), 29–40.

Balech, E. (1995). *The Genus Alexandrium Halim (Dinoflagellata)*. Sherkin Island: Sherkin Island Marine Station.

Balech, E., and Tangen, K. (1985). Morphology and taxonomy of toxic species in the *tamarensis* group (Dinophyceae): *Alexandrium excavatum* (Braarud) comb. nov. and *Alexandrium ostenfeldii* (Paulsen) comb. nov. *Sarsia* 70, 333–343. doi: 10.1080/00364827.1985.10419687

Boczar, B., Beitler, M., Liston, J., Sullivan, J., and Cattolico, R. (1998). Paralytic shellfish toxins in *Protogonyaulax tamarensis* and *Protogonyaulax catenella* in axenic culture. *Plant Physiol.* 88, 1285–1290. doi: 10.1104/pp.88.4.1285

Borkman, D. G., Smayda, T. J., Tomas, C. R., York, R., Strangman, W., and Wright, J. (2012). Toxic *Alexandrium peruvianum* (Balech and de Mendiola) Balech and Tangen in Narragansett bay, Rhode Island (USA). *Harmful Algae* 19, 92–100. doi: 10.1016/j.hal.2012.06.004

Brandenburg, K., de Senerpont Domis, L., Wohlrab, S., Krock, B., Uwe, J., Van Scheppingen, Y., et al. (2017). Combined physical, chemical and biological factors shape Alexandrium ostenfeldii blooms in the Netherlands. *Harmful Algae* 64, 146–153. doi: 10.1016/j.hal.2017.02.004

Butterwick, C., Heaney, S. I., and Talling, J. F. (2005). Diversity in the influence of temperature on the growth rates of freshwater algae, and its ecological relevance. *Freshw. Biol.* 50, 29–300. doi: 10.1111/j.1365-2427.2004.01317.x

Cembella, A., Lewis, N., and Quilliam, M. (2000). The marine dinoflagellate *Alexandrium ostenfeldii* (Dinophyceae) as the causative organism of spirolide shellfish toxins. *Phycologia* 39, 67–74. doi: 10.2216/i0031-8884-39-1-67.1

Cembella, A. D., Bauder, A. G., Lewis, N. I., and Quilliam, M. A. (2000). "Population dynamics and spirolide composition of the toxigenic dinoflagellate *Alexandrium ostenfeldii,*" in *Coastal Embayments of Nova Scotia*, eds G. M. Hallegraeff, S. I., Blackburn, J. S. Bolch, and R. J. Lewis (Hobart: Intergovernmental Oceanographic Commission), 173–176.

Cembella, A. D. (2018). "Chapter 16 harmful algal species fact sheets," in *Harmful Algal Blooms: A Compendium Desk Reference*, eds S. Alexandrium, J. Shumway, and S. Morton (Hoboken, NJ: Wiley-Blackwell).

Cembella, A. D., Bauder, A. G., Lewis, N. I., and Quilliam, M. A. (2001). Association of the gonyaulacoid dinoflagellate *Alexandrium ostenfeldii* with spirolide toxins in size-fractionated plankton. *J. Plankton Res.* 23, 1413–1419. doi: 10.1093/plankt/23.12.1413

Cembella, A. D., Lewis, N. I., and Quilliam, M. A. (1999). Spirolide composition in micro-extracted pooled cells isolated from natural plankton assemblages and from cultures of the dinoflagellate *Alexandrium ostenfeldii*. *Nat. Toxins* 8, 197–206. doi: 10.1002/1522-7189(200009/10)7:5<197::AID-NT62>3.0.CO;2-H

Collos, Y., Vaquer, A., Laabir, M., Abadie, E., Laugier, T., Pastoureaud, A., et al. (2007). Contribution of several nitrogen sources to growth of *Alexandrium catenella* during blooms in Thau lagoon, southern France. *Harmful Algae* 6, 781–789. doi: 10.1016/j.hal.2007.04.003

Edler, L., and Elbrächter, M. (2010). "The utermöhl method for quantitative phytoplankton analysis," in *Microscopic and Molecular Methods for Quantitative Phytoplankton Analysis*, eds B. Karlson, C. Cusack, and E. Bresnan (Paris: UNESCO).

Elferink, S., Neuhaus, S., Wohlrab, S., Toebe, K., Voß, D. Gottschling, M., Lundholm, N., et al. (2017). Molecular diversity patterns among various phytoplankton size-fractions in West Greenland in late summer. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 121, 54–69. doi: 10.1016/j.dsr.2016.11.002

European Union Reference Laboratory for Marine Biotoxins [EURLMB] (2015). *EU Harmonized Standard Operating Procedure for Determination of Lipophilic Marine Biotoxins in Molluscs by LC-MS/MS. Version 5*. Available at: http://www.aecosan.msssi.gob.es/CRLMB/docs/docs/metodos_analiticos_de_desarrollo/EU-Harmonised-SOP-LIPO-LCMSMS_Version5.pdf

Franco, J. M., Paz, B., Riobo, P., Pizarro, G., Figueroa, R., Fraga, S., et al. (2006). "First report of the production of spirolides by *Alexandrium peruvianum* (Dinophyceae) from the Mediterranean Sea," in *Proceedings of the Abstracts 12th International Conference on Harmful Algae, Copenhagen,* Denmark

Fritz, L., and Triemer, R. E. (1985). A rapid simple technique utilizing Calcofluor White M2R for the visualization of dinoflagellate thecal plates. *J. Phycol.* 21, 662–664. doi: 10.1111/j.0022-3646.1985.00662.x

García-Altarez. M., Casanova, A., Bane, V., Diogène, J., Furey, A., and De la Iglesia, P. (2014). Confirmation of pinnatoxins and.spirolides in shellfish and passive samplers from Catalonia (Spain) by liquid chromatography coupled with tripe quadrupole and high resolution hybrid tandem mass spectrometry. *Mar. Drugs* 12, 3706–3732. doi: 10.3390/md12063706

García-Mendoza, E., Cáceres-Martínez, J., Rivas, D., Fimbres-Martínez, M., Sánchez-Bravo, Y., Vásquez-Yeomans, R., et al. (2018). Mass mortality of cultivated northern bluefin tuna *Thunnus thynnus* orientalis associated with *Chattonella marina* in Baja California, Mexico. *Front. Mar. Sci.* 5:454. doi: 10.3389/fmars.2018.00454

García-Mendoza, E., Sánchez-Bravo, Y., Turner, A., Blanco, J., O'Neil, A., Mancera-Flores, J., et al. (2014). Lipophilic toxins in cultivated mussels (*Mytilus galloprovincialis*) from Baja California, México. *Toxicon* 90, 111–123. doi: 10.1016/j.toxicon.2014.07.017

Garneau, M. E., Schnetzer, A., Counway, P. D., Jones, A. C., Seubert, E. L., and Caron, D. A. (2011). Examination of seasonal dynamic of the toxic dinoflagellate *Alexandrium catenella* at Redondo beach, California by quantitative PCR. *Appl. Environ. Microbiol.* 77, 7669–7680. doi: 10.1128/AEM.06174-1

Gerssen, A., Mulder, P. P., McElhinney, M. A., and Boer, J. (2009). Liquid chromatography-tandem massspectrometry method for the detection of marine lipophilic toxins under alkaline conditions. *J. Chromatogr.* 1216, 421–430. doi: 10.1016/j.chroma.2008.12.099

Gill, S., Murphy, M., Clausen, J., Richard, D., Quilliam, M., MacKinnon, S., et al. (2003). Neural injury biomarkers of novel shellfish toxins, spirolides: a pilot study using immunochemical and transcriptional analysis. *Neurotoxicology* 24, 593–604. doi: 10.1016/S0161-813X(03)00014-7

Gribble, K., Keafer, B., Quilliam, M., Cembella, A., Kulis, D., Manahan, A., et al. (2005). Distribution and toxicity of *Alexandrium ostenfeldii* (Dinophyceae) in the Gulf of Maine, USA. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 52, 2745–2763. doi: 10.1016/j.dsr2.2005.06.018

Hakanen, P., Suikkanen, Frazén, J., and Frazén, H. (2012). Bloom and toxin dynamics of *Alexandrium ostenfeldii* in shallow embayment at the SW coast of Finland, northern Baltic Sea. *Harmful Algae* 15, 91–99. doi: 10.1016/j.hal.2011.12.002

Hall, T. (1999). BioEdit: a user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Sym. Ser.* 41, 95–98.

Hargraves, P. E., and Maranda, L. (2002). Potentially toxic of harmful microalgae from the northeast coast. *Northeastern Nat.* 9, 81–120. doi: 10.1656/1092-6194(2002)009[0081:PTOHMF]2.0.CO;2

Horner, R. A., Garrison, D. L., and Plumley, F. G. (1997). Harmful algal blooms and red tide problems on the U.S *west coast. Limnol. Oceanogr.* 42, 1076–1088. doi: 10.4319/lo.1997.42.5_part_2.1076

Hu, T., Burton, I. W., Cembella, A. D., Curtis, J. M., Quilliam, M. A., Walter, J. A., et al. (2001). Characterization of spirolides A, C, and

13-desmethyl C, new marine toxin isolated from toxic plankton and contaminated shellfish. *J. Nat. Prod.* 64, 308–312. doi: 10.1021/np000416q

Hu, T., Curtis, J. M., Walter, J. A., and Wright, J. L. (1996). Characterization of biologically inactive spirolides E and F: identification of the spirolide pharmacophore. *Tetrahedron Lett.* 37, 7671–7674. doi: 10.1016/0040-4039(96)01721-2

Hu, T., Curtis, J. M., Oshima, Y., Quilliam, M. A., Walter, J. A., Watson-Wright, W. M., et al. (1995). Spirolides B and D, two novel macrocycles isolated from the digestive glands of shellfish. *J. Chem. Soc. Chem. Commun.* 2159–2161. doi: 10.1039/C39950002159

Jacox, M. G., Alexander, M. A., Mantua, N. J., Scott, J. D., Hervieux, G., Webb, R. S., et al. (2018). Forcing of multiyear extreme ocean temperatures that impacted California current living marine resources in 2016. *Bull. Amer. Meteor. Soc.* 99, S27–S33. doi: 10.1175/BAMS-D-17-0119.1

Jensen, M, Ø., and Moestrup, Ø. (1997). Autoecology of the toxic dinoflagellate *Alexandrium ostenfeldii*: life history and growth at different temperatures and salinities. *Eur. J. Phycol.* 32, 9–312. doi: 10.1080/09541449710001719325

Kremp, A., Lundholm, T., Drebler, N., Erler, K., Gerdts, G., Eirtovaara, S., et al. (2009). Bloom forming *Alexandrium ostenfeldii* (Dinophyceae) in shallow waters of the Aland Archipelago Northern Baltic Sea. *Harmful Algae* 8, 318–328. doi: 10.1016/j.hal.2008.07.004

Kremp, A., Tahvanainen, P., Litaker, W., Krock, B., Suikkanen, S., Leaw, C. P., et al. (2014). Phylogenetic relationships, morphological variation and toxin patterns in the *Alexandrium ostenfeldii* (Dinophyceae) complex: implications for species boundaries and identities. *J. Phycol.* 50, 81–100. doi: 10.1111/jpy.12134

Krock, B., Tillman, U, John, U., and Cembella, A. (2008). LC-MS/MS aboard ship: tandem mass spectrometry in the search for phycotoxins and novel toxigenic plankton from the North Sea. *Anal. Bioanal. Chem.* 392, 797–803. doi: 10.1007/s00216-008-2221-7

Lund, J. W. G., Kipling, C., and Le Cren, E. D. (1958). The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. *Hydrobiology* 11, 143–170. doi: 10.1007/BF00007865

MacKenzie, L., White, D., Oshima, Y., and Kapa, J. (1996). The resting cyst and toxicity of *Alexandrium ostenfeldii* (Dinophyceae) in New Zealand. *Phycologia* 35, 148–155. doi: 10.2216/i0031-8884-35-2-148.1

MacKinnon, S., Cembella, A., Quilliam, M., LeBlanc, P., Lewis, N., Hardstaff, W., et al. (2004). "The characterization of two new spirolides isolated from Danish strains of the toxigenic dinoflagellate *Alexandrium ostenfeldii*," in *Proceedings of the 10th International Conference on Harmful Algae, Florida Fish and Wildlife Conservation Commission*, eds K. A. Steidinger, J. H. Landsberg, C. R. Tomas, and G. A, Vargo (Paris: Intergovernmental Oceanographic Commission of UNESCO).

Maeda Martínez, A. N. (2008). "Estado actual del cultivo de bivalvos en México," in *Estado Actual del Cultivo y Manejo de Moluscos Bivalvos y su Proyección Futura: Factores que Afectan su Sustentabilidad en América Latina*, eds A. Lovatelli, A. Farías, and I. Uriarte (Puerto Montt: Taller Técnico Regional de la FAO), 91–100.

Martín-Morales, E., Mariño, C., Arévalo, F., Correa, J., Moroño, A., and Blanco, J. (2013). "Marine and freshwater toxins analysis," in *Proceedings of the Fourth Joint Symposium and AOAC Task Force Meeting*, Baiona. doi: 10.13140/RG.2.1.2912.6647

McClatchie, S., Goericke, R., Leising, A., Auth, T. D., Bjorkstedt, E., Robertson, R., et al. (2016). State of the California current 2015–16: comparisons with the 1997–98 El Niño. *Calif. Coop. Ocean Fish. Invest. Rep.* 57, 5–61.

Moestrup, Ø., and Hansen, P. J. (1988). On the occurrence of the potentially toxic dinoflagellates *Alexandrium tamarense* (=*Gonyaulax excavata*) and *A. ostenfeldii* in Danish and Faroese waters. *Ophelia* 28, 195–213. doi: 10.1080/00785326.1988.10430813

Otero, A., Chapela, M. J., Atanassoya, M., Vieites, J. M., and Cabado, A. G. (2011). Cyclic imines: chemistry and mechanism of action: a review. *Chem. Res. Toxicol.* 11, ox1817–ox1829. doi: 10.1021/tx200182m.

Peña-Manjarrez, J. L., Helenes, J., Gaxiola-Castro, G., and Orellana-Cepeda, E. (2005). Dinoflagellate cysts and bloom events at Todos Santos Bay, Baja California, México, 1999–2000. *Continental Shelf Res.* 25, 1375–1393. doi: 10.1016/j.csr.2005.02.002

Penna, A., Bertozzini, E., Battocchi, C., Galluzzi, L., Giacobbe, M., Vila, M., et al. (2007). Monitoring of HAB species in the Mediterranean sea through molecular methods. *J. Plankton Res.* 29, 19–38. doi: 10.1093/plankt/fbl053

Percy, L., Morris, S., Higman, W., Stone, D., Hardstaff, W. R., Quilliam, M. A., et al. (2004). "Identification of *Alexandrium ostenfeldii* from the Fal Estuary, UK; morphology, molecular taxonomy and toxin composition," in *Proceedings of the XI International Conference on Harmful Algal Blooms*, Cape Town, 209.

Regueiro, J., Rossignoli, A. E., Alvarez, G., and Blanco, J. (2011). Automated online solid-phase extraction coupled to liquid chromatography tandem mass spectrometry for determination of lipophilic marine toxins in shellfish. *Food Chem.* 129, 533–540. doi: 10.1016/j.foodchem.2011.04.054

Richard, D., Arsenault, E., Quilliam, M.A., and Cembella, A.D. (2001). "Investigations into the toxicology and pharmacology of spirolides, a novel group of shellfish toxins," in *Harmful Algal Blooms 2000*, eds G. M. Hallegraeff, S. I. Blackburn, C. J. Bolch, and R. J. Lewis (Paris: International Oceanographic Commission), 383–386.

Rundberger, T., Aasen, J. A., Selwood, A. L., and Miles C. O. (2011). Pinnatoxins and spirolides in Norwegian blue mussels and seawater. *Toxicon* 58, 700–711. doi: 10.1016/j.toxicon.2011.08.008

Sánchez, S., Villanueva, P., and Carbajo, L. (2004). "Distribution and concentration of *Alexandrium peruvianum* (Balech and de Mendiola) in the Peruvian coast (03°24'–18°20' LS) between 1982–2004," in *Proceedings of the XI International Conference on Harmful Algal Blooms*, Cape Town.

Sánchez-Bravo, Y. (2013). *Concentración de Toxinas Lipofílicas en el Mejillón Mediterráneo (Mytilus galloprovincialis) Asociadas a la Presencia del Dinoflagelado del Género Dinophysis Durante Enero a Diciembre del 2012, en la Bahía de Todos Santos, B.C, México*. Tesis de licenciatura, Universidad Autónoma de Baja California, México City.

Schwarz, E. (2011). *Molecular and Morphological Characterization of Alexandrium species (Dinophyceae) from the East Coast, USA*. Master's thesis, University of North Carolina Wilmington, Wilmington, NC.

Sleno, L., Chalmers, M. L., and Volmer, D. A. (2004). Structural study of spirolide marine toxins by mass spectrometry. Part II. Mass spectrometric characterization of unknown spirolides and related compounds in a cultured phytoplankton extract. *Anal. Bioanal. Chem.* 378, 977–986. doi: 10.1007/s00216-003-2296-0

Stocker, T. F., Qin, D., Plattner, G. K., Tignor, M., Allen, S. K., Boschung, J., et al. (2013). Climate change 2013: the physical science basis. *Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge: Cambridge University Press, 1535.

Suikkanen, S., Kremp, A., Hautala, H., and Krock, B. (2013). Paralytic shellfish toxins or spirolides? The role of environmental and genetic factors in toxin production of the *Alexandrium ostenfeldii* complex. *Harmful Algae* 26, 52–59. doi: 10.1016/j.hal.2013.04.001

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. (1997). The ClustalX windows interfase: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acid Res.* 25, 4876–4882. doi: 10.1093/nar/25.24.4876

Touzet, N., Franco, J. M., and Raine, R. (2008). Morphogenetic diversity and biotoxin composition of *Alexandrium* (Dinophyceae) in Irish coastal waters. *Harmful Algae* 7, 782–797. doi: 10.1016/j.hal.2008.04.001

Touzet, N., Keady, E., Raine, R., and Maher, M. (2009). Evaluation of taxa-species real time PCR, whole-cell FISH and morphotaxonomy analyses for the detection and quantification of the toxic microalgae *Alexandrium minutum* (Dinophyceae), global clade ribotype. *FEMS Microbiol. Ecol.* 67, 329–341. doi: 10.1111/j.1574-6941.2008.00627.x

Trainer, V., Moore, L., Bill, B., Adams, N., Harrington, N., Borchert, J., et al. (2013). Diarrheic shellfish toxins and other lipophilic toxins of human health concern in Washington state. *Mar. Drugs* 11, 1816–1817. doi: 10.3390/md11061815

Van de Waal, D. B., Tillmann, U., Martens, H., Krock, B., Van Scheppingen, Y., and John, U. (2015). Characterization of multiple isolates from an *Alexandrium ostenfeldii* bloom in the Netherlands. *Harmful Algae* 49, 94–104. doi: 10.1016/j.hal.2015.08.002

Van Wagoner, R. M., Misner, I., Tomas, C. R., and Wright, J. L. C. (2011). Occurrence of 12- methylgymnodimine in a spirolide producing dinoflagellate *Alexandrium peruvianum* and the biogenic implications. *Tetrahedron Lett.* 52, 4243–4246. doi: 10.1016/j.tetlet.2011.05.137

Wohlrab, S., Iversen, M. H., and John, U. (2010). A molecular and co-evolutionary context for grazer induced toxin production in *Alexandrium tamarense*. *PLoS One* 5:e15039. doi: 10.1371/journal.pone.0015039

Wu, H., Yao, J., Guo, M., Tan, M., Zhou, D., and Zhai, Y. (2015). Distribution of marine lipophilic toxins in shellfish products collected from the Chinese market. *Mar. Drugs* 13, 4281–4295. doi: 10.3390/md13074281

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Paredes-Banda, García-Mendoza, Ponce-Rivas, Blanco, Almazán-Becerril, Galindo-Sánchez and Cembella. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Marine Science

ORIGINAL RESEARCH
published: 18 December 2018
doi: 10.3389/fmars.2018.00487

Population Dynamics of Benthic-Epiphytic Dinoflagellates on Two Macroalgae From Coral Reef Systems of the Northern Mexican Caribbean

*Elda Damaris Irola-Sansores[1], Benjamín Delgado-Pech[1], Ernesto García-Mendoza[2], Erick J. Núñez-Vázquez[3], Aramis Olivos-Ortiz[4] and Antonio Almazán-Becerril[1]**

[1] Laboratorio de Ecología Costera, Centro de Investigación Científica de Yucatán, A.C. Unidad de Ciencias del Agua, Cancún, Mexico, [2] Laboratorio FICOTOX, Departamento de Oceanografía Biológica, Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, Mexico, [3] Laboratorio de Toxinas Marinas y Aminoácidos, Centro de Investigaciones Biológicas del Noroeste, La Paz, Mexico, [4] Centro Universitario de Investigaciones Oceanológicas, Universidad de Colima, Manzanillo, Mexico

OPEN ACCESS

Edited by:
Jorge I. Mardones, Instituto de Fomento Pesquero (IFOP), Chile

Reviewed by:
Sai Elangovan S, National Institute of Oceanography (CSIR), India
Conrad Sparks, Cape Peninsula University of Technology, South Africa

***Correspondence:**
Antonio Almazán-Becerril
almazan@cicy.mx

Specialty section:
This article was submitted to Marine Ecosystem Ecology, a section of the journal Frontiers in Marine Science

Received: *03 September 2018*
Accepted: *30 November 2018*
Published: *18 December 2018*

Citation:
Irola-Sansores ED, Delgado-Pech B, García-Mendoza E, Núñez-Vázquez EJ, Olivos-Ortiz A and Almazán-Becerril A (2018) Population Dynamics of Benthic-Epiphytic Dinoflagellates on Two Macroalgae From Coral Reef Systems of the Northern Mexican Caribbean. Front. Mar. Sci. 5:487. doi: 10.3389/fmars.2018.00487

In the present study the abundance of epiphytic dinoflagellates was evaluated at two coral reef sites of the natural protected areas, Arrecife de Puerto Morelos and Isla Contoy, located in the northern Mesoamerican Reef System of the Mexican Caribbean. Abundances were monitored from April to December, 2015 on two genera of macroalga belonging to different functional groups: *Dictyota* and *Amphiroa*. In general, the total abundance of dinoflagellates was higher in Puerto Morelos on both macroalgae. *Ostreopsis* cf. *marina* and *O. heptagona* were the dominant species. Relative abundance of these species varied from 8 to 99% of total epiphytic dinoflagellates. Maximum abundances at Puerto Morelos were registered in April, with 33,801 cells·g^{-1} on *Dictyota* and 6,264 cells·g^{-1} on *Amphiroa*. In Isla Contoy the maximum abundance was 16,006 cells·g^{-1} and it was detected on *Dictyota* during December. Other dinoflagellate genera were more abundant during the warmer period (May–September) in both locations. *Prorocentrum* was the second most abundant genus and was represented by six species (*P. hoffmannianum*, *P. lima*, *P. belizeanum*, *P. elegans*, *P. emarginatum,* and *P. rathymum*). The maximum pooled abundance of *Prorocentrum* species was 4,144 cells·g^{-1} on *Dictyota* in August. *Coolia* spp. did not reach abundances higher than 1,000 cells·g^{-1} and *Gambierdiscus* spp. only exceeded 100 cells·g^{-1} during August. Mean water temperature in Isla Contoy was significantly lower than that of Puerto Morelos during the entire study period. There was a negative correlation between the water temperature and the abundance of *O.* cf. *marina*. The dominance of *Ostreopsis* in the Caribbean is significant because of its capability to produce palytoxin analogs and its potential role in ciguatera fish poisoning outbreaks in the region. This is the first study that reports blooms of *Ostreopsis* in Mexican Caribbean coral reefs, a fact that emphasizes the significance of this genus at global scale.

Keywords: *Ostreopsis*, *Dictyota*, ciguatera, Caribbean Sea, coral reef

INTRODUCTION

There is the general assumption that ciguatera fish poisoning (CFP) outbreaks are linked to benthic dinoflagellate blooms, especially of toxic species of *Gambierdiscus* genus (Chinain et al., 1999; Turquet et al., 2001). However, the ecological processes that occur between the benthic harmful algae blooms and intoxication in humans are not as clear as planktonic harmful algae blooms (HABs), which can be more easily detected, especially those that color the water. For a beter understanding of the ciguatera phenomenon, it is necessary to increase the knowledge of the factors that control the abundance of toxin-producing epiphytic dinoflagellates, such as the influence of environmental variability, host-epiphytic dynamics, herbivory patterns on the macroalgae substrates, trophic relationships between vectors and even the preferences of human consumption on top predator fishes.

Most research has focused on the environmental variability and host-epiphytic dynamics through the analysis of the experimental responses of benthic dinoflagellates to variations of temperature, irradiance, nutrient concentration and salinity (Bomber et al., 1988; Chinain et al., 1999; Hales et al., 1999; Anderson et al., 2008; Lartigue et al., 2009; Kibler et al., 2015), as well as the substrate preference of some dinoflagellate species (Lobel et al., 1988; Bomber et al., 1989; Nakahara et al., 1996; Parsons and Preskitt, 2007; Parsons et al., 2011; Rains and Parsons, 2015), and the monitoring of dinoflagellate abundance on several substrates and macroalgae (Morton and Faust, 1997; Vila et al., 2001; Okolodkov et al., 2007, 2014; Moreira et al., 2012; Cohu et al., 2013). Also, other features such as depth, the water motion and habitat structure have been studied in relation to abundance and occurrence of the benthic dinoflagellates (Richlen and Lobel, 2011; Cohu et al., 2013; Boisnoir et al., 2018; Yong et al., 2018).

These investigations demonstrate that in many cases, benthic dinoflagellate abundances are influenced by increased temperature (Tester et al., 2010; Kibler et al., 2015). On this basis, seasonal variability of water temperature has received special attention as a predictive variable of dinoflagellate abundance and ciguatera outbreaks (Chinain et al., 1999; Tosteson, 2004; Chateau-Degat et al., 2005; Gingold et al., 2014). Although temperature could be the most important variable that explains changes in the abundance of benthic dinoflagellates, other factors could account for the dominance of a particular species. Particularly, macroalgal species could control the population dynamics of associated dinoflagellates. Macroalgae could promote or inhibit dinoflagellate growth through providing shelter or surface for attachment (Bomber et al., 1989; Parsons et al., 2011; Rains and Parsons, 2015).

The historical relationship between dinoflagellates and macroalgae may be facing a nonreversible change due to the replacement of macroalgae coastal communities as consequence of eutrophication and climate change (McCook, 1999). The impact of these phenomena on benthic communities is especially evident in tropical coral reefs ecosystems (Hoegh-Guldberg et al., 2007). These ecosystems constitute a common habitat for toxin-producing dinoflagellates (Yasumoto et al., 1980; Chinain et al., 1999). Hard corals are key species in these ecosystems because they function as shelter for a variety of species. Coralline macroalgae are associated with the coral in healthy coral reefs (Björk et al., 1995). However, coral cover is decreasing dramatically and their skeletons are being colonized by fleshy opportunistic macroalgae (Kohler and Kohler, 1992). This implies that macroalgae cover can reach high cover percentages and, in turn, constitute an extensive substratum for epiphytic dinoflagellates. Therefore, it is necessary to understand if the replacement of coralline macroalgae communities with fleshy macroalgae communities also changes the epiphytic dinoflagellate assemblage inhabiting them.

In the Mexican Caribbean coast, the CFP is a syndrome with a relatively high incidence. The main vector of ciguatera toxins is the great barracuda (*Sphyraena barracuda*) (Arcila-Herrera et al., 1998), but other fishes have also been involved in outbreaks. The presence of several potentially toxic species of the genera *Gambierdiscus*, *Prorocentrum*, *Ostreopsis*, *Coolia,* and *Amphidinium* has been registered in the Mexican Caribbean (Hernandez-Becerril and Almazan-Becerril, 2004; Almazán-Becerril et al., 2015, 2016).

In the coral reefs of the Mexican Caribbean fleshy macroalgae cover is higher than 50% and may reach 90% at some sites (Delgado-Pech, 2016). *Dictyota* is one of the dominant genera and may account for the 80% of the total macroalgae cover at some sites and periods. This implies that, as dominant macroalgae, *Dictyota* could increase the density of dinoflagellates in absolute terms (cells·m^2).

The aim of this work is to characterize taxonomic composition and abundance of dinoflagellate community in two functionally distinct algae: *Dictyota* and *Amphiroa* at two sites located at the northern portion of the Mesoamerican Coral Reef Barrier. Each genera of macroalgae represent different states of coral reef health. *Dictyota* represents an impacted coral reef and *Amphiroa* a healthy one.

MATERIALS AND METHODS

Study Area

This study was carried out from April to December 2015 at two reef sites located in the northern Mexican Caribbean: Isla Contoy (IC) and Puerto Morelos (PM). Both sites are protected natural areas of the Mesoamerican Reef Barrier System (**Figure 1**). Ixlaché reef is located 1 km south of IC. It is a small reef 800 m long and 60 m wide. Depth varies between 0.5 and 3 m, macroalgae cover is 60%, and is influenced by coastal currents, waves, tides, and winds predominantly from the southeast and polar fronts. On the other hand, the Bonanza reef site is part of the PM reef; it is a shallow area of <2 m depth and macroalgae cover is higher than 75%.

Substrate Selection

Two macroalgae species were selected for this study, each representing a distinct functional group (Littler et al., 1983; Steneck and Deither, 1994). *Amphiroa* is a slow-growing rate coralline articulated macoalgae present at relatively low cover in the sites of study. *Dictyota* is fast-growing rate fleshy, foliose macroalgae with a high cover. Both sites selected for this study

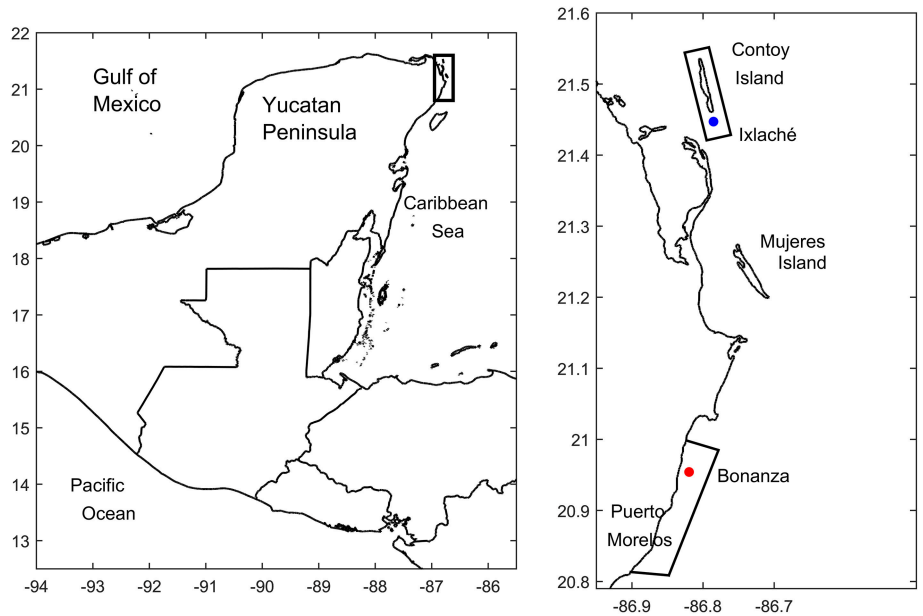


FIGURE 1 | Zone of study. Blue point corresponds to the Ixlaché Reef located at the Parque Nacional Isla Contoy, and the red point corresponds to La Bonanza reef at the Parque Nacional Arrecife de Puerto Morelos. Natural Protected Areas are enclosed with a frame.

are macroalgae-dominated reefs where *Dictyota*, *Turbinaria*, *Lobophora,* and *Laurencia* are very common, although patches of the coralline algae *Amphiroa* still remain.

Collection of Macroalgae Samples

At each site, 10 specimens of each genus of macroalgae were collected. The distance between collected specimens was at least 10 m according to Lobel et al. (1988). Collected macroalgae were stored in plastic bags with seawater and placed into a cooler with water of the site to maintain the temperature. Algae were fixed with formaldehyde at 4% final concentration and stored until analysis.

In the laboratory, macroalgae samples were strongly shaken during 2 min to detach epiphytic dinoflagellates. Macroalgae were separated from the seawater and the excess of water was removed with absorbent paper. Macroalgae were weighed and fixed in formaldehyde. A total of 400 samples were processed.

Environmental Variables

Water temperature was recorded every 3 h during the study period. HOBO Pro-v2 sensors were placed at the reef sites anchored at the level of the macroalgae mats. For nutrient determinations, water samples were collected with a 50 ml syringe, previously washed with a 10% HCl solution, connected to a filter holder containing a Whatman® membrane filter of 0.22 μm size pore and 2.5 mm diameter. Filtered seawater was recovered into a 30 ml Nalgene® polypropylene bottles that were stored in a cooler with ice. Once in the laboratory the samples were stored at −80°C. The quantification of NO_x^- ($NO_3^- + NO_2^-$) and PO_4^{3-} was performed by using a Skalar® segmented flux autoanalyzer following the techniques described by Strickland and Parsons (1972) and Grasshoff et al. (1983).

Abundance and Identification of Epiphytic Dinoflagellates

The abundance of epiphytic dinoflagellates was estimated with the help of a Carl Zeiss inverted microscope model Axiovert 40 CFL at 10X magnification using a Sedgewick-Rafter counting chamber. Abundance is expressed in cells·g^{-1} of wet weight macroalgae (Reguera et al., 2016).

For dinoflagellate species identification the following specialized literature was used: Fukuyo (1981), Faust (1990, 1993, 1994, 1995, 1997, 1999), Faust et al. (1996), Litaker et al. (2009), and Almazán-Becerril et al. (2015, 2016).

Dinoflagellate observations and measurements were made with an Axio Imager A2 Zeiss microscope at 40X magnification using the differential interference contrast and epifluorescence techniques. Photographs of the specimens were taken with an AxioCam ICc1 digital camera coupled to the microscope. The image processing was carried out with the software Axiovision®.

Statistic Analysis

The non-parametric Mann–Whitney *U*-test ($\alpha = 0.05$) was used to compare the dinoflagellate abundance at each sampling date between sites, and the non-parametric Wilcoxon rank test ($\alpha = 0.05$) was used to evaluate the potential differences between sites during the whole period of sampling by paired data. The Spearman rank correlation was applied to describe the community structure on basis of the associations between species in both macroalgae. The association of temperature and nutrient concentration was assessed using the same correlation test.

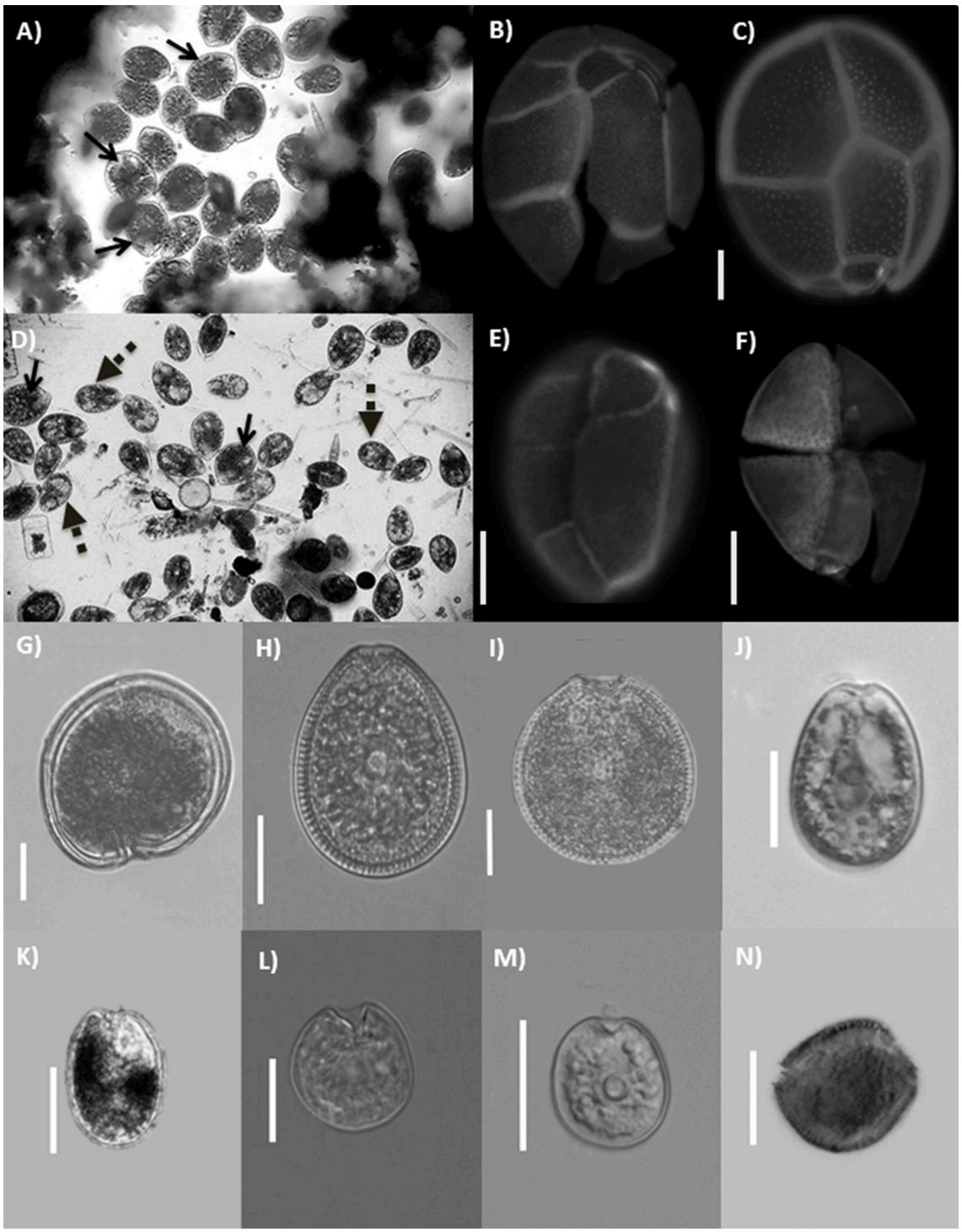


FIGURE 2 | Species of benthic dinoflagellates found in the study zone. **(A)** *Ostreopsis marina* bloom from IC. **(B)** Epifluorescence images of *O. marina* epitheca and **(C)** hipotheca. **(D)** *Ostreopsis heptagona* bloom from PM. Note the presence of some cells of *O. marina* (arrows). **(E)** Epifluorescence images of *O. heptagona* epitheca and **(F)** hipotheca. **(G)** *Gambierdiscus* sp. **(H)** *Prorocentrum hoffmannianum*. **(I)** *Prorocentrum belizeanum*. **(J)** *Prorocentrum lima*. **(K)** *Prorocentrum rathymum*. **(L)** *Prorocentrum emarginatum*. **(M)** *Prorocentrum* cf. *levis*. **(N)** *Coolia* sp. Scale bar = 20μm.

TABLE 1 | Mean abundance (in cells·g^{-1}) by date of the main taxonomic groups of benthic dinoflagellates on the macroalgae *Dictyota*.

Date	Coolia	Gamb	Ohep	Omar	Pbel	Plev	Pemar	Phoff	Plim	Prhat	Total
PUERTO MORELOS											
Apr17	803 (186)	94 (29)	14,129 (4,677)	19,672 (6,192)	90 (30)	98 (32)	377 (88)	1,188 (323)	1,544 (492)	475 (97)	38,471 (11,420)
May08	263 (77)	23 (16)	20,606 (3,910)	8,132 (1,086)	64 (20)	5 (5)	73 (30)	683 (157)	1281 (327)	516 (82)	31,645 (4,852)
May 29	75 (27)	26 (21)	18,876 (4,876)	967 (343)	41 (18)	13 (9)	115 (49)	621 (127)	1595 (461)	74 (23)	22,403 (5,667)
Jun22	260 (142)	26 (18)	307 (99)	1,559 (537)	71 (23)	61 (54)	178 (85)	544 (172)	811 (338)	142 (32)	3,958 (1,442)
Jul09	369 (112)	50 (12)	179 (50)	922 (272)	51 (19)	20 (6)	63 (25)	373 (85)	1728 (743)	154 (44)	3910 (1,163)
Aug10	673 (136)	180 (59)	11,412 (1,656)	2,713 (643)	35 (13)	100 (30)	178 (48)	799 (245)	2,523 (848)	610 (94)	19,221 (3,081)
Aug28	260 (65)	90 (28)	8,318 (1,295)	172 (35)	146 (47)	77 (21)	136 (40)	724 (181)	1,527 (412)	609 (261)	12,059 (2,034)
Sep17	495 (130)	94 (23)	252 (74)	147 (12)	63 (25)	110 (30)	51 (18)	751 (137)	2405 (253)	479 (73)	4,846 (464)
Nov04	164 (56)	18 (13)	109 (31)	1,574 (365)	28 (8)	8 (6)	21 (11)	74 (20)	534 (124)	59 (18)	2,590 (480)
Dec02	42 (13)	15 (8)	14 (8)	1,917 (519)	13 (6)	3 (3)	9(4)	59 (13)	211 (52)	3 (3)	2,285 (546)
ISLA CONTOY											
Apr17	777 (215)	25 (6)	0	548 (161)	35 (12)	29 (10)	46 (15)	181 (59)	117 (33)	84 (20)	1,843 (511)
May08	279 (74)	3 (2)	0	7,446 (1681)	20 (7)	5 (3)	17 (7)	136 (29)	117 (20)	7 (4)	8,031 (1,721)
May29	60 (24)	10 (10)	0	220 (98)	31 (22)	0	80 (19)	66 (22)	59 (21)	0	528 (156)
Jun22	169 (41)	19 (5)	0	2,094 (399)	60 (14)	3 (2)	151 (25)	98 (21)	50 (10)	21 (8)	2,665 (417)
Jul09	79 (44)	3 (3)	0	937 (519)	22 (8)	5 (3)	56 (16)	194 (57)	130 (25)	19 (9)	1,445 (578)
Aug10	457 (149)	67 (15)	0	72 (24)	82 (17)	27 (14)	125 (23)	337 (33)	245 (46)	45 (11)	1,457 (202)
Aug28	648 (165)	70 (23)	7 (6)	144 (31)	45 (11)	34 (19)	199 (34)	252 (49)	180 (47)	39 (14)	1,619 (241)
Sep17	615 (124)	75 (20)	10 (5)	247 (75)	64 (17)	93 (23)	182 (44)	185 (34)	148 (28)	41 (20)	1,658 (250)
Nov04	184 (52)	30 (13)	6 (6)	581 (140)	7 (5)	0	28 (11)	75 (20)	76 (21)	10 (6)	997 (168)
Dec02	11 (6)	5 (3)	284 (84)	15,721 (3,677)	25 (11)	2 (2)	2 (2)	59 (20)	49 (16)	3 (3)	16,160 (3,770)

The number between parentheses represents the standard error.

TABLE 2 | Mean abundance (in cells·g^{-1}) by date of the main taxonomic groups of benthic dinoflagellates on the macroalgae *Amphiroa*.

Date	*Coolia*	*Gamb*	*Ohep*	*Omar*	*Pbel*	*Plev*	*Pemar*	*Phoff*	*Plim*	*Prhat*	Total
PUERTO MORELOS											
Apr17	160 (47)	4 (4)	4,625 (960)	1,639 (382)	9 (7)	16 (5)	16 (6)	92 (26)	192 (32)	28 (7)	6,781 (1,334)
May08	36 (12)	5 (4)	7,109 (832)	1,199 (208)	22 (5)	3 (3)	5 (4)	130 (22)	212 (110)	81 (30)	8,804 (964)
May29	6 (5)	3 (3)	849 (314)	9 (6)	3 (2)	2 (2)	8 (4)	27 (8)	63 (22)	8 (4)	978 (335)
Jun22	16 (4)	3 (2)	191 (47)	377 (65)	1 (1)	0	7 (4)	59 (16)	76 (14)	5 (3)	734 (114)
Jul09	49 (16)	7 (5)	122 (33)	428 (138)	24 (11)	0	25 (9)	159 (72)	422 (156)	9 (8)	1,243 (361)
Aug10	66 (22)	48 (35)	1,029 (237)	227 (62)	38 (19)	11 (7)	37 (19)	126 (24)	358 (51)	25 (9)	1,964 (372)
Aug28	54 (28)	11 (5)	3,150 (1,087)	196 (75)	16 (8)	16 (9)	0	163 (49)	361 (120)	44 (18)	4,010 (1,329)
Sep17	44 (14)	13 (5)	246 (70)	61 (30)	13 (5)	4 (3)	3 (3)	116 (31)	607 (122)	14 (6)	1,119 (230)
Nov04	39 (13)	28 (7)	69 (21)	533 (118)	13 (5)	5 (5)	5 (5)	55 (15)	202 (54)	8 (4)	956 (186)
Dec02	47 (20)	71 (35)	347 (139)	2,665 (864)	0	0	7 (5)	18 (10)	88 (30)	20 (20)	3,263 (1,020)
ISLA CONTOY											
Apr17	2 (2)	3 (2)	0	623 (303)	4 (1)	3 (2)	2 (1)	17 (3)	26 (8)	4 (2)	684 (315)
May08	123 (75)	0	0	4,425 (1,504)	24 (20)	2 (2)	13 (9)	157 (77)	213 (106)	8 (8)	4,965 (1,737)
May29	6 (2)	0	0	28 (10)	0	0	18 (4)	17 (7)	3 (2)	1 (1)	73 (11)
Jun22	21 (5)	7 (4)	0	144 (31)	5 (3)	3 (2)	44 (12)	38 (18)	27 (16)	1 (1)	290 (71)
Jul09	44 (12)	11 (6)	0	85 (37)	17 (6)	0	33 (9)	57 (12)	100 (42)	1 (1)	347 (85)
Aug10	111 (20)	13 (7)	0	85 (18)	47 (12)	4 (4)	98 (20)	131 (17)	76 (25)	0	565 (78)
Aug28	206 (44)	6 (5)	0	14 (14)	13 (7)	54 (22)	28 (13)	79 (16)	45 (9)	0	445 (78)
Sep17	276 (89)	14 (7)	5 (5)	221 (91)	12 (6)	43 (14)	46 (13)	82 (20)	41 (12)	3 (3)	743 (196)
Nov04	115 (44)	46 (24)	22 (10)	1,105 (261)	21 (11)	6 (6)	0	48 (31)	47 (17)	0	1,410 (326)
Dec02	18 (7)	0	24 (13)	793 (165)	0	0	3 (3)	28 (16)	31 (24)	0	897 (179)

The number between parentheses represents the standard error.

RESULTS

Dinoflagellate Species

We recorded species of the genera *Gambierdiscus*, *Coolia*, *Prorocentrum, Ostreopsis*, *Amphidinium*, *Bysmatrum,* and *Sinophysis*. Identification of the species of *Gambierdiscus* and *Coolia* is difficult based only on the characters observed (size and shape of the cells) during the evaluation of abundance. Therefore, hereafter the *Gambierdiscus* and *Coolia* specimens will be referred as *Gambierdiscus* spp. and *Coolia* spp. Five species of *Ostreopsis* were observed but only two of them accounted for more of the 95% of the abundance of this genus. These two morphotypes of *Ostreopsis*, were clearly differentiated during the counting and were designated as *O.* cf. *marina* and *O. heptagona*. Finally, six *Prorocentrum* species were found: *P. belizeanum*, *P. emarginatum*, *P. hoffmannianum*, *P. lima*. *P.* cf. *levis*, and *P. rathymum*. *Gambierdiscus* spp. *Coolia* spp., *Ostreopsis* cf. *marina*, *O. heptagona,* and the six *Prorocentrum* species were the most abundant groups found in the samples, therefore, the analysis was constrained to these taxa. **Figure 2** shows the main species recorded in the zone of study.

Abundance Variability

Taking into account the entire sampling period, total abundance of epiphytic dinoflagellates was higher at PM on both algae (**Tables 1**, **2**; **Figure 3**). However, when the total abundance and the abundance by group were compared at each sampling date, it became evident that the numeric differences were not always significant for all groups (**Table 3**). Total dinoflagellate abundances on *Amphiroa* were significantly higher at PM during all sampling dates ($p < 0.05$, Mann–Whitney test) except for Sep17 and Nov09 when no differences were detected. In *Dictyota* total abundances were higher in PM during the entire study period, except on Jun22 when no differences were detected.

In PM, the maximum mean abundance recorded in *Dictyota* was 38,471 ± 11,420 cells·g^{-1} on Apr16. In the case of *Amphiroa,* the maximum abundance was 8,803 ± 964 cells·g^{-1} and was recorded on May08. At IC the maximum density of

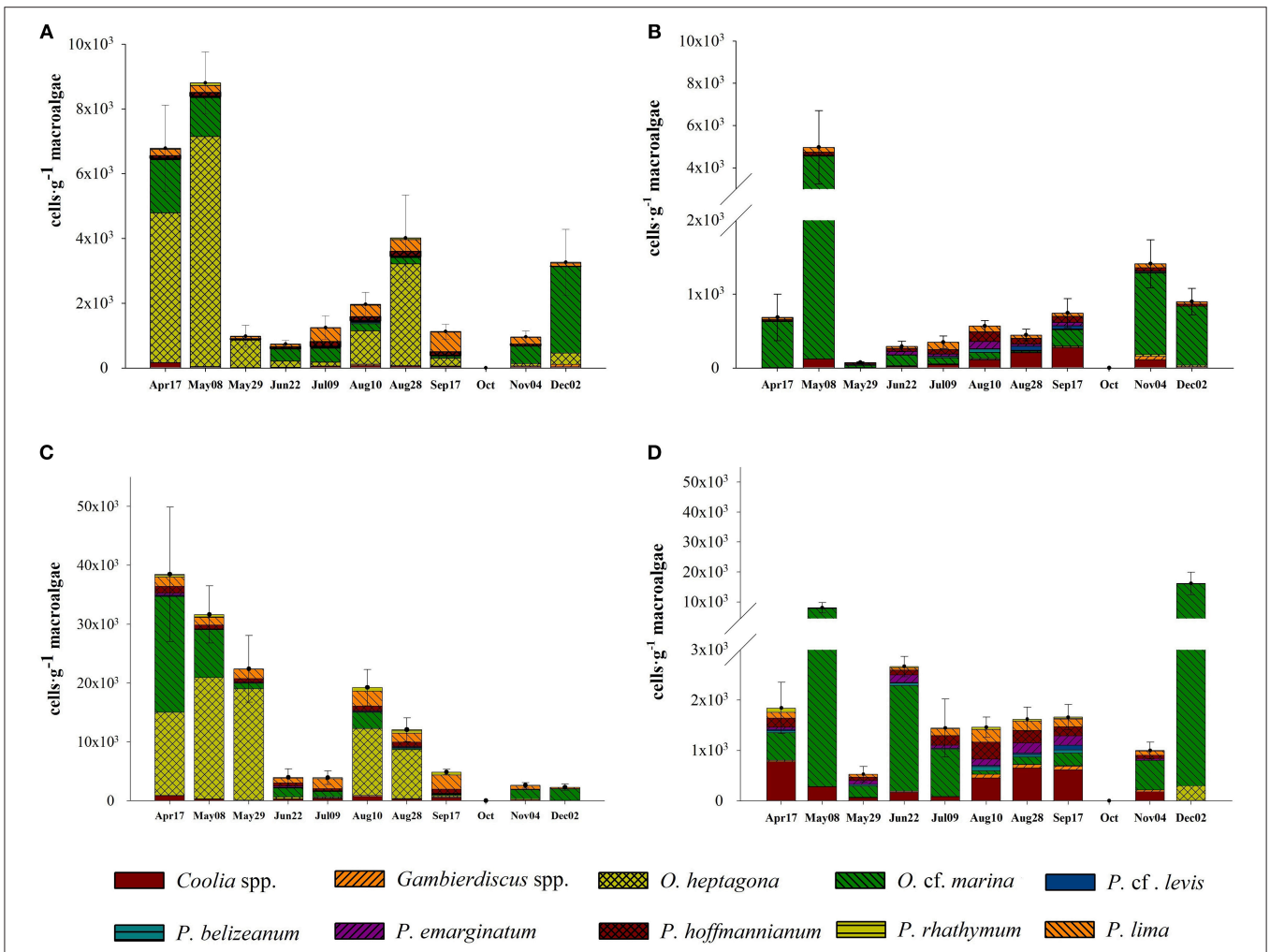


FIGURE 3 | Variability of total benthic dinoflagellate abundance on **(A)** *Amphiroa* at Puerto Morelos and **(B)** Isla Contoy, and on *Dictyota* at **(C)** Puerto Morelos and **(D)** Isla Contoy.

TABLE 3 | *P*-values of the abundances comparisons of the main taxonomic groups of epiphytic dinoflagellates at each sampling date between sites (Mann–Whitney test, $\alpha = 5\%$).

	Apr17	May08	May29	Jun22	Jul09	Aug10	Aug28	Sep17	Nov04	Dec02
Amphiroa										
Total	<0.001	<0.05	<0.001	<0.001	<0.01	<0.01	<0.001	ns	ns	<0.01
Omar	<0.05	ns	ns	<0.001	ns	<0.05	<0.001	ns	ns	<0.05
Ohep	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05	<0.05
Ptot	<0.001	ns	<0.05	ns	<0.05	ns	ns	<0.001	ns	ns
Gamb	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Coolia	<0.001	ns	ns	ns	ns	ns	**<0.001**	**<0.01**	ns	ns
Dictyota										
Total	<0.001	<0.002	<0.001	ns	<0.05	<0.001	<0.001	<0.001	<0.01	<0.001
Omar	<0.001	ns	ns	ns	ns	<0.001	ns	ns	0.02	**<0.001**
Ohep	<0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01
Ptot	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	ns
Gamb	ns	ns	ns	ns	<0.001	ns	ns	ns	ns	ns
Coolia	ns	ns	ns	ns	<0.05	ns	ns	ns	ns	ns

The abundances were higher at PM except in the case of the values indicated in bold.

dinoflagellates on *Dictyota* occurred on Dec02 (16,160 ± 3,770 cells·g^{-1}), and on *Amphiroa* the highest abundance was 16,160 ± 3,770 cells·g^{-1} in May08. In PM, the maximum individual abundance was registered on *Dictyota* during Apr16 (112,293 cells·g^{-1}).

Dominant species during most of the period were *Ostreopsis heptagona* and *O.* cf. *marina* Therefore, the variability of total abundance of epiphytic dinoflagellates were due the variation in the contribution of these species at each site. In fact, the contribution of *Ostreopsis* species varied linearly with total abundance (**Figures 4A,B**).

The contribution of *O.* cf. *marina* was important at both sites, but *O. heptagona* was important only in PM because this species was absent at IC during almost the entire sampling period. *O.* cf. *marina* and *O. heptagona* reached maximum densities of 53,231 and ∼46,462 cells·g^{-1} on a *Dictyota* sample during Apr16.

The contribution of *Gambierdiscus* spp. to total abundance was marginal on both macroalgae and at both sites: from 0 to 3.26% on *Amphiroa*, and from 0 to 4.6% on *Dictyota*. The highest abundance of this group on *Dictyota* was found at PM on Aug10 (178 ± 60 cells·g^{-1}); and on *Amphiroa* the maximum abundance was observed on Dec02 (71 ± 38 cells·g^{-1}).

The most abundant species of *Prorocentrum* were *P. hoffmannianum* and *P. lima* on both algae, representing the largest fraction of this genus (50–94%). *Prorocentrum* was important (65% of total abundance) when *Ostreopsis* abundances were low. Maximum abundance of all *Prorocentrum* species was registered at PM on *Dictyota* (4,144 cells·g^{-1}).

The contribution of *Coolia* spp. to total abundance was lowest at PM. This genus did not present a contribution higher than 10% on both algae. In contrast, in IC on some dates it reached more than 30% on both macroalgae. The abundance of this group was lower than 1,000 cells·g^{-1}on, and its abundance on *Amphiroa* was always lower than 300 cells·g^{-1}.

Associations Between Species

There were more correlations between species on *Dictyota* than on *Amphiroa* in both sites (**Table 4**). *Prorocentrum lima, P. belizeanum,* and *P. hoffmannianum* were correlated with each other on *Amphiroa* at both sites but not on *Dictyota*. Also *P. rathymum* was correlated with *O. heptagona* on *Amphiroa* at PM. Given that this species was almost absent at IC, the correlation did not exist at this site. At Isla Contoy, *P. hoffmannianum,* and *P.* cf. *levis* were correlated with *Coolia* spp. The association between *P. lima* and *P. hoffmannianum* was the only association detected in both algae at both sites. On *Dictyota*, the community structure was more complex because it included the correlations of *Coolia* with *Gambierdiscus*, and each of these genera with the *Prorocentrum* species. The negative correlations of *O. marina* (with *Gambierdiscus*, *P. emarginatum,* and *P. hoffmannianum*) were particularly important, but this only occurred at IC.

Temperature and Nutrients

The temperature varied from 24.04 to 30.20°C at IC and from 26.68 to 29.7°C at PM. Warmer temperatures were registered between August and September, and April was the coldest month in both sites (**Figure 5**). Over the entire sampling period, mean water temperature was slightly lower at IC (28.25°C) than at PM (28.74°C). Although small, this difference was significant ($p < 0.05$, Wilcoxon test). On Apr17, the temperature was 1.46°C higher at PM (27.90°C) than IC (26.44°C). Over the rest of the sampling period, the temperature differences between sites were lower than 1°C. After May29, the temperature decreased almost 1°C at both sites, followed by a gradual increase until the maximum value in August–September. This behavior is common in the zone and is related to the influence of the polar fronts coming from the north from December to February, although during the year of study, polar fronts occurred until July. For example, the event which occurred during June 12–19 was intense enough to decrease the temperature recorded on Jun22.

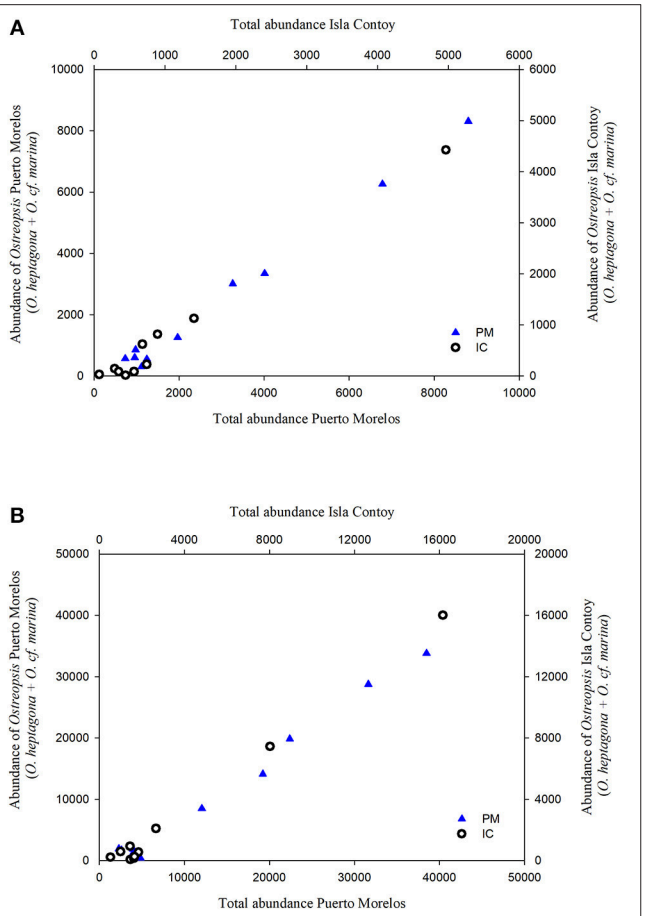


FIGURE 4 | Relationship between the contribution of *Ostreopsis* species abundance in cells·g^{-1} (*O. marina* + *O. heptagona)* with total abundance on *Amphiroa* **(A)** and *Dictyota* **(B)**.

High NO_x^- (NO_3^- +NO_2^-) concentrations were measured on both sites with mean values of 8.0 and 7.8 μmol·l^{-1} for PM and IC, respectively, but no significant differences were detected along the entire period of study. Conversely, PO_4^{-3} concentration was different between sites: 2.3 μmol·l^{-1} at IC and 2.4 μmol·l^{-1} in PM ($p < 0.05$, Wilcoxon test). NO_3^- and PO_4^{-3} showed a high correlation at PM ($r = 0.95$) but not for IC. Temperature did not correlate with nutrients at PM nor IC ($p > 0.05$, Spearman rank correlation).

Effect of Environmental on Dinoflagellate Abundances

The correlation between temperature and the abundance of *O.* cf. *marina* is shown in **Figure 6**. At both sites and on both algae this species showed a negative correlation with temperature ($p < 0.05$, Spearman rank correlation). Maximum abundances were detected at temperatures between 27 and 28°C, independent of the macroalgae substrate. In contrast, *Prorocentrum lima* and *P. emarginatum* were positively correlated with temperature at IC and only on *Dictyota:* $r = 0.66$ and $r = 0.79$, respectively. A negative correlation between *O. heptagona* and PO_4^{-3} concentration was found at PM on both algae ($r = -0.67$ and $r = 0.7$ on *Amphiroa* and *Dictyota,* respectively). Correlations with NO_3^- only were observed at IC with the abundances of *P. emarginatum*, *P. rathymun,* and *Coolia* spp. on *Dictyota* (**Table 5**).

DISCUSSION

Taxonomic Composition of Epiphytic Dinoflagellates

Describing the taxonomic composition of epiphytic dinoflagellates is more complicated now than in the past because at present there are more described species for each of the benthic genera. Pioneer studies on benthic dinoflagellates considered all specimens with the typical shape of *Gambierdiscus* to be *G. toxicus*, or all those individuals similar to *Coolia* as *C. monotis*. However, Faust (1995) and Litaker et al. (2009) increased to four the richness of the genus *Gambierdiscus* in the Caribbean: *G. belizeanus, G. caribaeus*, *G. carolinianus,* and *G. carpenteri*. Also the number of *Coolia* species increased after reports of the presence of *C. tropicalis*, *C. santacroce, C. malayensis,* and *C. areolata* (Faust, 1995; Karafas et al., 2015; Almazán-Becerril et al., 2016). Since the morphological identification of each of these species involves the inspection and analysis of the thecal plates, which is not a practical procedure given the high number of samples to analyse, it was necessary to join the species of each genus and consider them as taxonomic groups, although the ecological behavior of individual species of the same genus remain unclear.

Prorocentrum has the highest richness among the benthic-epiphytic thecate dinoflagellates, and the Caribbean harbors a great fraction of these species (Faust, 1990, 1993, 1994, 1997), however, just some of them are common in the macroalgae samples which allow an easier identification of the specimens. *Prorocentrum lima*, *P. hofmmaniannum*, *P. belizeanum*. *P. rathymum,* and *P. emarginatum* are well-recognized under light microscope as different species.

Ostreopsis taxonomy represents a challenge due to the lack of specific morphological characters for species discrimination (Penna et al., 2005; Parsons et al., 2012), however, in this study two *Ostreopsis* species were identified as *O. heptagona* and *O.* cf. *marina*. In the first case, the contact between the plates 4′ and 4″ (according to the interpretation of Besada et al., 1982) was an adequate characteristic to identify this species. The other species was referred as *O.* cf. *marina* because the original description of this species made by Faust (1999) matches with the characteristics of the specimens observed in this study. A closely related morphological species is *O. lenticularis*, also reported for the Caribbean (Ballantine et al., 1988; Moreira et al., 2012, 2017), which has nearly the same shape (Fukuyo, 1981; Faust, 1999). However, the size reported for this species in the literature is consistently lower than the size of the specimens observed in this study. Therefore, the discrimination of the specimens of these two species under microscope during counting was based on the size and shape of the specimens and was ratified by plate analysis of some cells in several samples. As consequence, the

TABLE 4 | Correlations between dinoflagellate species on the same macroalgae.

	Amphiroa		*Dictyota*	
	Puerto Morelos	**Isla Contoy**	**Puerto Morelos**	**Isla Contoy**
P. belizeanum-P. lima	0.74*	0.89***		
P. belizeanum-P. hoffmannianum	0.81**	0.84**		
P. belizeanum-P. rhathymum				0.66*
P. belizeanum-P. emarginatum			0.67*	0.76*
P. hoffmannianum-P. lima	0.77**	0.84**	0.66*	0.95***
P. hoffmannianum-P. emarginatum			0.67*	
P. hoffmannianum-P. rhathymum			0.77**	0.75*
P. hoffmannianum-P. cf. *levis*			0.79**	0.79**
P. lima-P. cf. *levis*			0.74*	0.77**
P. rhathymum-P. cf. *levis*				0.79**
P. rhathymum-P. lima				0.66*
P. rhathymum-O. heptagona	0.84**			
Coolia spp.-*P. hoffmannianum*		0.84**	0.80**	0.66*
Coolia spp.-*P.* cf. *levis*		0.68*	0.75*	0.81**
Coolia spp.-*P. lima*			0.69*	0.69*
Coolia spp.-*P. rhathymum*			0.65*	0.83**
Coolia spp.-*Gambierdiscus* spp.			0.83**	0.66*
Gambierdiscus spp.-*P. hoffmannianum*			0.87**	
Gambierdiscus spp.-*P. lima*			0.85**	
Gambierdiscus spp.-*P. belizeanum*				0.64*
Gambierdiscus spp.-*P. rhathymum*			0.71*	0.63*
Gambierdiscus spp.-*P.* cf. *levis*			0.94***	
Gambierdiscus spp.-*P. emarginatum*				0.68*
O. heptagona-P. emarginatum			0.65*	
O. heptagona-P. hoffmannianum			0.65*	
O. marina-Gambierdiscus spp.				−0.71*
O. marina-P. emarginatum				−0.69*
O. marina-P. lima				−0.70*

Numbers indicate the Rho value of the Spearman rank correlation test.
**p < 0.05, **p < 0.01, ***p < 0.001.*

taxonomic composition of epiphytic dinoflagellates cannot be unequivocally determined by using the standard methodology (rough morphometric discrimination), and for purposes of practicality and comparability it is necessary to work with this "schematic" community.

The taxonomic composition of the epiphytic dinoflagellate community on the surveyed algae of this study is quite similar to those reported in similar studies in the Caribbean (Morton and Faust, 1997; Delgado et al., 2006; Boisnoir et al., 2018) and other regions of the North Atlantic like the south of the Gulf of Mexico (Okolodkov et al., 2007, 2014) or Florida (Bomber et al., 1989) and even in the Pacific (Parsons and Preskitt, 2007; Richlen and Lobel, 2011) although each species (or genus) did not contribute in the same proportion to the total abundance. For example, Delgado et al. (2006) found that *P. lima* and *G. toxicus* were more abundant than *O. lenticularis* and *C. monotis*, whereas Boisnoir et al. (2018) reported that *Ostreopsis* spp. and *Prorocentrum* spp. were numerically more abundant than *Coolia* and *Gambierdiscus*. In the Gulf of Mexico, Okolodkov et al. (2007) found that depending of the site and the date *P. lima* or *C. monotis* could dominate the community, and in the Yucatan coast, *Prorocentrum* species, particularly *P. rathymum* was the dominant species on seagrass meadows present in Yucatan coast (Okolodkov et al., 2014).

Abundance and Seasonal Patterns

The results of this study demonstrate that all the taxonomic groups were present on both algae at both localities, excepting *O. heptagona* which was almost absent at IC with only a few cells observed between September and December. As the distribution of this species comprises the Gulf of Mexico, Florida and the Caribbean (Bomber et al., 1989; Faust et al., 1996), its low abundance at IC during the period of study could be the effect of a local characteristic of the site, because at the same time a high number of specimens were detected at PM.

The short period of sampling of this study was not enough to determinate seasonal patterns for any species of dinoflagellate (although positive significant correlations were found between temperature and *P. lima* and *P. emarginatum*) and it appears that more time is necessary to characterize seasonal variability. For

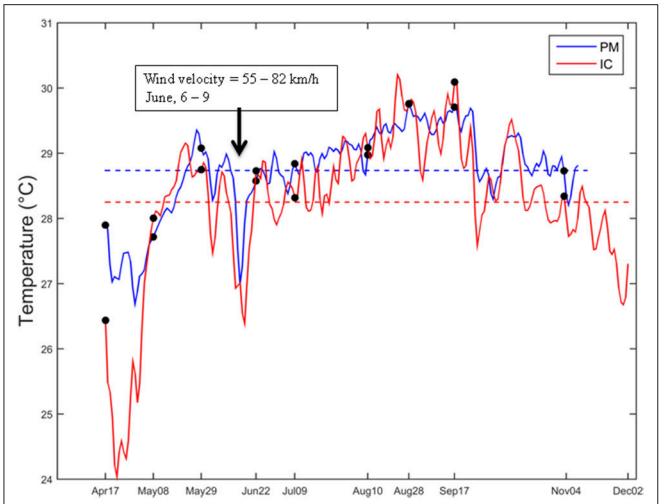


FIGURE 5 | Variability of the water temperature at Puerto Morelos (blue line), and Isla Contoy (red line). Dotted lines indicate the mean water temperature of each series. The black arrow indicates a period of an intense polar front occurred in the zone during 6–9 June (winds 55–87 km/h). These events have the potential to remove a great fraction of macroalgae in the zone. Data of temperature were acquired every 3 h. Temperature series were filtered and binned to have only one data per day. Sensors were placed at the same depth (∼1.5 m) in both sites to make data comparable.

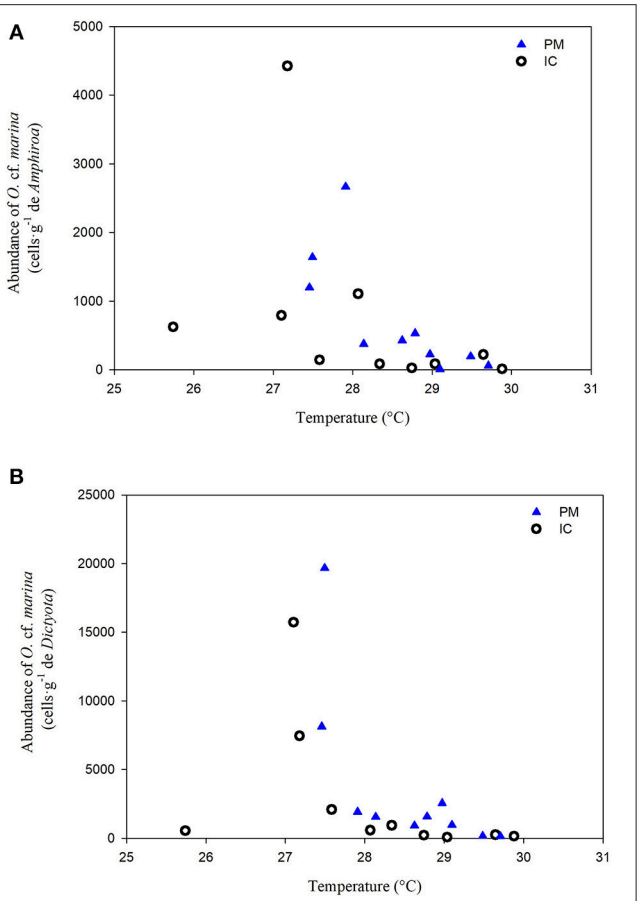


FIGURE 6 | Relationship between *Ostreopsis marina* abundance and water temperature on **(A)** *Amphiroa* and **(B)** *Dictyota*. The highest abundance of this species occurred between 27–28°C in both sites on both macroalgae.

example, Ballantine et al. (1988) found evidence of seasonality in *Gambierdiscus* and *O. lenticularis* in Puerto Rico in a 3-year study. Also, Chinain et al. (1999) used a 5-year time series to describe the seasonality of *Gambierdiscus* spp. at Tahiti, and Chateau-Degat et al. (2005) used an 8-year study in French Polynesia to describe the seasonal behavior of *Gambierdiscus*. Higher abundances were observed during April and May 08 on both algae in PM, and in May 08 for both algae at IC, additionally the higher abundance in *Dictyota* at IC was detected on Dec02.

These fluctuations were dominated by the contribution of *Ostreopsis* species which was largely variable and increased linearly with total abundance. The high abundance of *Ostreopsis* species was also reported at the Caribbean in Cuba (Moreira et al., 2012, 2017) and Puerto Rico (Ballantine et al., 1988) where *O. lenticularis* reached higher abundances than the reported in this study (**Table 6**). When the abundance of *Ostreopsis* species was removed from the data, the contribution of *Coolia* spp., *P. lima,* and *P. hoffmannianum* were more important, while the abundance of *Gambierdiscus* spp. and the rest of *Prorocentrum* species was almost marginal. *Prorocentrum lima* is a conspicuous member of benthic dinoflagellate communities in the Caribbean and Gulf of Mexico where it attaches to a variety of algae and seagrasses and at some sites could be the dominant species with maximum abundances above 10,000 cells·g^{-1} on phaeophyte algae (Delgado et al., 2006), and up to 29,756 cells·g^{-1} of *Thalassia testudinum* (Okolodkov et al., 2007). Also, at Florida Keys, Bomber et al. (1989) found that *P. lima* was more abundant on green algae *Penicillus capitatus* and *Avrainvillea nigricans* as compared to other brown or red algae species.

The case of *Gambierdiscus* abundance is remarkable because the species of this genus and those of *Fukuyoa* are thought to be primarily responsible for the toxin production involved in ciguatera intoxications. Although there is an intrinsic difficulty in comparing abundances between different studies based on number of cells normalized by wet weight of macroalgae due to the high variability of weight to surface area ratios between the different groups of host macroalgae (Lobel et al., 1988), there are some reports of high abundances of *Gambierdiscus* spp. on *Dictyota* in the Caribbean. For example, Ballantine et al. (1988) reported a maximum abundance slightly higher than 2,000 cells·g^{-1} in Puerto Rico and Morton and Faust (1997) found 400 cells·g^{-1} in Belize. Also, Bomber *et al.* (1989) reported a maximum mean value of 8,191 ± 300 cells·g^{-1} in Florida. In contrast, the *Gambierdiscus* abundances found on *Dictyota* in this study were similar to the values reported by Lobel et al. (1988) at the Caribbean Island St. Barthélemy (5–56 cells·g^{-1}). The factors that control the abundance of *Gambierdiscus* populations in the zone remain to be solved.

In the Caribbean high abundances of *Ostreopsis lenticularis* has been reported for Puerto Rico (Ballantine et al., 1988) and Cuba (Moreira et al., 2012, 2017), but no reports of high densities exist for *O. marina* and *O. heptagona* in this region. **Table 5** shows that maximum densities recorded for *Ostreopsis* species in the

TABLE 5 | Correlation of epiphytic dinoflagellates with temperature and nutrients.

	Amphiroa						*Dictyota*					
	Isla Contoy			Puerto Morelos			Isla Contoy			Puerto Morelos		
	T	NO_x^-	PO_4^{3-}	T	NO_x^-	PO_4^{3-}	T	NO_x^-	PO_4^{3-}	T	NO_x^-	PO_4^{3-}
Omar	−0.69*			−0.87**			−0.74*			−0.79**		
Pemar	0.63*						0.79**	0.75*				
Plim							0.66*					
Prhat							0.82**					
Ohep						−0.67*						−0.70*
Coolia								0.64*				

*$p < 0.05$, **$p < 0.01$.

TABLE 6 | Abundances from the literature of *Ostreopsis* species on the macroalgae *Dictyota* in the Caribbean basin.

Site	Species	Maximum abundance[a]	Date of maximum abundance	Number of replicates	Period of study	References
La Parguera, Puerto Rico	*O. lenticularis*	1.6×10^4/$4.4 \times 10^{4*}$	September 1985	4–5	October 1983–June 1986	Ballantine et al., 1988
Coral Reef off Belize	*O. lenticularis*	4×10^2	–	–	May–June 1994	Morton and Faust, 1997
Cienfuegos Bay, Cuba	*O. lenticularis*	5.3×10^5	February 2012	2	May 2011–March 2012	Moreira et al., 2017
Cienfuegos Bay, Cuba	*O. lenticularis*	7.9×10^4	June 2010	2	January–September 2010	Moreira et al., 2012
Puerto Morelos, México	*O. heptagona*	2.1×10^4/4.8×10^4	May 2015	10	April–December 2015	This study
Puerto Morelos, México	*O.* cf. *marina*	2.0×10^4/5.3×10^4	April 2015	10	April–December 2015	This study

[a]*Mean maximum abundance values/*maximum abundance in any sample.*

Caribbean vary from 10^2 to 10^5 cells·g^{-1} in *Dictyota*. Indeed, the highest abundance was recorded in Cuba (531,000 cel·g^{-1}) that is two times the maximum value reported by Ballantine et al. (1988) for the same species at Laurel Reef site, in Puerto Rico (235,803 cells·g^{-1}). High abundances have been reported for *O. siamensis* (Shears and Ross, 2009) at New Zealand coasts (1.4×10^6 cells·g^{-1} of *Carpophyllum plumosum*) and for *O.* cf. *ovata* at the Mediterranean (Cohu et al., 2013) where the maximum abundance recorded was 8.54×10^6 cells·g^{-1} of *Dictyota* spp. These results demonstrate that at temperate and tropical latitudes *Ostreopsis* species can reach high abundances, emphasizing the potential of *Ostreopsis* species to produce large benthic blooms globally (Rhodes, 2011).

Community Structure on *Dictyota* and *Amphiroa*

In terms of abundance, both algae are used as substrate by all species. However, in terms of community structure, correlation between species can be used to know if the substrate offers common advantages for different species, or if the increase of one species implies the decrease of another. The results of this study suggest that the structure of the epiphytic dinoflagellate community is different on each of the host algae *Amphiroa* and *Dictyota*, and there are even differences in the same alga between sites. These findings suggest that *Prorocentrum* species and *Coolia* constitute the basic structure on *Amphiroa*, and on *Dictyota* the structure is given by *Prorocentrum*, *Gambierdiscus* and *Coolia* that share the habitat. In addition, *O. marina* presents habitat separation with *Prorocentrum* and *Gambierdiscus*. A negative correlation between *Ostreopsis* and *Prorocentrum* was reported by Richlen and Lobel (2011) in the Pacific, but Boisnoir et al. (2018) found the contrary in the Eastern Caribbean. Also, the habitat separation between *Gambierdiscus* and *Ostreopsis* has been reported by Bomber et al. (1989) in Florida, but in the Caribbean, Ballantine et al. (1988) did not find evidence of correlation between these genera. The dominance of *Ostreopsis* over the other genera of dinoflagellates could be related to its preference to sites with little water motion, as these are shallow sites (Richlen and Lobel, 2011; Cohu et al., 2013; Boisnoir et al., 2018)

The Effect of Environment on the Population Dynamics

The distance between study sites is nearly 50 km; both sites are shallow and located in the northern region of the Mesoamerican Barrier Reef System. Therefore it is reasonable to suppose that these sites also undergo the same climatological and oceanographic conditions, generating similar abundances and taxonomic compositions of dinoflagellate populations. However, the results showed some important differences. For example, the differences in temperature are low but significant and this could be explained by the climate patterns. The region presents three seasons: (1) the season influenced by polar fronts (locally called "nortes") between December and February, (2) the dry season between March to May, and (3) the rainy season between June

and November. The presence of a polar front can occur year-round (Henry, 1979), but its intensity is higher during winter. During 2015 the cold fronts were intense even during July (2015). **Figure 5** shows the temperature series in the study sites. Both series have the same behavior but the magnitude of the variations is higher at IC than PM. The main effect of the polar fronts on the shallow aquatic ecosystems is the abrupt decrease of water temperature, but also the generation of strong movement of the surface layer of water. The energy of polar fronts decreases as they flow southward, therefore their effect on the coral reef systems should be higher at IC than PM. Since the energy of polar fronts removes host macroalgae, the turnover of dinoflagellate populations could be higher at IC, preventing their accumulation and resulting in higher abundances at PM. As the nutrient concentration remains high year-round, *Dictyota* can recover its biomass and abundance in short periods, providing surface for the attachment of *Ostreopsis*, which also grows rapidly (Ballantine et al., 1988). This implies that *Dictyota* and *Ostreopsis* are opportunistic genera exploiting an expanding habitat produced by the phase shift in the coral reefs.

Implications of *Ostreopsis* Blooms in the Caribbean Coral Reefs

The presence of *Ostreopsis* blooms on *Dictyota* along the Caribbean coasts has some ecological implications. First, toxicity has been reported in populations of *O. lenticularis* from Cuba and Puerto Rico (Ballantine et al., 1988; Moreira et al., 2012), as well as *O. heptagona* (Norris et al., 1985); in addition, some samples of *O.* cf. *marina* taken after the sampling period of this study showed toxicity on mouse bioassays and hemolytic activity (unpublished data). In fact, *Ostreopsis* species produce palytoxin-like compounds which are involved in human intoxications and possibly mass mortality of vertebrates and invertebrates (Ramos and Vasconcelos, 2010; Faimali et al., 2012; Patocka et al., 2017). Secondly, *Dictyota* is a macroalgae favored by the changes in the coral reef systems. In the sites studied these algae can form mats of several tens of square meters, at times reaching nearly 80% of cover (Delgado-Pech, 2016). However, the low palatability of *Dictyota* to larger benthic herbivores reduces grazing (Cruz-Rivera and Villareal, 2006) and prevents the flux of toxins to higher trophic levels. *Dictyota* mats are persistent, growing on other calcareous and coralline algae, corals and any other hard surface. Still, their attachment to the substrate is not firm enough to avoid detachment by the strong waves formed under the influence of polar fronts. During these events, the cover of *Dictyota* and the abundance of *Ostreopsis* decrease in the benthic environment, although, in the case of *Ostreopsis*, the cells (and their toxins) could remain in the water column and possibly enter the planktonic food web. If the coral-algal phase shift in the Caribbean results in dominance of *Dictyota* and other fleshy macroalgae, toxic *Ostreopsis* species could increase their abundance and distribution and cases of ciguatera and other syndrome of benthic origin could become a more serious public health problem in the region.

CONCLUSIONS

The taxonomic composition of epiphytic dinoflagellates inhabiting the macroalgae from the northern of the Mesoamerican Coral Reef Barrier was comprised of species of the genera *Ostreopsis*, *Prorocentrum*, *Coolia,* and *Gambierdiscus*.

Ostreopsis was the dominant genus in the sites studied. Particularly, *O.* cf. *marina* and *O. heptagona* reached maximum densities of 53,000 and 48,000 cells·g^{-1} on *Dictyota*. However, the period of sampling was not enough to detect seasonal trends of the dinoflagellate species.

Total abundances were higher in PM on both algae. These differences could be attributed to the intensity of environmental variables. For example, the lower temperature and the higher effect of polar fronts in IC could prevent the accumulation of epiphytic dinoflagellates.

O. marina showed a negative correlation with temperature between 27 and 30°C. On the contrary *P. lima* and *P. emarginatum* showed a positive correlation with temperature at IC on both macroalgae.

Nutrient concentrations NO_x^- and PO_4^{3-} were higher in both sites during the whole period of study, but in the most of the cases their concentration was not correlated with the abundance of dinoflagellates, excepting *O. heptagona* which showed a negative correlation with PO_4^{3-} concentration. On the contrary, *P. emarginatum*, *P. rathymum,* and *Coolia* spp. were positively correlated whit NO_x^- on *Dictyota* at IC.

The structure of epiphytic dinoflagellates was different between algae. There were more correlations between species on *Dictyota* than *Amphiroa*. The association between *P. lima* and *P. hoffmannianum* was the only one detected in both algae and in both sites.

The high cover of *Dictyota* in the study zone and the high abundance of *O.* cf. *marina* and *O. heptagona* on this macroalgae could represent a risk for human and marine fauna health if production of toxins is confirmed in this species.

The report of *Ostreopsis* blooms in the Mexican Caribbean coasts and the reports of blooms of this genus in other sites along the Caribbean basin emphasize the importance of this genus worldwide.

AUTHOR CONTRIBUTIONS

AA-B conceived and designed the study, wrote, and edited the document. EI-S and BD-P collected data, processed samples, wrote and edited the document, and built the figures and tables. EG-M and EN-V interpreted results, wrote, and edited the document. AO-O quantified the nutrient concentrations, interpreted the results, and wrote and edited the document.

FUNDING

CONACyT Fellowship number 308153. CONANP, Convenio PROMOBI/PNAPM/01/2015. CONABIO, Project MQ001. Red Temática sobre Florecimientos Algales Nocivos (RedFAN) CONACyT 2015-2017 projects.

ACKNOWLEDGMENTS

We thank to the National Protected Areas Comission (CONANP), specifically to the Parque Nacional Arrecife de Puerto Morelos and Parque Nacional Isla Contoy, and particularly, to the director of the areas Dra. Mary Carmen García Rivas. EI-S and BD-P thank to the Posgrado en Ciencias del Agua of CICY and to the RedFAN of CONACyT. Finally we thanks to Gabriela Resendiz for the figures of the study zone and temperature series, Jennifer Méndez for the epifluorescence images and to Eva Kozak for her support with english language.

REFERENCES

Almazán-Becerril, A., Escobar-Morales, S., Irola-Sansores, E. D., and Delgado-Pech, B. (2016). "Morfología y taxonomía de los géneros *Ostreopsis* y *Coolia* en el Caribe Mexicano," in *Florecimientos Algales Nocivos en México*, eds E. García-Mendoza, S. Quijano-Scheggia, A. Olivos-Ortiz, and E. J. Núñez-Vázquez (Ensenada: CICESE), 378–393.

Almazán-Becerril, A., Escobar-Morales, S., Rosiles-González, G., and Valadez, F. (2015). Benthic-epiphytic dinoflagellates from the northern portion of the Mesoamerican Reef System. *Bot. Mar.* 58, 115–128. doi: 10.1515/bot-2014-0093

Anderson, D. M., Burkholder, J. M., Cochlan, W. P., Glibert, P. M., Gobler, C. J., Heil, C. A., et al. (2008). Harmful algal blooms and eutrophication: examining linkages from selected coastal regions of the United States. *Harmful Algae* 8, 39–53. doi: 10.1016/j.hal.2008.08.017

Arcila-Herrera, H., Castello-Navarrete, A., Mendoza-Ayora, J., Montero-Cervantes, L., González-Franco, M. F., and Brito-Villanueva, W. O. (1998). Ten cases of ciguatera fish poisoning in Yucatan. *Rev. Inv. Clin.* 50, 149–152.

Ballantine, D. L., Tosteson, T. R., and Bardales, A. T. (1988). Population dynamics and toxicity of natural populations of benthic dinoflagellates in southwestern Puerto Rico. *J. Exp. Mar. Biol. Ecol.* 119, 201–212. doi: 10.1016/0022-0981(88)90193-1

Besada, E. G., Loeblich, L. A., and Loeblich, A. R. III. (1982). Observations on tropical, benthic dinoflagellates from ciguatera-endemic areas: *Coolia*, *Gambierdiscus*, and *Ostreopsis*. *Bull. Mar. Sci.* 32, 723–735.

Björk, M., Mohammed, S. M., Björklund, M., and Smesi, A. (1995). Coralline algae, important coral-reef builders threatened by pollution. *Ambio* 21, 502–505.

Boisnoir, A., Pascal, P. Y., Cordonnier, S., and Lemée, R. (2018). Depth distribution of benthic dinoflagellates in the Caribbean Sea. *J. Sea Res.* 135, 74–83. doi: 10.1016/j.seares.2018.02.001

Bomber, J. W., Guillard, R. R. L., and Nelson, W. G. (1988). Roles of temperature, salinity, and light in seasonality, growth, and toxicity of ciguatera causing *Gambierdiscus toxicus* Adachi *et* Fukuyo (Dinophyceae). *J. Exp. Mar. Biol. Ecol.* 115, 53–65. doi: 10.1016/0022-0981(88)90189-X

Bomber, J. W., Rubio, M. G., and Norris, D. R. (1989). Epiphytism of dinoflagellates associated with the disease ciguatera: substrate specificity and nutrition. *Phycologia* 28, 360–368. doi: 10.2216/i0031-8884-28-3-360.1

Chateau-Degat, M. L., Chinain, M., Cerf, N., Gingras, S., Hubert, B., and Dewailly, E. (2005). Seawater temperature, *Gambierdiscus* spp. variability and incidence of ciguatera poisoning in French Polynesia. *Harmful Algae* 4, 1053–1062. doi: 10.1016/j.hal.2005.03.003

Chinain, M., Germain, M., Paulliac, S., and Legrand, A.-M. (1999). Seasonal abundance and toxicity of the dinoflagellate Gambierdiscus spp. (Dinophyceae), the causative agent of ciguatera in Tahiti French Polynesia. *Mar. Biol.* 135, 259–267. doi: 10.1007/s002270050623

Cohu, S., Mangialajo, L., Thibaut, T., Blanfuné, A., Marro, S., and Lemée, R. (2013). Proliferation of the toxic dinoflagellate *Ostreopsis* cf. ovata in relation to depth, biotic substrate and environmental factors in the North West Mediterranean Sea. *Harmful Algae* 24, 32–44. doi: 10.1016/j.hal.2013.01.002

Cruz-Rivera, E., and Villareal, T. A. (2006). Macroalgal palatibility and the flux of ciguatera toxins through marine food webs. *Harmful Algae* 5, 497–525. doi: 10.1016/j.hal.2005.09.003

Delgado, G., Lechuga-Devéze, C. H., Popowski, G., Troccoli, L., and Salinas, C. A. (2006). Epiphytic dinoflagellates associated with ciguatera in the northwestern coast of Cuba. *Rev. Biol. Trop.* 54, 299–310. doi: 10.15517/rbt.v54i2.13870

Delgado-Pech, B. (2016). *Comunidades de Macroalgas en Arrecifes Coralinos de Quintana Roo: Composición Taxonómica y Patrones de Colonización*. Master's thesis, Centro de Investigación Científica de Yucatán, Cancún.

Faimali, M., Giussani, V., Piazza, V., Garaventa, F., Corrà, C., Asnaghi, V., et al. (2012). Toxic effects of harmful benthic dinoflagellate *Ostreopsis ovata* on invertebrate and vertebrate marine organisms. *Mar. Environ. Res.* 76, 97–107. doi: 10.1016/j.marenvres.2011.09.010

Faust, M. A. (1990). Morphologic details of six benthic species of *Prorocentrum* (Pyrrophyta) from a mangrove island, Twin Cays, Belize, including two new species. *J. Phycol.* 26, 548–558. doi: 10.1111/j.0022-3646.1990.00548.x

Faust, M. A. (1993). Three new benthic species of *Prorocentrum* (Dinophyceae) from Twin Cays, Belize: *P. maculosum* sp. nov., *P. foraminosum* sp. nov., and *P. formosum* sp. nov. *Phycologia* 32, 410–418. doi: 10.2216/i0031-8884-32-6-410.1

Faust, M. A. (1994). Three new benthic species of *Prorocentrum* (dinophyceae) from Carrie Bow Cay, Belize: *P. sabulosum* sp. nov., *P. sculptile* sp. nov., and *P. arenarium* sp. nov. *J. Phycol.* 30, 755–763. doi: 10.1111/j.0022-3646.1994.00755.x

Faust, M. A. (1995). Observation of sand-dwelling toxic dinoflagellates (Dinophyceae) from widely differing sites, including two new species. *J. Phycol.* 31, 996–1003. doi: 10.1111/j.0022-3646.1995.00996.x

Faust, M. A. (1997). Three new benthic species of *Prorocentrum* (dinophyceae) from Belize: *P. norrisianum* sp. nov., *P. tropicalis* sp. nov., and *P. reticulatum* sp. nov. *J. Phycol.* 33, 851–858. doi: 10.1111/j.0022-3646.1997.00851.x

Faust, M. A. (1999). Three new *Ostreopsis* species (Dinophyceae): *O. marina* sp. nov., *O. belizeanus* sp. nov., and *O. caribbeanus* sp. nov. *Phycologia* 38, 92–99. doi: 10.2216/i0031-8884-38-2-92.1

Faust, M. A., Morton, S. L., and Quod, J. P. (1996). Further SEM study of marine dinoflagellates: the genus *Ostreopsis* (Dinophyceae). *J. Phycol.* 32, 1053–1065 doi: 10.1111/j.0022-3646.1996.01053.x

Fukuyo, Y. (1981). Taxonomical study on benthic dinoflagellates collected in coral reefs. *Bull. Jpn. Soc. Sci. Fish.* 47, 967–978. doi: 10.2331/suisan.47.967

Gingold, B. D., Strickland, M. J., and Hess, J. J. (2014). Ciguatera fish poisoning and climate change: analysis of national poison data in the United States, 2001-2011. *Environ. Health Perspect.* 122, 580–586. doi: 10.1289/ehp.1307196

Grasshoff, K., Ehrhardt, M., and Kremling, K. (1983). *Methods of Seawater Analysis, 2nd Edn*. Weinheim: Verlag Chemie.

Hales, S., Weinstein, P., and Woodward, A. (1999). Ciguatera (fish poisoning), El Niño, and Pacific sea surface temperatures. *Ecosyst. Health* 5, 20–25. doi: 10.1046/j.1526-0992.1999.09903.x

Henry, W. K. (1979). Some aspects of the fate of cold fronts in the Gulf of Mexico. *Mon. Weather Rev.* 107, 1078–1082.

Hernandez-Becerril, D. U., and Almazan-Becerril, A. (2004). El género *Gambierdiscus* (Dinophyceae) en el Caribe Mexicano. *Rev. Biol. Trop.* 52, 77–87. doi: 10.15517/RBT.V52I1.26521

Hoegh-Guldberg, O., Mumby, P. J., Hooten, A. J., Steneck, R. S., Greenfield, P., Gomez, E., et al. (2007). Coral reefs under rapid climate change and ocean acidification. *Science* 318, 1737–1742. doi: 10.1126/science.1152509

Karafas, S., York, R., and Tomas, C. (2015). Morphological and genetic analysis of the *Coolia monotis* species complex with the introduction of two new species, *Coolia santacroce* sp. nov. and *Coolia* palmyrensis sp. nov. (Dinophyceae). *Harmful Algae* 46, 18–33. doi: 10.1016/j.hal.2015.05.002

Kibler, S. R., Tester, P. A., Kunkel,. K. E., Moore, S. K., and Litaker, R. W. (2015). Effects of ocean warming on growth and distribution of dinoflagellates associated with ciguatera fish poisoning in the Caribbean. *Ecol. Model.* 316, 194–2010. doi: 10.1016/j.ecolmodel.2015.08.020

Kohler, S. T., and Kohler, C. C. (1992). Dead bleached coral provides new surfaces for dinoflagellates implicated in ciguatera fish poisonings. *Environ. Biol. Fish.* 35, 413–416 doi: 10.1007/BF00004993

Lartigue, J., Jester, E. L., Dickey, R. W., and Villareal, T. A. (2009). Nitrogen source effects on the growth and toxicity of two strains of the ciguatera-causing dinoflagellate *Gambierdiscus toxicus*. *Harmful Algae* 8, 781–791. doi: 10.1016/j.hal.2008.05.006

Litaker, R. W., Vandersea, M. W., Faust, M. A., Kibler, S. R., Chinain, M., Holmes, M. J., et al. (2009). Taxonomy of *Gambierdiscus* including four new species, *Gambierdiscus caribaeus, Gambierdiscus carolinianus, Gambierdiscus carpenteri* and *Gambierdiscus ruetzleri* (Gonyaulacales, Dinophyceae). *Phycologia* 48, 344–390. doi: 10.2216/07-15.1

Littler, M. M., Littler, D. S., and Taylor, P. R. (1983). Evolutionary strategies in a tropical barrier reef system: functional-form groups of marine macroalgae. *J. Phycol*. 19, 229–237. doi: 10.1111/j.0022-3646.1983.00229.x

Lobel, P. S., Anderson, D. M., and Durand-Clement, M. (1988). Assessment of ciguatera dinoflagellate populations: sample variability and algal substrate selection. *Biol. Bull*. 175, 94–101. doi: 10.2307/1541896

McCook, L. J. (1999). Macroalgae, nutrients and phase shifts on coral reefs: scientific issues and management consequences for the Great Barrier Reef. *Coral Reefs* 18, 357–367. doi: 10.1007/s003380050213

Moreira, A., Fernandes, L., Escarrá, R., Díaz, L., Rodríguez, F., Riobo, P., et al. (2017). "Notes on morphology, phylogeny and toxicity of a dominant community of toxic benthic dinoflagellates from southern-central coast of Cuba," in *Marine and Freshwater Harmful Algae. Proceedings of the 17th International Conference on Harmful Algae*, eds L. O. A. Proença and M. Hallegraeff (Florianópolis: International Society for the Study of Harmful Algae 2017), 88–91.

Moreira, A., Rodríguez, F., Riobó, P., Franco, J. M., Martínez, N., Chamero, D., et al. (2012). Notes on *Ostreopsis* sp. from southern-central coast of Cuba. *Cryptol. Algol*. 33, 217–224. doi: 10.7872/crya.v33.iss2.2011.217

Morton, S. L., and Faust, M. A. (1997). Survey of toxic epiphytic dinoflagellates from the Belizean barrier reef ecosystem. *Bull. Mar. Sci*. 61, 899–906.

Nakahara, H., Sakami, T., Chinain, M., and Ishida, Y. (1996). The role of macroalgae in epiphytism of the toxic dinoflagellate *Gambierdiscus toxicus* (Dinophyceae). *Phycol. Res*. 44, 113–117. doi: 10.1111/j.1440-1835.1996.tb00385.x

Norris, D. R., Bomber, J. W., and Balech, E. (1985). "Benthic dinoflagellates associated with ciguatera from the Florida Keys. I. *Ostreopsis heptagona* sp. nov.," in *Toxic Dinoflagellates*, eds D. M. Anderson, A. W. White, and D. G. Baden (New York, NY: Elsevier Scientific), 39–44.

Okolodkov, Y. B., Campos-Bautista, G., Gárate-Lizárraga, I., González-González, J. A. G., Hoppenrath, M., and Arenas, V. (2007). Seasonal changes of benthic and epiphytic dinoflagellates in the Veracruz reef zone, Gulf of Mexico. *Aquat. Microb. Ecol*. 47, 223–237. doi: 10.3354/ame047223

Okolodkov, Y. B., Virgilio, F. D. C. M., Castillo, J. A. A., Trujillo, A. C. A., Espinosa-Matías, S., and Silveira, A. H. (2014). Seasonal changes in epiphytic dinoflagellate assemblages near the northern coast of the Yucatan Peninsula, Gulf of Mexico. *Acta Bot. Mex*. 107, 121–151. doi: 10.21829/abm107.2014.204

Parsons, M. L., Aligizaki, K., Bottein, M. Y. D., Fraga, S., Morton, S. L., Penna, A., et al. (2012). *Gambierdiscus* and *Ostreopsis*: Reassessment of the state of knowledge of their taxonomy, geography, ecophysiology and toxicology. *Harmful Algae* 14, 107–129. doi: 10.1016/j.hal.2011.10.017

Parsons, M. L., and Preskitt, L. B. (2007). A survey of epiphytic dinoflagellates from the coastal waters of the island of Hawaii. *Harmful Algae* 6, 658–669. doi: 10.1016/j.hal.2007.01.001

Parsons, M. L., Settlemier, C. J., and Ballauer, J. M. (2011). An examination of the epiphytic nature of *Gambierdiscus toxicus*, a dinoflagellate involved in ciguatera fish poisoning. *Harmful Algae* 10, 598–605. doi: 10.1016/j.hal.2011.04.011

Patocka, J., Nepovimova, E., Wu, Q., and Kuka, K. (2017). Palytoxin congeners. *Arch. Toxicol*. 92, 143–156. doi: 10.1007/s00204-017-2105-8

Penna, A., Vila, M., Fraga, S., Giacobbe, M. G., Andreoni, F., Riobó, P., et al. (2005). Characterization of *Ostreopsis* and *Coolia* (dinophyceae) isolates in the western Mediterranean Sea based on morphology, toxicity and internal transcribed spacer 5.8 s rDNA sequences. *J. Phycol*. 41, 212–225. doi: 10.1111/j.1529-8817.2005.04011.x

Rains, L. K., and Parsons, M. L. (2015). *Gambierdiscus* species exhibit different epiphytic behaviors toward a variety of macroalgal hosts. *Harmful Algae* 49, 29–39. doi: 10.1016/j.hal.2015.08.005

Ramos, V., and Vasconcelos, V. (2010). Review: palytoxin and analogs: biological and ecological effects. *Mar. Drugs* 8, 2021–2037 doi: 10.3390/md8072021

Reguera, B., Alonso, R., Moreira, A., Méndez, S., and Dechraoui-Bottein, M. Y. (2016). *Guide for Designing and Implementing a Plan to Monitor Toxin-Producing Microalgae, 2nd Edn*. IOC Manuals and Guides, No. 59. Paris; Vienna: Intergovernmental Oceanographic Commission (IOC) of United Nations Educational Scientific and Cultural Organization (UNESCO) and International Atomic Energy Agency (IAEA).

Rhodes, L. (2011). World-wide occurrence of the toxic dinoflagellates genus *Ostreopsis* Schmidt. *Toxicon* 3, 400–407. doi: 10.1016/j.toxicon.2010.05.010

Richlen, M. L., and Lobel, P. S. (2011). Effects of depth, habitat, and water motion on the abundance and distribution of ciguatera dinoflagellates at Johnston Atoll, Pacific Ocean. *Mar. Ecol. Prog. Ser*. 421, 51–66. doi: 10.3354/meps08854

Shears, N. T., and Ross, P. M. (2009). Blooms of benthic dinoflagellates of the genus *Ostreopsis*; an increasing and ecologically important phenomenon on temperate reefs in New Zealand and worldwide. *Harmful Algae* 8, 916–925. doi: 10.1016/j.hal.2009.05.003

Steneck, R. S., and Deither, M. N. (1994). A functional group approach to the structure of algal-dominated communities. *Oikos* 69, 476–498. doi: 10.2307/3545860

Strickland, J. D., and Parsons, T. R. (1972). *A Practical Handbook of Seawater Analysis*. Ottawa, ON: Fisheries Research Board of Canada.

Tester, P. A., Feldman, R. L., Nau, A. W., Kibler, S. R., and Litaker, W. R. (2010). Ciguatera fish poisoning and sea surface temperatures in the Caribbean Sea and the West Indies. *Toxicon* 56, 698–710. doi: 10.1016/j.toxicon.2010.02.026

Tosteson, T. R. (2004). Caribbean ciguatera: a changing paradigm. *Rev. Biol. Trop*. 52, 109–113. doi: 10.15517/RBT.V52I1.26524

Turquet, J., Quod, J. P., Ten-Hage, L., Dahalani, Y., and Wendling, B. (2001). "Example of a *Gambierdiscus toxicus* flare-up following the 1998 coral bleaching event in Mayotte Island (Comoros, southwest Indian Ocean)," in *Harmful Algal Blooms 2000*, eds G. M. Hallegraeff, S. I. Blackburn, C. J. Bolch, and R. J. Lewis (Hobart, TAS: Intergovernmental Oceanographic Commission of UNESCO), 50–53

Vila, M., Garcés, E., and Masó, M. (2001). Potentially toxic epiphytic dinoflagellate assemblages on macroalgae in the NW Mediterranean. *Aquat. Microb. Ecol*. 26, 51–60. doi: 10.3354/ame026051

Yasumoto, T., Inoue, A., Ochi, T., Fujimoto, K., Oshima, Y., Fukuyo, Y., et al. (1980). Environmental studies on a toxic dinoflagellate responsible for ciguatera. *Bull. Jpn. Soc. Sci. Fish*. 46, 1397–1404. doi: 10.2331/suisan.46.1397

Yong, H. L., Mustapa, N. I., Lee, L. K., Lim, Z. F., Tan, T. H., Usup, G., et al. (2018). Habitat complexity affects benthic harmful algae assemblages in the reef of Raka Island, Malasya. *Harmful Algae* 78, 56–68. doi: 10.1016/j.hal.2018.07.009

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Irola-Sansores, Delgado-Pech, García-Mendoza, Núñez-Vázquez, Olivos-Ortiz and Almazán-Becerril. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Marine Science

ORIGINAL RESEARCH
published: 17 January 2019
doi: 10.3389/fmars.2018.00532

Population Genetic Structure at the Northern Edge of the Distribution of *Alexandrium catenella* in the Patagonian Fjords and Its Expansion Along the Open Pacific Ocean Coast

Javier Paredes[1,2], Daniel Varela[2,3], Camila Martínez[2], Andrea Zúñiga[2], Karen Correa[2,4], Adrián Villarroel[2] and Bianca Olivares[2,4]*

[1] Programa de Doctorado en Biología y Ecología Aplicada, Departamento de Biología Marina, Facultad de Ciencias del Mar, Universidad Católica del Norte, Coquimbo, Chile, [2] Centro i~mar, Universidad de Los Lagos, Puerto Montt, Chile, [3] Centre for Biotechnology and Bioengineering, Santiago, Chile, [4] Instituto de Fomento Pesquero (IFOP), Centro de Estudios de Algas Nocivas (CREAN), Puerto Montt, Chile

OPEN ACCESS

Edited by:
Juan Jose Dorantes-Aranda, Institute for Marine and Antarctic Studies (IMAS), Australia

Reviewed by:
Uwe John, Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung, Helmholtz-Gemeinschaft Deutscher Forschungszentren (HZ), Germany
Shauna Murray, University of Technology Sydney, Australia

***Correspondence:**
Javier Paredes
jaelpame@gmail.com;
javier.paredes@ulagos.cl

Specialty section:
This article was submitted to Marine Ecosystem Ecology, a section of the journal Frontiers in Marine Science

Received: *01 August 2018*
Accepted: *31 December 2018*
Published: *17 January 2019*

Citation:
Paredes J, Varela D, Martínez C, Zúñiga A, Correa K, Villarroel A and Olivares B (2019) Population Genetic Structure at the Northern Edge of the Distribution of Alexandrium catenella in the Patagonian Fjords and Its Expansion Along the Open Pacific Ocean Coast. Front. Mar. Sci. 5:532. doi: 10.3389/fmars.2018.00532

In southern Chile, *Alexandrium catenella* is the main species generating harmful algal blooms (HABs) and over time it has expanded its range since it was first recorded in the Magallanes region in 1972. In 2016 a severe bloom of an *Alexandrium* species occurred, which was notable for its intensity and geographical extent, extending into new areas to the north of the Patagonian fjords including areas along the open Pacific Ocean coast. Given the exceptional nature of this event, we verified the taxonomic classification of the species that generated the bloom and evaluated the influence of the range expansion process on its genetic structure and population diversity. This was achieved by isolating clones collected in 2014 from cyst beds located at the northern limit of its then known distribution, along with clones isolated from the water samples taken during the 2016 bloom. These clones were characterized genetically with LSU rDNA and AFLPs molecular markers. Phylogenetic analyses showed that all clones were aggregated in the Group I of the *A. tamarense* species complex, which confirmed that *A. catenella* was the species responsible for the 2016 bloom. High genetic diversity was observed within populations though there were no significant differences between populations. Furthermore, genetic structure showed an isolation by distance relationship among populations, and several analyses consistently indicated a high divergence among the population groups derived from both cysts and vegetative cells. Despite this study not finding the patterns expected for species range expansion (i.e., diversity gradient and/or high population divergence), the genetic diversity and structure indicated that these were influenced by the geographic distance effect and the physical restrictions to gene flow, along with the demographic processes that occurred during the distinct phases of the life cycle of *A. catenella*.

Keywords: *Alexandrium catenella*, south Chile, range expansion, genetic population, bloom, Patagonian fjords, Pacific Ocean coast

INTRODUCTION

Around the world, factors such as climate change and eutrophication of coastal waters have favored the recurrence and severity of harmful algal blooms (HABs), increasing the probability that harmful species will expand their ranges to new areas (Wells et al., 2015). Species of the *A. tamarense* species complex are among the most prominent HAB species (Anderson et al., 2012b; Murray et al., 2015). These species, following a trend shown by other harmful algae (Anderson et al., 2012a), have over recent decades expanded their geographic ranges in both the northern and southern hemispheres (Penna et al., 2005; Persich et al., 2006).

Underlying the process of species range expansion are the colonization-extinction dynamics of the populations, whether a proportion of the migrating population will survive or not and successfully reproduce in the new area (e.g., Sexton et al., 2009). Theoretically, it is expected that the range expansion process will generate a gradient in genetic diversity and marked genetic differences between populations, due to a fraction of the migrants establishing in the new area changing due to the effects of genetic drift (Excoffier et al., 2009; Sexton et al., 2009). The genetic characterization of populations using AFLPs (Amplified fragment length polymorphisms) has permitted the study of genetic diversity and differentiation in several harmful species, with an important advantage over microsatellite markers due to the high amount of genome-wide loci observed (Rengefors et al., 2017) and its power in identifying weak genetic population differentiation (Alpermann et al., 2009). Although, AFLPs disadvantages include variation in the precision of sizing of fragments, and the assumption that bands of the same size are homologous, might not always be right, especially at higher levels of divergence (John et al., 2004; Fry et al., 2009). For instance, AFLP population characterizations have permitted the examination of the range expansion process of species such as *Gonyostomum semen* and *Alexandrium ostenfeldii,* which show no evidence of diversity gradients, but do show levels of population divergence and isolation by distance (Tahvanainen et al., 2012; Sassenhagen et al., 2015). However, in *A. ostenfeldii*, Brandenburg et al. (2018) recently described a contrasting genetic and phenotypic variability between a highly diverse population established in the Baltic Sea approximately at 3000 – 8000 BP and the low variability of the populations that colonized the Netherlands localities in 2012, that represent examples of established and founder population respectively. Furthermore, genetic characterization using AFLPs and microsatellites of *Alexandrium* populations have shown the presence of high levels of genetic diversity, which is related in turn to high phenotypic variability, factors which are relevant to their adaptive potential (Alpermann et al., 2010; John et al., 2015; Kremp et al., 2016; Brandenburg et al., 2018). In the case of *A. tamarense* species complex, the population genetics characterization using microsatellites and AFLPs, have revealed that the population divergence could have been generated by demographic changes that have occurred over time, in the populations of cysts in the sediments, and/or in the population of vegetative cells in the water column, and as a result of reduced gene flow. For example, it has been hypothesized that the vegetative cells generated in a bloom would come from diverse banks of cysts, formed by different cohorts, that have accumulated in the sediment over time (Alpermann et al., 2009). In addition, during the bloom the environmental conditions could favor changes in allele frequency, due to selective pressures on certain genotypes, which could then be amplified by asexual reproduction (Alpermann et al., 2009; Dia et al., 2014). Finally, the potential migration of vegetative cells could be limited by oceanographic barriers (Casabianca et al., 2012) or restricted by geographic distance (Nagai et al., 2007), generating high levels of differentiation between populations.

The taxonomic identification of species comprising the *A. tamarense* species complex is fundamental not just for monitoring programs and implementing management strategies (John et al., 2014), but also, in order to verify that the genetic analyses of populations are made with conspecific individuals. Over time various authors have discussed the taxonomic classification of the species of the *A. tamarense* species complex which occurs in southern Chile, proposing a range of nomenclatural alternatives, such as *A. tamarense* (Jedlicki et al., 2012), *A. fundyense* (John et al., 2014; Mcneill et al., 2014; Wang et al., 2014) or *A. catenella* (Cordova and Müller, 2002). However, this discussion was recently concluded with priority being given to the name *A. catenella* (Willem, 2017). This classification was based in large part on the homology of ribosomal genetic characters (rDNA) rather than morphology, as morphology can be highly plastic and variable within the species (John et al., 2014). For example, historically the capacity to form chains has been used as a character for distinguishing between the species of the *A. tamarense* species complex. However, evidence indicates that the formation of chains is influenced by environmental variables such as a high pH and low pCO_2 (Mardones et al., 2016b). On the other hand, the phylogenetic analysis with rDNA molecular markers consistently produces five clades, where clones are separated, including those with a sympatric distribution (John et al., 2014).

In southern Chile *Alexandrium catenella* is the principal species causing HABs, resulting in serious ecological, social and economic impacts throughout the Patagonian fjords, where this species has expanded its range northward (Varela et al., 2012). Since 1972, when it was first identified in the Magallanes region (ca. 56°S), monitoring programs have registered the northward expansion of the species range distribution, arriving in the Aysén region in 1992, and in the waters of the southern end of Chiloé Island (ca. 43°S), Los Lagos region, in 2002 (Molinet et al., 2003; Varela et al., 2012). Until 2015 the blooms of *A. catenella* were restricted to the fjords and channels between Magallanes and southern Chiloé Island (Molinet et al., 2003; Mardones et al., 2010; Varela et al., 2012). However, a severe bloom of *A. catenella* occurred during 2016 that extended from the central-northern region of Aysén (45° 27′S) to the Chiloé inland sea, and for the first time, the bloom occurred in the open Pacific Ocean along the west coast of Chiloé Island (**Figure 1**; Buschmann et al., 2016).

The exceptional bloom of 2016 was surprising due to its wide geographical distribution, extending into new areas along the open coast of the Pacific Ocean and Chiloé inland sea

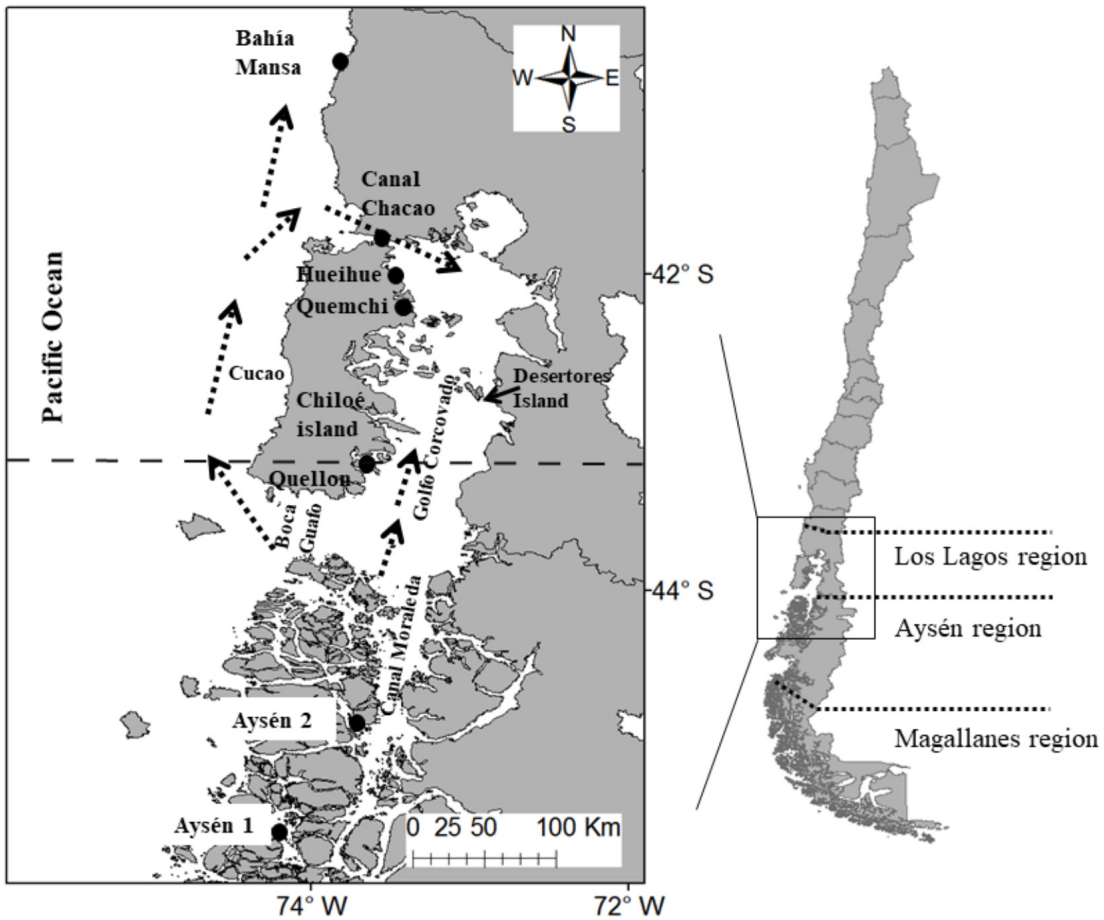


FIGURE 1 | Localities from where the clones were isolated (black circles): Aysen 1, Aysén 2, and Quellón, the populations from northern limit of the known distribution of *A. catenella* up to 2015, where the clones were isolated from cysts beds in 2014. Quemchi, Hueihue, Canal Chacao, Bahía Mansa, the populations isolated from vegetative cells of the 2016 bloom, isolate from opportunistic sampling. Arrows show a hypothesized route of the spread of the 2016 bloom. Horizontal dotted lines illustrate both the political boundary of the regions **(right)** and the horizontal segmented line show distribution limit of the species up to 2015 **(left)**.

(**Figure 1**). The event chronology indicated that the first evidence of the bloom was recorded around November–December of 2015 in the central-northern area of the Aysén region (45°23′S). Later, during January–February of 2016 the highest abundances of vegetative cells were observed in the southern area of the Chiloé inland sea (Buschmann et al., 2016). Until then, the bloom had followed the same pattern of geographical distribution observed during the severe blooms that occurred in 2002 and 2009 (Molinet et al., 2003; Mardones et al., 2010). However, from the first week of March 2016 the bloom extend northward, through the Chiloé inland sea up to Desertores Island (42° 41′ S), from where high levels of paralytic shellfish poison (PSP) were detected in mollusks (Buschmann et al., 2016). By April, the bloom declined in the Chiloé inland sea, but surprisingly the highest concentrations of PSP in mollusks were found along the exposed Pacific coast of Chiloé Island (42°37′S). This was the first evidence of the spread of the bloom into the open Pacific Ocean. Subsequently, PSP increased rapidly in mollusks living further north along the exposed coast, vegetative cells abundance also increased in areas beyond the Patagonian fjords (up to 39°42′S). Finally, by mid-May, both the cellular abundance and PSP concentrations began to decline rapidly in most areas (Buschmann et al., 2016; Hernández et al., 2016).

Given the exceptional nature of the 2016 bloom, several hypotheses have been proposed to explain the origin and dynamics of the range expansion, including doubt as to whether the species that caused the bloom were the same species that have historically affected the Patagonian fjords. Thus, the objective of the current investigation was to verify the taxonomic classification of the species that generated this exceptional bloom and evaluate the influence of the range expansion process on its genetic structure and population diversity. In order to achieve this, rDNA large subunit (LSU) and AFLPs molecular markers were used for the genetic characterization of clones. This was done with two groups of samples; the first group were vegetative cells isolated from cysts collected in 2014 from sediments located at the northern limit of its known distribution; and the second group were vegetative cells isolated from samples taken from the bloom expansion areas observed during the 2016 bloom in waters of the open Pacific

Ocean coast and from the waters of the Chiloé inland sea (**Figure 1**).

MATERIALS AND METHODS

Sampling and Cultivation of Clones

The samples used for the genetic characterization of the exceptional bloom of 2016 were collected in April, during the peak of the event, from central-northern area of its distribution, and they were compared with samples collected previously, in 2014, from sites near the origin of the same bloom event. In 2014 sediment samples containing *A. catenella* cysts were collected by SCUBA divers from the Aysén 1, Aysén 2 and Quellón localities (Populations from the northern limit of the then known species distribution (**Figure 1** and **Table 1**). The sediment samples were transported to the laboratory, Centro i∼mar Universidad de Los Lagos, where the cysts were isolated using the methodology described by Varela et al. (2012). The cysts were then placed in multi-welled cultivation plates with L1 medium and exposed to conditions that facilitate germination (i.e., 12°C, 30 psu, 35 ± 5 μmol photons m^{-2} s^{-1}, and a photoperiod of 16:8 h light/dark). From each well where cysts germinated, a single cell was selected and transferred to a new well (48-well plate) with the objective of generating clonal cultures. The single cell was isolated using an inverted microscope (Olympus CKX 42) by an extended Pasteur pipette (Anderson and Kawachi, 2005) and then deposited in a well with an area of 0.64 cm^2 (48-well plate) with 500 μl of L1. Subsequently, the well was carefully examined using an inverted microscope to verify the successful transfer. Additionally, in April of 2016, seawater samples were collected during the *A. catenella* bloom at Quemchi, Hueihue, Canal Chacao, and Bahía Mansa localities (**Figure 1** and **Table 1**). These samples were part of an opportunistic sampling that attempted to determine the extent of the 2016 bloom beyond its historic distribution. Seawater samples were also transported to the laboratory (Centro i∼mar) where vegetative cells were isolated and maintained following the same methodology and environmental conditions described above. Moreover, after 24 days of culturing, cell characteristic were determined (N = 50 per locality), i.e., viability in culture (number of well with cells alive), cell density, growth rate and the number of the cells forming chains (*c.f.* Paredes et al., 2016). Finally, cell isolates, both from cysts and vegetative cells, once they began to multiply, were transferred first to culture tubes, then to 250 ml conical flasks, from which cells were harvested for DNA extraction. Thus, all the strains utilized in this study were established as clonal cultures and maintained under the same laboratory conditions (i.e., 12°C, 30 psu, 35 ± 5 μmol photons m^{-2} s^{-1}, photoperiod of 16:8 h light/dark, and L1 cultivation medium).

Taxonomic Identification of the Clones

To verify the taxonomic classification of the clones a total of 96 clones isolated from the 2016 bloom and the cysts were characterized using rDNA LSU (D1D2) molecular marker as described by Scholin et al. (1994). DNA extractions were performed with the DNeasy plant mini Kit (Qiagen, Hilden, Germany) using 250 ml of culture harvested in the exponential phase of growth, and concentrated by centrifugation. The amplification of the DNA was performed using a thermocycler (Px2 Thermal Cycler, Thermo Electron Corporation, Waltham, MA, United States) with the following settings: one cycle of 5 min at 95°C; 30 cycles of 45 s at 95°C, 45 s at 56°C and 1 min at 72°C; and a final elongation for 10 min at 72°C. The amplifications were verified using an agarose gel (1.4%), and then, the PCR products were sent to biotechnological company Macrogen® for sequencing. The obtained sequences were evaluated visually and the homology with the sequences available in GenBank was explored. Finally, a phylogenetic reconstruction was performed using the sequences generated by John et al. (2014) which were obtained from the **Supplementary Material** (i.e., **Supplementary Figure 1**). Alignments of the SSU, ITS and LSU sequences for the phylogenetic analyses presented in this study. 1-s2.0-S1434461014001011-mmc1.doc). The reconstruction was performed with the maximum likelihood (ML) method, with a bootstrap analysis (1000 replicates) which evaluated the robustness of the nodes, and using an alignment of 826 base pair (bp). These analyses were performed with the software PAUP (Swofford, 2002). To perform the ML reconstruction, previous DNA sequences were analyzed with the MODELTEST 3.7 software which determined the most appropriate model of nucleotide substitution for the data (Possada and Crandall, 1998). Thus, the GTR + G + I model was selected with the following

TABLE 1 | *Alexandrium catenella* samples information.

Populations	Coordinates		Isolation date	Isolated from	rDNA	AFLP
	S	W				
Aysén 1	45°29′13.29″	74°10′55.87″	2014	Cyst	7	10
Aysén 2	44°52′01.09″	73°35′42.70″	2014	Cyst	17	20
Quellón	43°06′04.86″	73°35′42.70″	2014	Cyst	7	18
Quemchi	42°07′56.56″	73°26′59.74″	2016	V. cell	12	24
Hueihue	41°55′47.06″	73°29′06.82″	2016	V. cell	10	16
Canal Chacao	41°46′57.85″	73°36′26.91″;	2016	V. cell	7	12
Bahia Mansa	40°36′27.05″	73°46′02.18″	2016	V. cell	8	8

rDNA, number of clones sequenced. AFPL, number de clones characterized.

corrections: a base frequency of A = 0.26130, C = 0.16530, G = 0.24910, T = 0.32430; and a substitution rate matrix of [A _ G] = 2.2885, [A _ T] = 0.7838, [C _ G] = 0.7838, [C _ T] = 3.3797, [A _ C] = 1, and [G _ T] = 1; with no proportion of invariable sites and an unequal distribution of rates at variable sites (gamma shape parameter of alpha = 1.2519).

Genotyping Using AFLP Analyses

The genetic characterization was performed with 150 clonal strains, using the AFLPs technique and 100 ng of DNA, as suggested by Vos et al. (1995) and Mardones et al. (2016a). DNA extractions were performed with the DNeasy Plant Mini Kit (Qiagen), using 250 ml of culture harvested in the exponential phase of growth and concentrated by centrifugation. The concentration and quality of the DNA (proportion 280/260 nm) were estimated using a NanoQuant Plate (Infinite M200 PRO). The primers EcoRI- 5′ GACTGCGTACCAATTCXXX 3′ and MseI-5′ GATGAGTCCTGAGTAAXXX 3′ were used for the selective amplifications with the following combinations of primers marked with Fam dyes: $EcoRI_{AAG}$ x $MseI_{CTA}$, $EcoRI_{AAG}$ x $MseI_{CTT}$, $EcoRI_{ACC}$ x $MseI_{CTA}$ and, $EcoRI_{ACC}$ x $MseI_{CTT}$. Selective amplifications were performed with the settings "touchdown" which included: 13 initial cycles with 30 s at 95°C, 45 s at 56°C (gradually reduced by 0.7°C per cycle), and 30 s at 72°C. This was followed by 25 cycles of 1 min at 95°C; 1 min at 56°C; 1 min at 72°C, and finally an elongation of 10 min at 72°C. The amplifications were verified in an agarose gel (1.4%) and then these were analyzed using an ABI PRISM 3500 sequencer (Applied Biosystems). Consequently, the AFLP fragments were visualized and edited with the software GeneMarker. In order to create a presence/absence matrix of co-migrating AFLPs fragment, the sequence profiles were examined visually considering a fragment detection range between 100 and 15000 relative fluorescence units (RFUs) and between 50 and 400 bp. In addition, the reproducibility of the amplifications was determined visually by repeating 20% of the samples selected at random.

Intraspecific Distance, Genetic Diversity, and Population Structure

The intraspecific distance was determined using Nei's genetic distance (Saitou and Nei, 1987) while the genetic diversity of the populations (H) was calculated according to Nei (1973). The genetic diversity differentiation between populations was evaluated by an ANOVA, performed in a generalized linear mixed model (GLMM) framework. The genetic diversity for each locus was estimated, and the populations were considered to be a random effect to account for the lack of independence between the samples. In addition, a negative binomial residuals model distribution was used, and the null hypothesis (H_0) was evaluated using a likelihood ratio test with a significance level (α) of 0.05 (Venables and Ripley, 2002). The estimation of genetic diversity per locus and population was performed with the ARLEQUIN 3.5.1.2 software (Excoffier et al., 2005) while the GLMM was performed using R software (R Core Team, 2017) and the package "lme4" (Bates et al., 2015).

The genetic structure was estimated using different analyses. Firstly, analysis of molecular variance (AMOVA) and the significance of the F_{ST}, $F_{SC,}$ and F_{CT} were tested using a non-parametric analysis with 10,000 permutations. For this analysis the following sources of variation were considered: (1) between groups, composed of isolated populations in the northern limit of distribution (derived from cysts) and the populations isolated from the bloom; (2) variation between populations within groups, and (3) variation within the population. Furthermore, the level of differentiation between populations was estimated using the fixation index F_{ST}. Both analyses were performed using the software ARLEQUIN 3.5.1.2 (Excoffier et al., 2005). Subsequently, in order to visualize the similarities/dissimilarities between populations, a Principal Component Analysis (PCA) was performed using a matrix of genetic distances between populations which was estimated using Nei (1978) method. This was done using the software GENALEX 6.5 with a binary model for haploid organisms (Peakall and Smouse, 2012). The identification of genetic populations and the level of admixture of each genotype, or proportion of genotype assigned to each population, were estimated using a Bayesian cluster analysis implemented in the software STRUCTURE 2.3.4. The simulation was performed using a correlated allele frequency model and an ancestry mixed model without using location as prior information (Falush et al., 2003). The simulations were conducted using values of K populations from 1 to 10; a burning length of 100,000 and 100,000 repetitions of Monte Carlo Markov Chains (MCMC), and the results were evaluated using likelihood of K populations [L(K)]. The evaluation of K populations and the visualization of the genotype assignments were made using the web application www.pophelper.com (Francis, 2016). Finally, the software ARLEQUIN was used to conduct a Mantel analysis to evaluate isolation by distance, which included the paired values of F_{ST} (Y matrix) and the geographic distance in Km (X matrix).

Disequilibrium of the Multilocus Linkage

The index of multilocus disequilibrium $\bar{r}_d$ (Agapow and Burt, 2001) was calculated for each population and significance was determined using 10,000 permutations. The values of the index vary between 0 and 1 indicating equilibrium and disequilibrium respectively. The analyses were performed with R software (R Core Team, 2017) and the package "Poppr" (Kamvar et al., 2014).

RESULTS

Taxonomic Identification

Only 68 sequences of molecular marker rDNA LSU (D1D2) were used (**Table 1**). The remaining sequences were eliminated after visual evaluations of the chromatograms which suggested ambiguity in the assignment of some nucleotides i.e., no clear nucleotide peak or nucleotide peak overlaying. The phylogenetic analysis indicated that sequences of the clones isolated from both the 2016 bloom and the northern limit formed part of the same clade, corresponding to the Group I of the *A. tamarense* species complex (Lilly et al., 2007; John et al., 2014) (**Figure 2**). Moreover, Chi-square test did not show

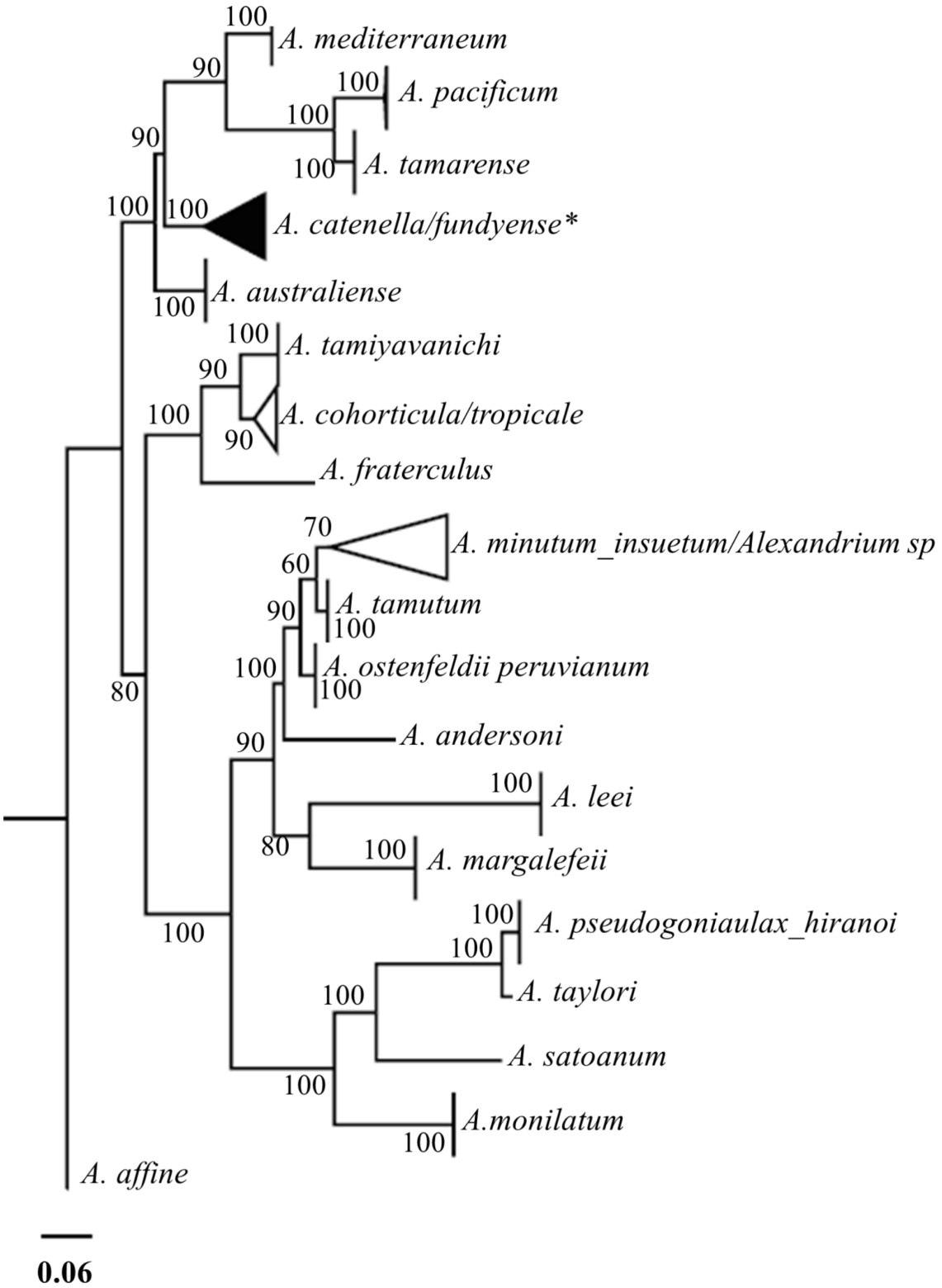


FIGURE 2 | Maximum likelihood phylogenetic reconstruction and bootstrap values using sequences generated by John et al. (2014) and sequences of the clones isolated from the northern limit of species distribution and from the 2016 bloom. Collapsed black triangle indicates the *A. catenella* clade (Group 1) and aggregation of all the clone sequences in this study. *Sequences of the morphotype *A. fundyense* are also included in this clade.

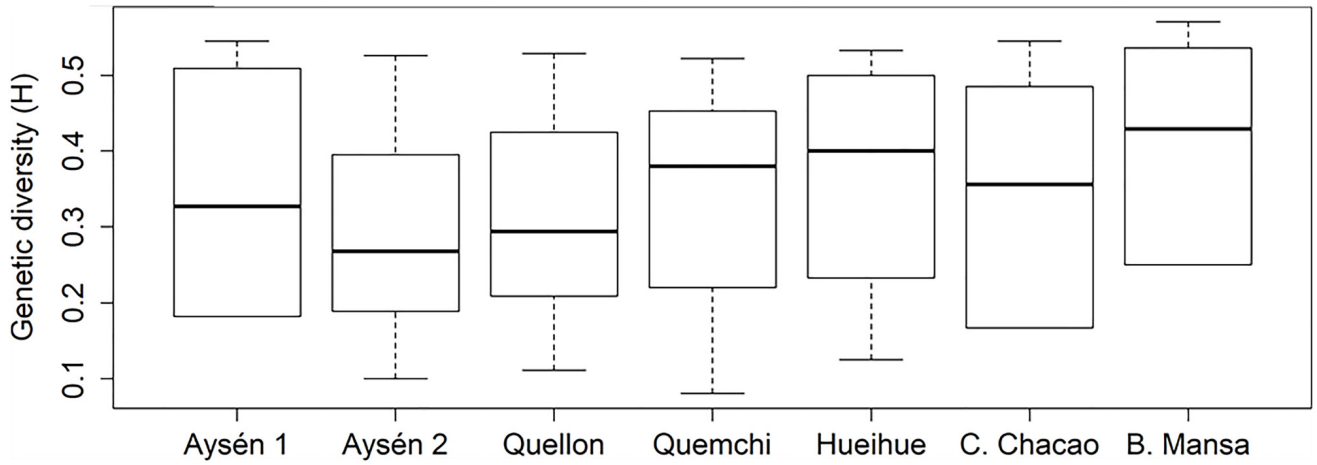


FIGURE 3 | Genetic diversity boxplot of *A. catenella* populations (GLMM, $p > 0.05$).

differences in the base frequencies among sequences used for the phylogenetic reconstruction ($\chi^2 = 62.243$, $p > 0.05$). The maximum genetic distance, according to maximum-likelihood distance, was observed between *A._pacificum*_ver v/s *A._leei_ver* strains (0.924). Meanwhile, among clones isolated from southern Chile the distance ranged between zero, for several pairwise clones isolated from closer and separate populations, and 0.069 between clones isolated from Bahía Mansa v/s Canal Chacao. Furthermore, in the clones isolated from the 2016 bloom, the number of cells in chains ranged from 2 to 8, cell viability from 0.396 to 0.875 probability, and growth rate from 0.140 to 0.331 div/day (c.f. Paredes et al., 2016). These responses were concordant with the characteristics of *A. catenella* strains previously isolated from southern Chile.

Intraspecific Genetic Distance and Population Diversity

Based on the evaluation of the banding patterns, following the RFU criteria and amplification reproducibility, 264 loci were used for the genetic evaluation, from a total of 108 clones distributed across seven populations (**Table 1**). Similar genotypes were not observed between clones. The genetic distance between clones varied between 0.100 and 0.708, with smallest distances between Hueihue and Canal Chacao; and the greatest distances observed between the clones sampled in Quemchi and Hueihue. On the other hand, the mean genetic diversity (H) had values that varied between 0.289 and 0.415, for Aysén 2 and Bahía Mansa populations respectively (**Figure 3**). Although, significant differences in the genetic diversity ($p > 0.05$, GLMM) were not observed among populations.

Genetic Structure

The genetic variability of *A. catenella* was significantly structured (AMOVA, $p < 0.05$, for each of the fixation indices; **Table 2**). Although most of the genetic variability was explained by variation within populations (90.450%), the genetic differences were significant between groups, explaining 6.730% of the genetic variation, and between populations within groups, which explained 2.820% of the variation (**Table 2**). The paired index of fixation F_{ST} was significant for the majority of comparisons between populations, with the highest values being observed for the comparisons made between the populations derived from cysts and those derived from vegetative cells collected in the 2016 bloom (**Table 3**). Of the comparisons, the population in Quemchi had the greatest differences compared with each of the three populations derived from cysts ($F_{ST} = 0.120 – 0.122$), these were followed by Bahía Mansa ($F_{ST} = 0.104 – 0.109$), Hueihue ($F_{ST} = 0.101 – 0.103$) and the population in the Canal Chacao ($F_{ST} = 0.076 – 0.093$). Among the populations derived from cysts the genetic differences were relatively similar, with the largest value observed between populations of Quellón and Aysén 2 ($F_{ST} = 0.032$). The paired values of F_{ST} between populations derived from the bloom were more heterogeneous. There were no significant differences in the values of F_{ST} between Bahía Mansa and the populations of the Canal Chacao and Hueihue, the same was true for the comparison between the Canal Chacao and Quemchi. However, there were significant differences in the values of F_{ST} for the comparisons between Quemchi and Hueihue ($F_{ST} = 0.048$), Quemchi and Bahía Mansa ($F_{ST} = 0.054$), and between Hueihue and Canal Chacao ($F_{ST} = 0.031$). These differences were relatively higher than those found for the comparisons between populations derived from cysts. PCA indicated segregation of the populations in at least three groups. Thus, the first coordinate explained 65.76% of the variation and showed evident genetic similarities between the populations isolated from cysts beds in 2014 (Aysén 1, Aysén 2 and Quellon) and populations isolated from 2016 bloom. Moreover, among these last populations the second coordinates, which explained 18.35% of the variation, showed genetic similarities between Bahía Mansa and Hueihue; and between Canal Chacao and Quemchi (**Figure 4**).

A similar pattern described with PCA was observed when $K = 3$ (**Supplementary Figure 1**) or $K = 5$ Bayesian cluster analysis was plotted, distinguishing clearly between the strains

TABLE 2 | Analysis of molecular variance (AMOVA).

Source of variation	Degree freedom	Sum of squares	Variance components	Percentage variation
Between populations	1	206	3	6.730
Between populations within groups	5	273	1	2.820
Within populations	101	3782	37	90.450
Total		4261	41	
Fixation indexs	F_{SC}: 0.030	$p < 0.05$		
	F_{ST}: 0.096	$p < 0.05$		
	F_{CT}: 0.067	$p < 0.05$		

TABLE 3 | Pair-wise F_{ST} parameters between populations (bold numbers = $p < 0.05$).

Populations	Aysén 1	Aysén 2	Quellón	Quemchi	Hueihue	Canal Chacao	Bahía Mansa
Aysén 1	0.000						
Aysén 2	**0.020**	0.000					
Quellón	**0.025**	**0.032**	0.000				
Quemchi	**0.122**	**0.120**	**0.121**	0.000			
Hueihue	**0.102**	**0.101**	**0.103**	**0.048**	0.000		
Canal Chacao	**0.076**	**0.093**	**0.081**	0.012	**0.031**	0.000	
Bahía Mansa	**0.104**	**0.108**	**0.109**	**0.054**	0.012	0.028	0.000

from cysts and those from the bloom population, and recognizing higher variability within the group of isolated from the bloom. However, the Bayesian cluster analyses suggested a $K = 5$ as the more probable number of clusters [L(K) = −13490, **Supplementary Figure 2**]. The strains derived from cysts, in the main, were assigned more or less in the same proportions in two subgroups (pink and light blue, **Figure 5**). In the case of the strains derived from the bloom, despite the higher degree of mixing in their assignment, it was also possible to distinguish between geographic populations. Thus, the majority of the strains from Quemchi and Canal Chacao presented a high proportion of assignment to a third subgroup (yellow, **Figure 5**). Meanwhile, the strains from Hueihue and Bahía Mansa presented a higher degree of heterogeneity, with individuals assigned to different subgroups (blue, red or pink, **Figure 5**) and others assigned more or less equally to the same subpopulations.

Isolation by Distance

There was a significant positive relationship between the genetic and geographic distances based on the results of the Mantel test ($p < 0.01$), with a correlation coefficient of 0.612 and determination of y by x of 0.375.

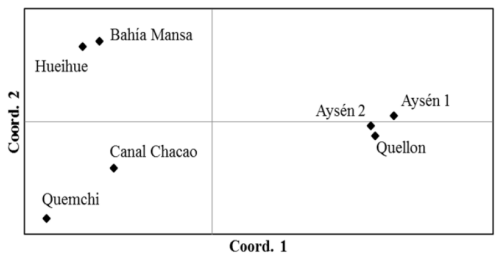


FIGURE 4 | Principal components analysis of *A. catenella* populations.

Disequilibrium of the Multilocus Linkage

The index of multilocus disequilibrium $\bar{r}_d$ gave significant values ($p < 0.05$) for all the populations (**Table 4**). The highest and lowest values were observed in the populations derived from the 2016 bloom, and ranged between 0.021 and 0.007 for Hueihue and Quemchi respectively. Between the populations derived from cysts intermediate values were observed, ranged between 0.010 and 0.015.

DISCUSSION

Phylogeny and Genetic Diversity

The phylogenetic reconstruction based on rDNA LSU sequences indicated that all the clones analyzed from the populations associated with the 2016 bloom, as well as those isolated from the northern limit of distribution, were aggregated in the Group I of the *A. tamarense* species complex, verifying that the species being studied was *A. catenella* (Lilly et al., 2007; John et al., 2014; Willem, 2017). The current taxonomic revision of *A. tamarense* species complex, supported more by ribosomal phylogeny than morphological characters (John et al., 2014), has merged into *A. catenella* species group some toxic strains previously referred to as *A. fundyense* or *A. tamarense* (John et al., 2014; Willem, 2017). As a result, the studies made by John et al. (2004), Nagai et al. (2007), Alpermann et al. (2009, 2010), Erdner et al. (2011), and Richlen et al. (2012) with the toxic strains of *A. tamarense* and *A. fundyense* now correspond to *A. catenella*, with *A. tamarense* and *A. fundyense* considered as morphotypes (*c.f.* John et al., 2014). In turn, the *A. tamarense* species is the only known non-toxic species among the species comprising the *A. tamarense*

TABLE 4 | Multilocus linkage disequilibrium index $\bar{r}_d$ per population and significance value (p).

Populations	Aysén 1	Aysén 2	Quellón	Quemchi	Hueihue	Canal Chacao	Bahía Mansa
$\bar{r}_d$	0.013	0.010	0.015	0.007	0.021	0.019	0.013
p	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$

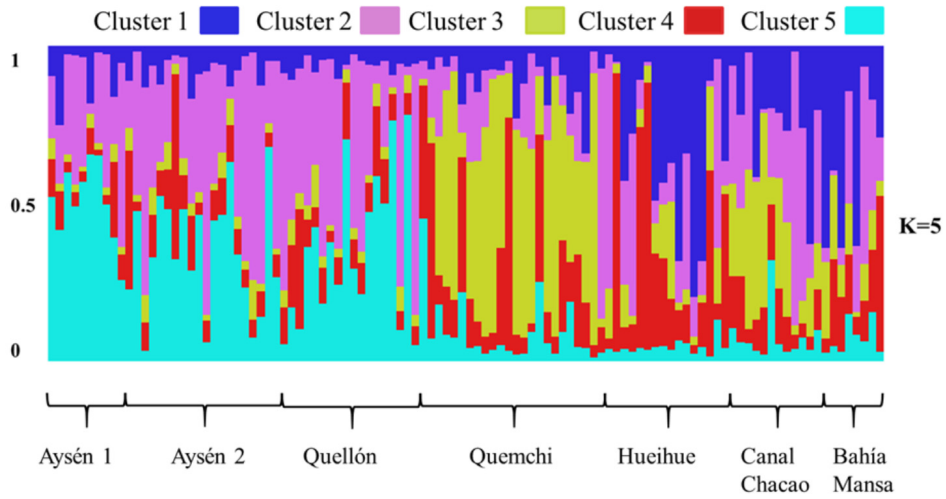


FIGURE 5 | Bayesian cluster analysis. The proportion of genotype membership to K population is illustrated by a bar plot with different colors. Below the graph are the names of the populations from which the clones were isolated.

species complex, which segregate in Group 3 (John et al., 2014). Similar to our results, clones isolated from the Patagonian fjords between 1994 and 2016 have been placed in the same Group I (Cordova and Müller, 2002; Aguilera-Belmonte et al., 2011; Varela et al., 2012; Mardones et al., 2016a). Moreover, several studies have consistently found PSP toxins in the clones characterized and in the shellfish after blooms where *A. catenella* was the trigger (Krock et al., 2007; Molinet et al., 2010; Aguilera-Belmonte et al., 2011; Varela et al., 2012; Buschmann et al., 2016). These facts reduce the possibility that other relevant species comprising the *A. tamarense* species complex are present in the Patagonian fjords, as has been suggested by other authors (e.g., Jedlicki et al., 2012), and they confirm that the diversity and variation in genetic populations observed in the present study are intraspecific. However, taking account the huge geographical extent of the 2016 bloom and the impossibility of sampling many localities in different times, we cannot discount the presence of the *A. ostenfeldii*. This species has been identified before in the Patagonian fjords in low densities in the same zones where the 2016 bloom initiated, and on some localities within the northward expansion of the bloom (e.g., Salgado et al., 2015; Pizarro et al., 2018).

In this study, the high genetic diversity observed among *A. catenella* populations, isolated from both cysts and vegetative cells, are consistent with the high genetic diversity reported in *A. catenella* populations in the northern hemisphere (Alpermann et al., 2010; Erdner et al., 2011). Mutation is the fundamental mechanism generating genetic variation in both the asexual and sexual phase of all organisms (Rengefors et al., 2017). In *Alexandrium* species the mutation can be produced during asexual division of vegetative cells in the water column, and also during genetic recombination of distinct cells (i.e., heterothallic mating; Mardones et al., 2016a) that generate cysts that sink to the seafloor (Brandenburg et al., 2018). In the growth season, these cysts hatch continuously and thereby supply new genotypes to the water column, a mechanism by which they contribute to the genetic diversity (Brandenburg et al., 2018). Possibly, the high genetic diversity observed within the *A. catenella* populations is an attribute that confers to the species the capacity to adapt to the variable and heterogeneous environmental conditions observed across the Patagonian fjords. There, latitudinal gradients in the physical-chemical variables explain the presence of ecoregion, bioclimatic region, and biogeographic patterns (Camus, 2001; Spalding et al., 2007). However, further studies are needed to understand if the genetic diversity of *A. catenella* is coupled with phenotypic variability, as some authors have observed in the northern hemisphere (e.g., Alpermann et al., 2010; Brandenburg et al., 2018).

The genetic diversity observed between the populations did not indicate a process of recent colonization. Even though the reduction in diversity and segregation are expected results for a species during range expansion (Excoffier et al., 2009; Sexton et al., 2009), in this study the high diversity detected using AFLP does not differ significantly among populations ($p < 0.05$, GLMM) and it was distributed mainly within them (90.450%, AMOVA). Similarly, in some HAB species that have expanded their distribution, gradients of genetic diversity between established and founder populations have not been

found (Tahvanainen et al., 2012; Lebret et al., 2013). Indeed, the genetic diversity of the *A. catenella* populations off the coasts of Japan, Korea, and the United States of America, estimated from samples taken in different months, years, localities, and life cycle phases, consistently show high values, even when population genetic divergence was found (Nagai et al., 2007; Erdner et al., 2011; Richlen et al., 2012). Although, when genetic differences in diversity and/or variability levels have been observed, they have occurred among populations separated by large geographic distances (Masseret et al., 2009) and when the colonization events have been separated by considerable periods of time (Brandenburg et al., 2018). In these latter cases, the gene flow limitation (Masseret et al., 2009) and the extended time to population diversification (Brandenburg et al., 2018) may have favored the maintenance of the diversity/variability gradient among populations. In a rapidly growing organism with a short generation time, it has been suggested that the huge population sizes could be considered as virtually infinite and genotypic diversity could thus be maintained during the bloom events, even if the organism primarily reproduces clonally (Dia et al., 2014). Thus, the lack of a genetic diversity gradient among *A. catenella* populations may be related to the potential observed in the genus *Alexandrium* for maintaining high genetic diversity even in a bloom, and the considerable gene flow among the populations, as is suggesting by the low and moderate pairwise F_{ST} values.

Population Structure

The observations that described the progressive expansion of *A catenella* indicate that the presence of this species in the Aysén region dates from 1992, but it was only in 2002 when an intense bloom that started in this region, reached further north up to Quellon on the south coast of Chiloé Island (Molinet et al., 2003). Considering the local oceanographic currents between the populations of Aysén and those around Quellón the dispersion among populations should be restricted (e.g., Sievers and Silva, 2003). Two strong surface (0–30 m) counter currents meet in the Golfo Corvocado and from here flow out through the Boca Guafo toward the Pacific Ocean (Sievers and Silva, 2003): one current flows south from Chiloé inland sea and, the other, flows north from the Canal Moraleda (**Figure 1**). Taking into account these currents and that the abundances of vegetative cells are mainly found in the surface waters, down to about 15 m (Molinet et al., 2003), it is thought that the gene flow between populations from both sides of the Boca Guafo should be limited, generating high levels of genetic differentiation. However, the lowest levels of differentiation (F_{ST} = 0.020–0.032) and similar patterns of genotype assignment to genetic population (Bayesian aggregation analysis) were observed between these populations, indicating that the current systems are not a barrier to gene flow. Indeed, the meteorological conditions that prevail during spring and summer, with the predominance of southerly winds, could be enough to push the vegetative cells northward, particularly during intense bloom events, such as those that occurred in 1998, 2002, and 2009 (Molinet et al., 2003; Mardones et al., 2010). The descriptions of these events indicated that they started in the central-northern areas of the Aysén region and then expanded to the north (Molinet et al., 2003; Mardones et al., 2010). Thus, successive bloom events could have favored the dispersal of the vegetative cells from Aysén toward the north, increasing gene flow and obscuring the evidence of an initial founder effect between the populations.

AMOVA, PCA, and Bayesian cluster analysis (K3 genetic population) indicated that the segregation of the genetic variance was important at least in two levels: between the populations coming from both the previous northern limit of the distribution and from the bloom of 2016, and also between the populations inside of each of these two groups. The differences between the former two groups of populations explained 6.730% of the variance in the AMOVA or 65.76% of the variance explained by the first axes (PCA), it also accounted for the greatest genetic differences (F_{ST} = 0.076–0.122). Within each of the two groups, smaller differences were observed between populations collected during the 2016 bloom (F_{ST} = 0.031–0.054) and the smallest differences were observed between the populations derived from cysts (F_{ST} = 0.020–0.032). In *A. catenella* populations significant values of F_{ST} have been observed in different geographical contexts: higher values comparing different geographical regions (F_{ST}: 0.281–0.308; John et al., 2004); or from moderate to high when comparing isolated subpopulations within the same region (F_{ST}: 0.07–0.36; Alpermann et al., 2009). Thus, the differentiation observed both between populations in the north of the distribution and those from the 2016 bloom, and within each of these groups, could be considered as subpopulations rather than clearly divergent populations.

The significant correlation between genetic and geographic distances of the populations ($p < 0.05$, Mantel Test) seems to support the assertion that differences observed between the populations from the northern limit of the distribution and those from the bloom of 2016 can be attributed, in part, to restrictions of the gene flow due to the limited dispersion of vegetative cells through the marine currents. Similar observations have been made for *A. catenella* in Japan, where the genetic structuring was correlated with geographic distance (Nagai et al., 2007), and also for *A. minutum*, where differentiation among samples from different locations in the Mediterranean Sea were attributed to the complex regional geography and hydrodynamics (Casabianca et al., 2012). Along over 300 km of exposed coast over which the great bloom of 2016 occurred (Buschmann et al., 2016), only some of the vegetative cells could have been advected by the unusual southern winds that were recorded throughout the month of March (Buschmann et al., 2016), limiting the dispersion to the other fraction of the population, and as a consequence, generating the observed differentiation.

Additionally, the differentiation and the changes in the genotype proportions assigned to ancestral populations, observed in the Bayesian aggregation analysis (K5 genetic population), between populations derived from cysts and those isolated from the bloom, could be the result of distinct population dynamics at each stage in the life cycle from which the samples came. The life cycle of *A. catenella* is characterized by alternating between a planktonic population of haploid cells that reproduce asexually and a benthic population of resting cysts

that results from the occasional sexual reproduction of planktonic populations (Anderson et al., 2012b). The heterothallic behavior of the gametes (Mardones et al., 2016a), and the implicit recombination during sexual reproduction (Anderson et al., 2012b), along with the capacity of cysts to remain viable in the sediments over long periods of time (years in some cases), means that banks of cysts in the sediments act as reservoirs of genetic diversity (Alpermann et al., 2009; Anderson et al., 2012b). This could explain, in part, the relatively homogeneous assignment of the genotypes within the subpopulations derived from cysts, containing individuals with allelic proportions of the five clusters from detected ancestral subpopulations. This level of genotypic admixture and the dominance of two clusters could be the result of the allelic recombination of ancestral populations, probably of different geographic origins. However, in these subpopulations there is no genetic evidence of successive cohorts of temporally differentiated cysts, as has been observed by Alpermann et al. (2009). Despite the few studies undertaken in this area, low concentrations of cysts are observed in the Patagonian fjords (Mardones et al., 2016a). Furthermore, rapid reductions in cysts density have been observed (Diaz et al., 2014), probably due to the relatively short period of mandatory dominance (Mardones et al., 2016a) and the germination potential over the year (Varela, personal observation). This all indicates that the banks of cysts are a dynamic reservoir, without the accumulation of many cohorts over time.

Within the populations from the 2016 bloom, the heterogeneity of assignment proportions of the genotypes in genetic subpopulations (Bayesian aggregation analysis) and the high levels of the linkage disequilibrium index provided evidence of the incidence of demographic factors on the population genetic divergence. The Bayesian aggregation analysis revealed a relative dominance of individuals with a greater proportion of alleles from one of the ancestral subpopulations, with the insertion of some individuals that could be considered migrants between populations. At one extreme of the differentiation was the population from Quemchi, with a greater number of individuals with a dominant allelic proportion (yellow cluster), than the other populations which appear to be in a state of transition. This apparent process of rapid genetic spatial differentiation over the development of the bloom has also been observed in *A. minutum* (Dia et al., 2014) and in *A. catenella* (Erdner et al., 2011); differentiation which could also be temporal (Richlen et al., 2012; Dia et al., 2014). In these cases, it has been argued that these rapid genetic changes may reflect selective effects that are nevertheless not strong enough to reduce the genetic diversity (Dia et al., 2014). On the other hand, if encystment occurs during the bloom process (Brosnahan et al., 2016), and it is induced by adverse environmental conditions, this could magnify the selective effects on the proportions of different genotypes, reducing or eliminating lineages which are less successful under the prevailing conditions (Erdner et al., 2011). Both combined effects, the rate of reproduction and the differential encystment, could potentially reduce gene flow between subpopulations, generating differentiation between populations whilst maintaining the diversity of the regional population (Erdner et al., 2011).

CONCLUSION

In this study we did not observe the patterns generated by a range expansion process i.e., diversity gradients and/or high inter-population divergence. However, the genetic diversity and structure indicated that these were influenced by the geographic distance effects and the physical restrictions to gene flow, along with the demographic processes that occur during the distinct phases of the life cycle. Thus, the characteristics of asexual and sexual reproduction (e.g., heterothallism), the participation of ancestral populations in the allele proportions of individuals, as well as the selective value of some clonal lines amplified by asexual reproduction or by differential encystment, could be account of the genetic diversity and genetic structure observed in *A. catenella* populations in southern Chile.

AUTHOR CONTRIBUTIONS

JP was responsible for the laboratory and statistical analyses, and generation of the manuscript. DV was responsible for the generation of the manuscript. JP, CM, AZ, KC, AV, and BO were responsible for the sampling collection, isolation of strains, and clones maintaining.

FUNDING

Financial support was provided by the Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT N° 1080548 and 1130954) and Chilean scholar fellowship Doctorado Nacional 2012 from the Comisión Nacional de Investigación Científica y Tecnológica (CONICYT), and also by Proyecto Interno FNI02/16 ULA CR8520.

ACKNOWLEDGMENTS

Special thanks to Dr. Federico Winkler and reviewer for significant comments to manuscript improvement, and to Dr. Matthiew Lee for English review. Moreover, thanks to Ma. Andrea Pérez Ríos from CTI Team (Competencias Transversales en Inglés) and Convenio Marco FID 1758 (Formación de Profesores 2017–2020, Universidad de Los Lagos) for the written English course imparted, which helped to substantially to the manuscript redaction.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2018.00532/full#supplementary-material

REFERENCES

Agapow, P.-M., and Burt, A. (2001). Indices of multilocus linkage disequilibrium. *Mol. Ecol. Notes* 1, 101–102. doi: 10.1046/j.1471-8278.2000.00014.x

Aguilera-Belmonte, A., Inostroza, I., Franco, J. M., Riobó, P., and Gómez, P. I. (2011). The growth, toxicity and genetic characterization of seven strains of *Alexandrium catenella* (Whedon and Kofoid) Balech 1985 (Dinophyceae) isolated during the 2009 summer outbreak in southern Chile. *Harmful Algae* 12, 105–112. doi: 10.1016/j.hal.2011.09.006

Alpermann, T. J., Beszteri, B., John, U., Tillmann, U., and Cembella, A. D. (2009). Implications of life-history transitions on the population genetic structure of the toxigenic marine dinoflagellate *Alexandrium tamarense*. *Mol. Ecol.* 18, 2122–2133. doi: 10.1111/j.1365-294X.2009.04165.x

Alpermann, T. J., Tillmann, U., Beszteri, B., Cembella, A. D., and John, U. (2010). Phenotypic variation and genotypic diversity in a planktonic population of the toxigenic marine Dinoflagellate *Alexandrium tamarense* (Dinophyceae). *J. Phycol.* 46, 18–32. doi: 10.1111/j.1529-8817.2009.00767.x

Anderson, D. M., Cembella, A. D., and Hallegraeff, G. M. (2012a). Progress in understanding harmful algal blooms: paradigm shifts and new technologies for research, monitoring, and management. *Annu. Rev. Mar. Sci.* 4, 143–176. doi: 10.1146/annurev-marine-120308-081121

Anderson, D. M., Alpermann, T. J., Cembella, A. D., Collos, Y., Masseret, E., and Montresor, M. (2012b). The globally distributed genus *Alexandrium*: multifaceted roles in marine ecosystems and impacts on human health. *Harmful Algae* 14, 10–35. doi: 10.1016/j.hal.2011.10.012

Anderson, R., and Kawachi, M. (2005). "Traditional Microalgae isolation techniques," in *Algal Culturing Techniques*, ed. R. A. Andersen (Cambridge, MA: Academic Press), 83–100. doi: 10.1016/B978-012088426-1/50007-X

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48. doi: 10.18637/jss.v067.i01

Brandenburg, K. M., Wohlrab, S., John, U., Kremp, A., Jerney, A., Krock, B., et al. (2018). Intraspecific trait variation and trade-offs within and across populations of a toxic dinoflagellate. *Ecol. Lett.* 21, 1561–1571. doi: 10.1111/ele.13138

Brosnahan, M. L., Velo-suárez, L., Ralston, D. K., Fox, S. E., Sehein, T. R., Shalapyonok, A., et al. (2016). Rapid growth and concerted sexual transitions by a bloom of the harmful dinoflagellate *Alexandrium fundyense* (Dinophyceae). *Limnol. Oceanogr.* 60, 2059–2078. doi: 10.1002/lno.10155

Buschmann, A., Farías, L., Tapia, F., Varela, D., and Vásquez, M. (2016). *Informe Final: Comisión Marea Roja (CMR)*. Alphen aan den Rijn: Wolters Kluwer.

Camus, P. A. (2001). Marine biogeography of continental Chile. *Rev. Chil. Hist. Nat.* 74, 587–617. doi: 10.4067/S0716-078X2001000300008

Casabianca, S., Penna, A., Pecchioli, E., Jordi, A., Basterretxea, G., and Vernesi, C. (2012). Population genetic structure and connectivity of the harmful dinoflagellate *Alexandrium minutum* in the Mediterranean Sea. *Proc. Biol. Sci.* 279, 129–138. doi: 10.1098/rspb.2011.0708

Cordova, J., and Müller, I. (2002). Use of PCR and partial sequencing of the large-subunit rRNA gene to identify *Alexandrium catenella* (Dinophyceae) from the South of Chile. *Harmful Algae* 1, 343–350. doi: 10.1016/S1568-9883(02)00066-5

Dia, A., Guillou, L., Mauger, S., Bigeard, E., Marie, D., Valero, M., et al. (2014). Spatiotemporal changes in the genetic diversity of harmful algal blooms caused by the toxic dinoflagellate *Alexandrium minutum*. *Mol. Ecol.* 23, 549–560. doi: 10.1111/mec.12617

Diaz, P. A., Molinet, C., Seguel, M., Diaz, M., Labra, G., and Figueroa, R. I. (2014). Coupling planktonic and benthic shifts during a bloom of *Alexandrium catenella* in southern Chile: implications for bloom dynamics and recurrence. *Harmful Algae* 40, 9–22. doi: 10.1016/j.hal.2014.10.001

Erdner, D. L., Richlen, M., McCauley, L. A. R., and Anderson, D. M. (2011). Diversity and dynamics of a widespread bloom of the toxic dinoflagellate *Alexandrium fundyense*. *PLoS One* 6:22965. doi: 10.1371/journal.pone.0022965

Excoffier, L., Foll, M., and Petit, R. J. (2009). Genetic consequences of range expansions. *Annu. Rev. Ecol. Evol. Syst.* 40, 481–501. doi: 10.1146/annurev.ecolsys.39.110707.173414

Excoffier, L., Laval, G., and Schneider, S. (2005). Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evol. Bioinform.* 1, 47–50. doi: 10.1177/117693430500100003

Falush, D., Stephens, M., and Pritchard, J. K. (2003). Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164, 1567–1587.

Francis, R. M. (2016). POPHELPER: an R package and web app to analyse and visualize population structure. *Mol. Ecol. Resour.* 17, 27–32. doi: 10.1111/1755-0998.12509

Fry, N., Savelkoul, P., and Visca, P. (2009). Amplified fragment length polymorphism analysis. *Methods Mol. Biol.* 551, 89–104. doi: 10.1007/978-1-60327-999-4_8

Hernández, C., Díaz, P. A., Molinet, C., and Seguel, M. (2016). Exceptional climate anomalies and north- wards expansion of paralytic shellfish poisoning outbreaks in Southern Chile. *Harmful Algae News* 54, 1–2.

Jedlicki, A., Fernández, G., Astorga, M., Oyarzún, P., Toro, J. E., Navarro, J. M., et al. (2012). Molecular detection and species identification of *Alexandrium* (Dinophyceae) causing harmful algal blooms along the Chilean coastline. *AoB Plants* 2012:ls033. doi: 10.1093/aobpla/pls033

John, U., Groben, R., Beszteri, B., and Medlin, L. (2004). Utility of Amplified Fragment Length Polymorphisms (AFLP) to analyse genetic species complex. *Protist* 155, 169–179. doi: 10.1078/143446104774199574

John, U., Litaker, R. W., Montresor, M., Murray, S., Brosnahan, M. L., and Anderson, D. M. (2014). Formal revision of the *Alexandrium tamarense* species complex (Dinophyceae) taxonomy: the introduction of five species with emphasis on molecular-based (rDNA) classification. *Protist* 165, 779–804. doi: 10.1016/j.protis.2014.10.001

John, U., Tillmann, U., Hülskötter, J., Alpermann, T. J., Wohlrab, S., and Van de Waal, D. B. (2015). Intraspecific facilitation by allelochemical mediated grazing protection within a toxigenic dinoflagellate population. *Proc. R. Soc. B Biol. Sci.* 282, 1–9. doi: 10.1098/rspb.2014.1268

Kamvar, Z. N., Tabima, J. F., and Gr, N. J. (2014). Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and /or sexual reproduction. *PeerJ* 2:e281. doi: 10.7717/peerj.281

Kremp, A., Oja, J., Letortorec, A. H., Hakanen, P., Tahvanainen, P., Tuimala, J., et al. (2016). Diverse seed banks favour adaptation of microalgal populations to future climate conditions. *Environ. Microbiol.* 18, 679–691. doi: 10.1111/1462-2920.13070

Krock, B., Seguel, C. G., and Cembella, A. D. (2007). Toxin profile of *Alexandrium catenella* from the Chilean coast as determined by liquid chromatography with fluorescence detection and liquid chromatography coupled with tandem mass spectrometry. *Harmful Algae* 6, 734–744. doi: 10.1016/j.hal.2007.02.005

Lebret, K., Kritzberg, E. S., and Rengefors, K. (2013). Population genetic structure of a microalgal species under expansion. *PLoS One* 8:e82510. doi: 10.1371/journal.pone.0082510

Lilly, E. L., Halanych, K. M., and Anderson, D. M. (2007). Species boundaries and global biogeography of the *Alexandrium tamarense* complex (Dinophyceae). *J. Phycol.* 43, 1329–1338. doi: 10.1111/j.1529-8817.2007.00420.x

Mardones, J. I., Bolch, C., Guzmán, L., Paredes, J., Varela, D., and Hallegraeff, G. M. (2016a). Role of resting cysts in Chilean *Alexandrium catenella* dinoflagellate blooms revisited. *Harmful Algae* 55, 238–249. doi: 10.1016/j.hal.2016.03.020

Mardones, J. I., Mu, M. N., and Hallegraeff, G. M. (2016b). Toxic dinoflagellate blooms of *Alexandrium catenella* in Chilean fjords: a resilient winner from climate change. *ICES J. Mar. Sci.* 74, 988–995. doi: 10.1093/icesjms/fsw164

Mardones, J., Clément, A., and Aparicio, C. (2010). Alexandrium catenella during 2009 in Chilean waters, and recent expansion to coastal ocean. *Harmful Algae News* 41, 8–9.

Masseret, E., Grzebyk, D., Nagai, S., Genovesi, B., Lasserre, B., Laabir, M., et al. (2009). Unexpected genetic diversity among and within populations of the toxic dinoflagellate *Alexandrium catenella* as revealed by nuclear microsatellite markers. *Appl. Environ. Microbiol.* 75, 2037–2045. doi: 10.1128/AEM.01686-08

Mcneill, J., Redhead, S. A., Wiersema, J. H., John, U., Litaker, W., Montresor, M., et al. (2014). (2302) proposal to reject the name *Gonyaulax catenella* (*Alexandrium catenella*) (Dinophyceae). *Taxon* 63, 932–933. doi: 10.12705/634.21

Molinet, C., Lafon, A., Lembeye, G., and Moreno, C. (2003). Spatial and temporal distribution patterns of blooms of *Alexandrium catenella* (Whedon & Kofoid) Balech 1985, on inland seas of northwest Patagonia, Chile. *Rev. Chil. Hist. Nat.* 76, 681–698. doi: 10.4067/S0716-078X2003000400011

Molinet, C., Niklitschek, E., and Seguel, M. (2010). Trends of natural accumulation and detoxification of paralytic shellfish poison in two bivalves from the Northwest Patagonian inland sea. *Rev. Biol. Mar. Oceanogr.* 45, 195–204. doi: 10.4067/S0718-19572010000200001

Murray, S., Uwe, J., and Kremp, A. (2015). "*Alexandrium* spp.: genetic and ecological factors influencing saxitoxin production and proliferation," in *Climate Change and Marine and Freshwater Toxins*, eds L. M. Botana, L. Carmen, and N. Vilariño (Berlin: Walter De Gruyter Incorporated), 125–154.

Nagai, S., Lian, C., Yamaguchi, S., Hamaguchi, M., Matsuyama, Y., Itakura, S., et al. (2007). Microsatellite markers reveal population genetic structure of the toxic dinoflagellate *Alexandrium tamarense* (Dinophyceae) in Japanese coastal waters. *J. Phycol.* 43, 43–54. doi: 10.1111/j.1529-8817.2006.00304.x

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci. U.S.A.* 70, 3321–3323. doi: 10.1073/pnas.70.12.3321

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89, 583–590.

Paredes, J., Andrea, Z., Bianca, O., Camila, M., and Daniel, V. (2016). "Vegetative cells of *Alexandrium catenella* from the first bloom reported in southern Chile along Pacific Ocean coast: growth attributes and genetic identification," in *Proceedings of the 17th International Conference on Harmful Algae Brazil*, (Florianópolis: ICHA).

Peakall, R., and Smouse, P. E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28, 2537–2539. doi: 10.1093/bioinformatics/bts460

Penna, A., Garcés, E., Vila, M., Giacobbe, M. G., Fraga, S., Lugliè, A., et al. (2005). *Alexandrium catenella* (Dinophyceae), a toxic ribotype expanding in the NW Mediterranean sea. *Mar. Biol.* 148, 13–23. doi: 10.1007/s00227-005-0067-5

Persich, G. R., Kulis, D. M., Lilly, E. L., Anderson, D. M., and Garcia, V. M. T. (2006). Probable origin and toxin profile of *Alexandrium tamarense* (Lebour) Balech from southern Brazil. *Harmful Algae* 5, 36–44. doi: 10.1016/j.hal.2005.04.002

Pizarro, G., Paz, B., Alarcón, C., Toro, C., Frangópulos, M., Salgado, P., et al. (2018). Winter distribution of toxic, potentially toxic phytoplankton, and shellfish toxins in fjords and channels of the Aysén region, Chile. *Lat. Am. J. Aquat. Res.* 46, 120–139. doi: 10.3856/vol46-issue1-fulltext-13

Possada, D., and Crandall, K. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14, 817–818. doi: 10.1093/bioinformatics/14.9.817

R Core Team (2017). *R: a Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Rengefors, K., Kremp, A., Reusch, T. B. H., and Wood, A. M. (2017). Genetic diversity and evolution in eukaryotic phytoplankton: revelations from population genetic studies. *J. Plankton Res.* 39, 165–179. doi: 10.1093/plankt/fbw098

Richlen, M. L., Erdner, D. L., McCauley, L. A. R., Libera, K., and Anderson, D. M. (2012). Extensive genetic diversity and rapid population differentiation during blooms of *Alexandrium fundyense* (Dinophyceae) in an isolated salt pond on Cape Cod, MA, USA. *Ecol. Evol.* 2, 2588–2599. doi: 10.1002/ece3.373

Saitou, N., and Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4, 406–425.

Salgado, P., Riobó, P., Rodríguez, F., and Bravo, I. (2015). Toxicon differences in the toxin pro fi les of *Alexandrium ostenfeldii* (Dinophyceae) strains isolated from different geographic origins: evidence of paralytic toxin, spirolide, and gymnodimine. *Toxicon* 103, 85–98. doi: 10.1016/j.toxicon.2015.06.015

Sassenhagen, I., Sefbom, J., Säll, T., Godhe, A., and Rengefors, K. (2015). Freshwater protists do not go with the flow: population structure in *Gonyostomum* semen independent of connectivity among lakes. *Environ. Microbiol.* 17, 5063–5072. doi: 10.1111/1462-2920.12987

Scholin, C. A., Herzog, M., Sogin, M., and Anderson, D. M. (1994). Identification of group-and strain-specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). II. Sequence analysis of a fragment of the LSU rRNA gene. *J. Phycol.* 30, 999–1011. doi: 10.1111/j.0022-3646.1994.00999.x

Sexton, J. P., McIntyre, P. J., Angert, A. L., and Rice, K. J. (2009). Evolution and ecology of species range limits. *Annu. Rev. Ecol. Evol. Syst.* 40, 415–436. doi: 10.1146/annurev.ecolsys.110308.120317

Sievers, H. A., and Silva, N. (2003). "Masas de agua y circulación en los canales y fiordos australes," in *Avances en el Conocimiento Oceanográfico de las Aguas Interiores Chilenas, Puerto Montt a Cabo de Hornos*, eds N. Silva and S. Palma (Valparaíso: Comité Oceanográfico Nacional).

Spalding, M. D., Fox, H. E., Allen, G. R., Davidson, N., Ferdaña, Z. A., Finlayson, M. A. X., et al. (2007). Marine ecoregions of the world: a bioregionalization of coastal and shelf areas. *Bioscience* 57, 573–583. doi: 10.1641/B570707

Swofford, D. (2002). *PAUP: Phylogenetic Analysis Using Parsimony*. Available at: http://phylosolutions.com/paup-test

Tahvanainen, P., Alpermann, T. J., Figueroa, R. I., John, U., Hakanen, P., Nagai, S., et al. (2012). Patterns of post-glacial genetic differentiation in marginal populations of a marine microalga. *PLoS One* 7:53602. doi: 10.1371/journal.pone.0053602

Varela, D., Paredes, J., Alves-de-Souza, C., Seguel, M., Sfeir, A., and Frangópulos, M. (2012). Intraregional variation among *Alexandrium catenella* (Dinophyceae) strains from southern Chile: morphological, toxicological and genetic diversity. *Harmful Algae* 15, 8–18. doi: 10.1016/j.hal.2011.10.029

Venables, W. N., and Ripley, B. D. (2002). *Modern Applied Statistics with S*. Berlin: Springer. doi: 10.1007/978-0-387-21706-2

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., et al. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res.* 23, 4407–4414. doi: 10.1093/nar/23.21.4407

Wang, L., Zhuang, Y., Zhang, H., Lin, X., and Lin, S. (2014). DNA barcoding species in *Alexandrium tamarense* complex using ITS and proposing designation of five species. *Harmful Algae* 31, 100–113. doi: 10.1016/j.hal.2013.10.013

Wells, M. L., Trainer, V. L., Smayda, T. J., Karlson, B. S. O., Trick, C. G., Kudela, R. M., et al. (2015). Harmful algal blooms and climate change: learning from the past and present to forecast the future. *Harmful Algae* 49, 68–93. doi: 10.1016/j.hal.2015.07.009

Willem, F. (2017). Report of the nomenclature committee for algae: 15. *Taxon* 66, 191–192. doi: 10.12705/661.16

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Paredes, Varela, Martínez, Zúñiga, Correa, Villarroel and Olivares. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Marine Science

MINI REVIEW
published: 23 January 2019
doi: 10.3389/fmars.2019.00001

Pyrodinium bahamense One the Most Significant Harmful Dinoflagellate in Mexico

*Lourdes Morquecho**

Centro de Investigaciones Biológicas del Noroeste, La Paz, Mexico

Pyrodinium bahamense produces saxitoxins and can cause paralytic shellfish poisoning (PSP). This species has caused more human illnesses and fatalities than any other toxic dinoflagellate in Mexico. The distribution of dinoflagellate cysts with their vegetative stage is broad, mainly along Mexican Pacific coasts from the central Gulf of California to Chiapas, as well as in the southern Gulf of Mexico and the Mexican Caribbean Sea on the Atlantic coast. *In vitro* germination of living cysts from the southern Gulf of California occurs under thermophilic (20–35°C) and euryhaline (20–35 ups) conditions. Blooms occurred typically during summer rainy season (June through September), inside of restricted shallow lagoons surrounded by mangrove forests. The data obtained so far on *P. bahamense* spatial and population variability in Mexican Pacific and the Gulf of Mexico, suggest a seasonal and latitudinal pattern. Also, in these regions, the abundance, seasonality, and species distribution tend to decrease from tropical to subtropical areas. The local strain toxicity has only been corroborated in one isolate from the southern Gulf of California, which exhibited a high saxitoxin concentration of 95 pg STX eq cell^{-1}. PSP outbreaks linked with *P. bahamense* in the Gulf of Tehuantepec from 1989 to 2007, caused at least ∼200 human cases, with 15 fatalities. This mini-review ends with a viewpoint of management and research strategies to better understand the factors that play essential roles in the bloom dynamics and toxicity of this species.

Keywords: *Pyrodinium bahamense*, Mexico, harmful blooms, saxitoxins, PSP

OPEN ACCESS

Edited by:
Jorge I. Mardones, Instituto de Fomento Pesquero (IFOP), Chile

Reviewed by:
Sai Elangovan S., National Institute of Oceanography (CSIR), India
Kathryn Coyne, University of Delaware, United States

***Correspondence:**
Lourdes Morquecho
lamorquecho@cibnor.mx

Specialty section:
This article was submitted to Marine Ecosystem Ecology, a section of the journal Frontiers in Marine Science

Received: *01 July 2018*
Accepted: *07 January 2019*
Published: *23 January 2019*

Citation:
Morquecho L (2019) Pyrodinium bahamense One the Most Significant Harmful Dinoflagellate in Mexico. Front. Mar. Sci. 6:1. doi: 10.3389/fmars.2019.00001

INTRODUCTION

Pyrodinium bahamense Plate is a tropical/subtropical euryhaline dinoflagellate that produces saxitoxins and can cause paralytic shellfish poisoning (PSP). This species has caused more human illnesses and fatalities than any other paralytic shellfish toxin (PST) producing dinoflagellate, with a spate of toxic blooms in the Indo-Pacific and the Pacific coast of Central America (Usup et al., 2012). *Pyrodinium bahamense*, along with toxic *Gymnodinium catenatum* Graham, have caused direct adverse consequences for human health, aquaculture industries, tourism, and ecosystem functions in Mexican coastal waters (Hernández-Becerril et al., 2007; García-Mendoza et al., 2016). *Pyrodinium bahamense* has particular importance since it has caused a significant impact on human health, mainly in southern Mexican Pacific. The effect of harmful algae could be increasing and expand as a consequence of increasing local marine eutrophication (Heisler et al., 2008) and ocean climate change (Hallegraeff, 2010).

Globally *P. bahamense* is mainly distributed in tropical areas of both hemispheres. The vegetative stage has been reported into three regions, the Caribbean Sea and Central America, Persian Gulf and the Red Sea, and the western Pacific; while cyst stage distribution is broader, primarily in tropical to subtropical coastal areas from both the Atlantic and Pacific regions, and the Caribbean Sea (Usup et al., 2012). *Pyrodinium bahamense*, is one of the most critical harmful algal bloom (HABs) organisms in South Asian coastal waters (Mertens et al., 2015).

Pyrodinium bahamense is characterized by a high bioluminescence (Seliger et al., 1971), a heterothallic sexual cycle (Wall and Dale, 1969), and a simple toxins profile (dc-STX, STX, neoSTX, B1 and B2) (Usup et al., 2012). In 1980, the species taxonomic status was raised to variety, based on morphological variations in the motile stage, the capability of PSP toxin production, and the geographic distribution (Steidinger et al., 1980). However, based on morphologic and phylogenetic analysis of specimens (motile and cyst stages) from 13 coastal areas of various tropical and subtropical waters, Mertens et al. (2015) demonstrated that there is no consistent criterion to separate unequivocally both varieties based only in morphology. Additionally, Mertens et al. (2015) revealed within the *Pyrodinium* clade, both Indo-Pacific and Atlantic-Caribbean ribotypes, suggesting that *P. bahamense* is a species complex.

According to Balech (1985), *P. bahamense* cells are polyhedral and irregularly rounded, with strong crests along the sutures and tend to be compressed when they are in chains. Most cells have a well-developed left antapical spine and a smaller right spine that is an extension of the sulcal list (**Figure 1A**). The theca has a granular surface and numerous trichocyst pores, there is a pore in the 4′ apical plate, and the apical pore complex consists of a comma-shaped granular closing plate and 9–14 pores. Cysts are spherical, of chorate type, with ∼152 oblate tubular processes of variable length and randomly arranged (**Figures 1D,E**). The colorless wall is bi-layered, with a scabrate outer surface and a smooth inner surface. In living cysts, the yellow-green cytoplasm encloses a red accumulation body (**Figure 1D**) and numerous starch grains (Morquecho et al., 2014). Vegetative stage is easy to identify with basic microscopy equipment, and it could hardly be confused with other species. By contrast, due to its similarity with the cysts of other dinoflagellates, the resting stage may be confused with other species.

Blooms of *P. bahamense* are generally aperiodic and unpredictable (Usup et al., 2012); nevertheless, on a large temporal scale, there is some evidence that significant blooms overlap with peaks of El Niño and La Niña cycles (Maclean, 1989; Usup and Azanza, 1998; Phlips et al., 2006). Even though, no relationship has been established between blooms and these cycles, the enhanced delivery of nutrients into the coastal waters could be a factor (Usup et al., 2012). Despite species seasonality that vary with local physiography, hydrography, and climate (Usup et al., 2012), blooms are more predictable at a smaller and local scale (Azanza and Taylor, 2001). Critical elements such as environmental factors, specifically removal of top-down predators and a change from eutrophic to oligotrophic conditions, likely promote the dominance and toxicity of *P. bahamense* in Florida (Walsh et al., 2011).

In this mini-review, the most significant information about *Pyrodinium bahamense* in Mexico since 1942 to date is summarized and analyzed. Aspects such as vegetative and cyst stage distribution, Mexican strains physiology and toxicology, as well as ecology and bloom dynamics, toxicity, human illnesses, and other significant impacts are discussed. Also, research priorities will be proposed to support the establishment of the guidelines for a transnational scientific approach, which is needed to coordinate and advance the understanding and management of HABs in coastal areas of Latin America.

METHODOLOGY USED IN MEXICO TO STUDY AND MONITOR TO *Pyrodinium bahamense*

In Mexico, *P. bahamense* studies focus on the vegetative stage taxonomy, occurrence, and distribution, as well as HABs recordings. Research on ecology, bloom dynamics, toxicology, and genetic characterization is minimal. Until now short-term studies have been undertaken, and there is no permanent scientific monitoring.

Basic methodologies such as water samplers (quantitative analysis), plankton nets (20 μm, qualitative analysis), as well as, segmented tubes are applied to obtain marine phytoplankton sample collection at the superficial level or along the water column. For sample preservation, both Lugol and neutralized formalin are used (Throndsen, 1978), and in few cases, the combination of these fixatives is considered to preserve samples for long periods. For quantitative analysis, light microscopy is used to estimate the cell density by the Utermöhl (Hasle, 1978) or Sedgewick-Rafter (Guillard, 1978) methods.

Concerning ambient variables, the record of *in situ* hydrological variables such as temperature and salinity are the most common, while nutrients rarely are considered. Basic water quality sonde or CTD is the regular multiparameter equipment to record these variables. Nutrients analysis is carried out with standard chemical analytical methods (Strickland and Parsons, 1972).

Research on *P. bahamense* cysts is mainly developed with palynological procedures (de Vernal et al., 2010). Marine sediments are collected with gravitational corers or boxes, and scuba dive is also used to collect surface sediments or corers by hand. Biological procedures to clean and concentrate cysts (Matsuoka and Fukuyo, 2000) has been used for identification, quantification, germination assays, and strains establishment.

The identification of species is carried out by different techniques such as light and scanning electron microscopy, which allows the morphological and theca arrangement observations. It is worth noting that although Mertens et al. (2015) showed that there was no basis for distinction of two species varieties of *P. bahamense*, some researchers still accept this taxonomic status.

For research purposes, shellfish toxicity is determined by standard mouse bioassay (AOAC, 1995), and high-performance liquid chromatography (HPLC-FD with pre-column oxidation) (AOAC, 2005; Lawrence et al., 2005). For food safety, health authorities use the standard AOAC mouse bioassay

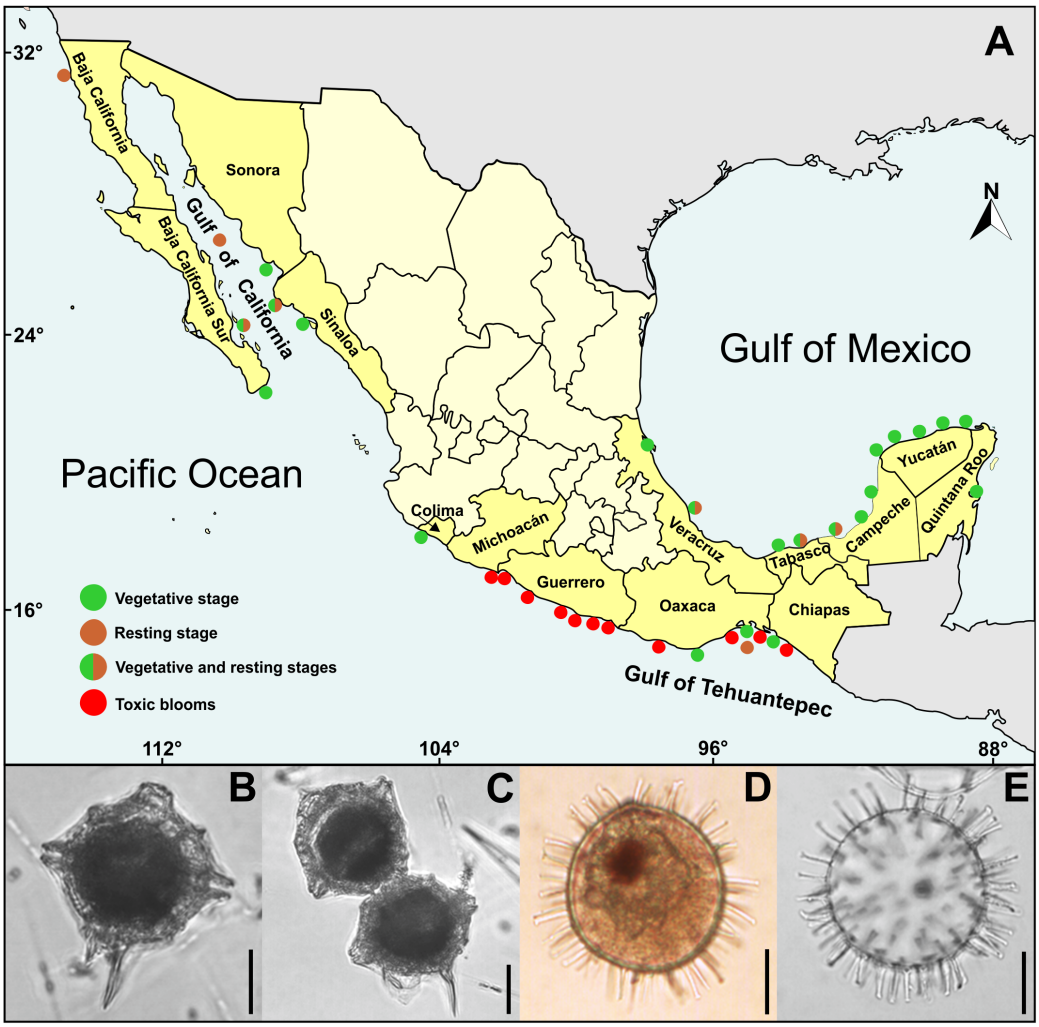


FIGURE 1 | *Pyrodinium bahamense* in Mexico: **(A)** Distribution map based on data documented by: Osorio-Tafall (1942); Martínez-Hernández and Hernández-Campos (1991); Saldate-Castañeda et al. (1991); Cortés-Altamirano et al. (1993); Gómez-Aguirre (1998a,b); Ronsón-Paulin (1999); Peña-Manjarrez et al. (2001, 2005); Licea et al. (2004); Terán-Suárez et al. (2006); Martínez-López et al. (2007); Morquecho (2008); Vásquez-Bedoya et al. (2008); Limoges et al. (2010, 2013); Gárate-Lizárraga and González-Armas (2011); Gárate-Lizárraga et al. (2011, 2013); Meave-del Castillo et al. (2012); Morquecho et al. (2012); Merino-Virgilio et al. (2014); Poot-Delgado et al. (2014); Alonso-Rodríguez et al. (2015); Maciel-Baltazar (2015); Poot-Delgado (2016); Campos-Campos et al. (2017), and Cuellar-Martínez et al. (2018). **(B)** Solitary vegetative cell. **(C)** Paired vegetative cells. **(D)** Whole living cyst with red accumulation body. **(E)** Empty cyst showing chorate processes. Scale bars = 20 μm.

(AOAC, 1995) and fast track probes (Scotia Rapid Tests) to estimate PSP toxins concentration in shellfish monthly. Phytoplankton samples are collected weekly and abundances $\geq 5 \times 10^3$ cells L^{-1} of *P. bahamense* are considered a potential indicator of toxins accumulation in shellfish[1].

GEOGRAPHIC DISTRIBUTION

The first report of *P. bahamense* on Chiapas coast, in southern Mexican Pacific (**Figure 1A**), came from Osorio-Tafall (1942). At that time, it was believed that the species had a distribution restricted to the Bahamas; however, at present, the vegetative stage (**Figures 1B,C**) is distributed in almost all coastal margin of Gulf of Mexico, Caribbean Sea, Gulf of California, and Mexican Pacific (**Figure 1A**). Cysts (**Figures 1D,E**) have been reported in limited areas, both Mexican Pacific as in the Gulf of Mexico (**Figure 1A**). However, it is highly probable that its distribution is as broad as that of vegetative cells, considering that *P. bahamense* cysts have been found in the central Gulf of California (Martínez-Hernández and Hernández-Campos, 1991), and in Baja California northern limit (Peña-Manjarrez et al., 2001, 2005) (**Figure 1A**). Despite these findings, a limited distribution of *P. bahamense* cysts has been reported (Martínez-Hernández and Hernández-Campos, 1991), restricted only to Guaymas basin phosphorite sediments, where it was

[1]https://www.gob.mx/cofepris/acciones-y-programas/marea-roja-76038

TABLE 1 | *Pyrodinium bahamense* blooms associated with human PSP outbreaks and wildlife mortalities in Mexican Pacific.

Localities	Month/year	*In situ* maximum dinoflagellate abundance (cells L^{-1})	Shellfish toxicity (μg STX eq kg^{-1})	Impacts	Reference
Oaxaca and Chiapas	12/1989	1.7×10^6	8,110 (*Crassostrea iridescens*) 5,420 (*Choromytilus palliopunctatus*)	99 human intoxications 3 fatalities	Saldate-Castañeda et al., 1991; Cortés-Altamirano et al., 1993
Oaxaca (Huatulco)	12/1990 to 01/1991	ND	ND	6 human fatalities	Cortés-Altamirano et al., 1995
Oaxaca	11/2001	1.14×10^6	14,560 (*Mytella arciformis*)	17 human intoxications 3 fatalities	Gárate-Lizárraga et al., 2008
Oaxaca (La Colorada and La Ventosa)	06/2010 to 02/2011	ND	ND	23 human intoxications	COFEPRIS, 2018
Guerrero	07–08/2010	ND	ND	16 human intoxications	COFEPRIS, 2018
Guerrero (Costa Grande)	12//2010	410×10^3	1,460–5,360 (rock oyster) 25,410 (unidentified clam)	12 human intoxications by ingesting raw and cooked clam	Gárate-Lizárraga et al., 2011
Guerrero (Bahía de Acapulco)	07/2010 to 01/2011	773.5×10^3	1,000–20,920	Precautionary closure of fisheries and seafood consumption	Gárate-Lizárraga et al., 2012; Meave-del Castillo et al., 2012
Guerrero (Costa Chica)	06–12/2010	194×10^3	460–7,890 (rock oyster) 520–4,410 (farmed rocky oysters)	Precautionary closure of fisheries and seafood consumption	Gárate-Lizárraga et al., 2013
Oaxaca (Santiago Astata and Puerto Escondido)	06/2009 to 06/2010	32×10^3	Toxins concentration exceeded the permissible limit (*Striostrea prismatica*)	Precautionary closure of fisheries and seafood consumption	Alonso-Rodríguez et al., 2015
Guerrero (Bahía de Acapulco, Costa Chica, and Costa Grande)	01–02/2016	ND	ND	Precautionary closure of fisheries and seafood consumption Mortality of turtles	COFEPRIS, 2018
Oaxaca (seven coastal municipalities)	02–03/2016	ND	ND	Precautionary closure of fisheries and seafood consumption Mortality of turtles and some cetaceans	COFEPRIS, 2018

ND, not documented.

the dominant morphotype (34%). This distribution is likely attributed to the scarcity of the vegetative stage along the Gulf of California, whereas its presence in the phosphorite, could indicate the influence of slightly hypersaline and tropical warmer water masses. To date, it has been demonstrated that cysts have a broader distribution in southern Gulf of California, primarily in restricted coastal lagoons, where it is the dominant morphotype (33–86%) (Morquecho et al., 2012; Cuellar-Martínez, 2018; Cuellar-Martínez et al., 2018).

In Gulf of Tehuantepec, and Gulf of Mexico *P. bahamense* cysts are also one of most dominant morphotype. For the northern part of the Gulf of Tehuantepec, the dinoflagellate dominates in the upper productivity zone associated with seasonal upwelling (Vásquez-Bedoya et al., 2008). This gulf has an average and a maximum water depth of ∼250 m and ∼1,000 m, respectively (Vásquez-Bedoya et al., 2008). During boreal winter (December through April), a strong but intermittent wind blows across central America from the Gulf of Mexico and the Caribbean driving upwelling events (Antoine et al., 1996; Pennington et al., 2006). During these events, the average 0–100 m NO_3 concentration is very high at 18.3 μmol l^{-1} (Pennington et al., 2006), and surface chlorophyll concentrations reaching as high as 2 mg Chl m^3 (Lluch-Cota et al., 1997; McClain et al., 2002). For the Gulf of Mexico, the annual temperature (approximately 25–28°C) is the most critical factor that controls nearshore cyst distribution and where *P. bahamense* achieve very high abundances, notably on the west Florida shelf and in the Mexican lagoons (Limoges et al., 2013).

ECOLOGY AND BLOOMS DYNAMICS

Pyrodinium bahamense usually blooms during the rainy summer (June through September), in restricted shallow lagoons surrounded by mangrove forests (Licea et al., 2004; Meave-del Castillo et al., 2012; Morquecho et al., 2012; Merino-Virgilio et al., 2014), with coastal underground drainage (Gómez-Aguirre, 1998b).

In the southern Gulf of Mexico, *P. bahamense* appears to have a continuous distribution and occurrence in a wide salinity range (3–38 ups) throughout the year, reaching densities of up to 1.5×10^6 cells L^{-1} Gómez-Aguirre (1998a). In contrast, in the southern Gulf of California, moderate ($63–151 \times 10^3$ cells L^{-1}), and short-term blooms are influenced by a short summer

rainy season (August–September), relatively high seawater temperature (25–32°C), typical salinity (31–36 ups), intense sunlight, and relatively high concentrations of ammonium (0.37–33.04 μM) and phosphates (0.68–2.87 μM); the last one, in turn, depending on rainfall and runoff and seems stronger on the eastern side of the gulf (Morquecho et al., 2012).

In southern Mexican Pacific and the Gulf of Tehuantepec, *P. bahamense* harmful blooms, with cell densities of up to 3×10^6 cells L^{-1}, have occurred from summer to winter (see **Table 1**) and have been associated with upwelling events (Cortés-Altamirano et al., 1993). The local winds "tehuanos", and currents systems can move the dinoflagellate towards Mexico along the Central Pacific Coast through the Costa Rica Current Flow and Mexican Western Current (Vargas-Montero et al., 2008).

In Mexican coasts, *P. bahamense* has co-occurred with the diatom *Pseudo-nitzschia* spp. (Morquecho, 2008), and other dinoflagellates such as *Ceratium furca, C. dens, Dinophysis caudata, G. catenatum, Margalefidinium polykrikoides, Polykrikos* sp., *Prorocentrum lima, P. gracile, Protoperidinium oceanicum* and *P. pellucidum* (Terán-Suárez et al., 2006; Gárate-Lizárraga et al., 2011; Meave-del Castillo et al., 2012). Interactions or environmental factors that may promote these co-occurrence has not yet been clarified. However, a similar co-occurrence between *P. bahamense* and *Pseudo-nitzschia* species has also been reported in the Indian River Lagoon, Florida (Phlips et al., 2004).

MEXICAN STRAINS TOXICITY AND CYSTS GERMINATION CHARACTERISTICS

Ecophysiological studies with regional *P. bahamense* strains are insufficient in México, and so far, strains from Isla San José, Gulf of California have only been studied. To elucidate the toxicity of vegetative cells grown from cyst germination, Morquecho et al. (2014) analyzed nine strains through fluorescence high-performance liquid chromatography, and only one exhibited toxicity with high saxitoxin concentration (95 pg STX eq $cell^{-1}$). Additionally, morphological features and size of cysts agreed with previous descriptions, particularly morphotypes found in the subtropical North Atlantic. Cysts germination exhibit thermophilic (20–35°C with the peak between 25 and 30°C) and euryhaline characteristics (salinities from 20 to 35 ups). Also, the *in vitro* germination is improved in growth medium enriched with terrestrial soil extract and selenium.

TOXICITY AND IMPACTS

Pyrodinium bahamense has caused more human illnesses and fatalities than any other PST producing dinoflagellate in Mexico (**Table 1**). Southeast Mexican Pacific has been the most affected area, particularly the Gulf of Tehuantepec, as well as Guerrero and Michoacán (**Figure 1** and **Table 1**). From 1989 to 2007 shellfish toxicity reached concentrations above the permissible limits for human consumption (800 μg STX eq kg^{-1}), and consequently caused 200 human cases, with 15 fatalities (Hernández-Becerril et al., 2007). By comparison, *Gymnodinium catenatum* is another toxic species linked with PSP deaths in Mexico, from 1989 to 2015 has caused, in some localities of the Gulf of California, 37 human PSP cases and three fatalities (Mee et al., 1986; Cortés-Altamirano and Núñez-Pasten, 1992; Núñez-Vázquez et al., 2016).

According to the registry of Federal Commission for the Protection against Sanitary Risks (COFEPRIS, by its acronym in Spanish) of the 118 HABs that have been reported from 2004 to 2014 in Mexico, 12% were linked to *P. bahamense*. The entity most affected was Oaxaca, followed by Guerrero and Chiapas (COFEPRIS, 2018). *Pyrodinium bahamense* HABs also have been linked to endangered marine fauna (sea turtles and cetaceans) with ecologic importance, leading in some cases to mass mortalities (**Table 1**), and the establishment of precautionary closures (COFEPRIS, 2018).

DISCUSSION AND CONCLUSION

The data obtained so far on *P. bahamense* spatial and population variability in Mexican Pacific and the Gulf of Mexico, suggest a seasonal and latitudinal pattern. From tropical to subtropical regions, the abundance, seasonality, and species distribution, tends to decrease. For example, the dinoflagellate may develop persistent moderate and massive toxic blooms in Guerrero, Oaxaca, and Chiapas coastal margin (**Figure 1**), which is characterized by a large-scale hydrological and atmospheric influence (**Table 1**); while in the southern Gulf of California, moderate blooms are restricted to coastal lagoons inhabited by mangroves, and develop only during the summer and the short-term rainy period. However, comparative research is needed on both coasts of Mexico to define precisely the hydrologic and climatic variables that are triggering *P. bahamense* blooms.

Epidemiological numbers of outbreaks of food poisoning related to *P. bahamense*, reveal that this dinoflagellate is the major source of PSP in Mexico. Monitoring for food safety on the coasts of Mexico, with particular emphasis on aquaculture areas or the exploitation of marine products, requires essential adjustments to validate and strengthen the management and decision-making database. Permanent records of hydrological variables, climate signals, harmful species abundance, seasonal variation, impacts in human and environmental health, as well as economic activities are essential to implement early warnings and minimize impacts.

Frequency and intensity of HABs as well as, the variation in phytoplankton composition toward toxic species have increased throughout the world (Fu et al., 2012). Recent studies suggest that ocean acidification, combined with the limitation of nutrients, or temperature changes, might dramatically increase the toxicity of some harmful groups (Fu et al., 2012). Mexico and Latin America are not immune to this problem as was discussed in this mini-review. *Pyrodinium bahamense* and other toxic dinoflagellates are significantly impacting human and environmental health, as well as having significant impact on economic activities.

The United States of America, the European Union, and other countries such as Australia and New Zealand, have managed

significant advances to counteract the impact of harmful microalgae. However, the information that we currently have on blooms dynamics, and toxicological and autecological characteristics of harmful species inhabiting Mexico and Latin America is insufficient. This prevents, in the short term, the establishment of management plans to minimize HABs impacts as well as the advance of the understanding and prediction of them.

Short-term perspectives about scientific research and monitoring activities related with *P. bahamense* populations in Mexico and Central America may include:

- Design and implement an interdisciplinary, specific and permanent monitoring program that consider meroplanktonic characteristic of the species and ecosystems comparison.
- Select as target ecosystems the coastal lagoons with and without mangrove populations. Usup et al. (2012) have questioned the significance of the presence or absence of mangrove forests in the distribution pattern of *P. bahamense*. Also, they have highlighted the importance of carrying out comparative studies between the western Pacific and tropical Atlantic populations, to understand better the factors that play essential roles in the bloom dynamics and toxicity of the species.
- Develop morphological, ecotoxicological, and genetic studies with strains from several geographical regions of Mexico and Central America, which are needed to accept or modify the current species taxonomic status, as well as, corroborate populations autoecology and toxicity.

AUTHOR CONTRIBUTIONS

The author participated in all the activities of research: data collection, analyses, interpretation of the results, and manuscript writing.

FUNDING

This work was supported by the CIBNOR project 20014 (Colección de Dinoflagelados Marinos).

ACKNOWLEDGMENTS

The author thank Dr. Andrea Murillo from Biotechnologika A2 for critically reading of the manuscript.

REFERENCES

Alonso-Rodríguez, R., Mendoza-Amézquita, E., Velásquez-López, S. A., Seim, J. A., and Martínez-Rodríguez, V. M. (2015). Florecimientos algales nocivos producidos por *Pyrodinium bahamense* en Oaxaca, México (2009–2010). *Salud. Publica. Mex.* 57, 343–351. doi: 10.21149/spm.v57i4.7578

Antoine, D., André, J. M., and Morel, A. (1996). Oceanic primary production. 2. Estimation at global scale from satellite (coastal zone colour scanner) chlorophyll. *Global Biogeochem. Cycles* 10, 57–69. doi: 10.1029/95GB02832

AOAC (1995). "Paralytic shellfish poison: biological method," in *Official Methods of Analysis of AOAC International*, ed. P. A. Cunniff (Gaithersburg, MD: AOAC International).

AOAC (2005). *Official Method 2005.06. Quantitative Determination of Paralytic Shellfish Poisoning Toxins in Shellfish Using Prechromatographic Oxidation and Liquid Chromatography with Fluorescence Detection*. Gaithersburg, MD: AOAC International.

Azanza, R. V., and Taylor, F. J. R. (2001). Are *Pyrodinium* blooms in the Southeast Asian region recurring and spreading? A view at the end of the millennium. *Ambio* 30, 356–364. doi: 10.1579/0044-7447-30.6.356

Balech, E. (1985). A redescription of *Pyrodinium bahamense* plate (Dinoflagellata). *Rev. Palaeobot. Palynol.* 45, 17–34. doi: 10.1016/0034-6667(85)90063-6

Campos-Campos, B., Cortés-Lara, M. C., and Rivas-Acuña, M. G. (2017). Microalgas planctónicas en la laguna costera «El Carmen», Cárdenas, Tabasco, México. *Kuxulkab* 23, 29–40. doi: 10.19136/kuxulkab.a23n46.2556

COFEPRIS. (2018). *Antecedentes de Presencia de Marea Roja en Costas Nacionales. Comisión Federal de Protección Contra Riesgos Sanitarios.* Available at: https://www.gob.mx/cofepris/documentos/presencia-de-marea-roja-en-costas-nacionales-durante-2003 [accessed September 27, 2018].

Cortés-Altamirano, R., Hernández-Becerril, D. U., and Luna-Soria, R. (1995). Mareas rojas en México: una revisión. *Rev. Latinoam. Microbiol.* 37, 343–352.

Cortés-Altamirano, R., Muñoz-Cabrera, L., and Sotomayor-Navarro, O. (1993). Envenenamiento paralítico por mariscos (PSP), causado por el dinoflagelado *Pyrodinium bahamense* var. compressum en la costa suroeste de México. *An. Inst. Cienc. del Mar y Limnol. UNAM* 20, 43–54.

Cortés-Altamirano, R., and Núñez-Pasten, A. (1992). Doce años (1979-1990) de registros de mareas rojas en la Bahía de Mazatlán, Sin., México. *An. Inst. Cienc. Mar Limnol. Univ. Nac. Auton. Mex.* 19, 113–121.

Cuellar-Martínez, T., Alonso-Rodríguez, R., Ruiz-Fernández, A. C., de Vernal, A., Morquecho, L., Limoges, A., et al. (2018). Environmental forcing on the flux of organic-walled dinoflagellate cysts in recent sediments from a subtropical lagoon in the Gulf of California. *Sci. Total Environ.* 621, 548–557. doi: 10.1016/j.scitotenv.2017.11.269

Cuellar-Martínez, T. C. (2018). *Distribución Temporal y Germinación de Quistes de Pyrodinium bahamense Plate, 1906 en Sedimentos Recientes de dos Lagunas Costeras del Golfo de California, México*. Ph.D, thesis, Universidad Nacional Autónoma de México-Posgrado en Ciencias del Mar y Limnología, Ciudad de México.

de Vernal, A., Henry, M., and Bilodeau, G. (2010). *Micropaleontological Preparation Techniques and Analyses. Les Cahiers du GEOTOP No 3, 3rd Edn.* Available at: https://www.geotop.ca/upload/files/laboratoires/laboratoire-de-micropaleontologie-et-palynologie-marine-uqam/Micropal_Methods_2010.pdf [accessed September 20, 2018].

Fu, F. X., Tatters, A. O., and Hutchins, D. A. (2012). Global change and the future of harmful algal blooms in the ocean. *Mar. Ecol. Prog. Ser.* 470, 207–233. doi: 10.3354/meps10047

Gárate-Lizárraga, I., Díaz-Ortiz, J. A., Pérez-Cruz, B., Alarcón-Romero, M. A., Chávez-Almazán, L. A., García-Barbosa, J. L., et al. (2011). A multi-species dinoflagellate bloom and shellfish toxicity in Costa Grande, Guerrero, Mexico (December, 2010). *CICIMAR Oceánides* 26, 67–71.

Gárate-Lizárraga, I., and González-Armas, R. (2011). Occurrence of *Pyrodinium bahamense* var. compressum along the southern coast of the Baja California Peninsula. *Mar. Pollut. Bull.* 62, 626–630. doi: 10.1016/j.marpolbul.2011.01.009

Gárate-Lizárraga, I., Pérez-Cruz, B., Días-Ortiz, J., and Band-Schmidt, C. J. (2008). Microalgas y biotoxinas marinas en las costas mexicanas. *Conversus* 9, 22–26.

Gárate-Lizárraga, I., Pérez-Cruz, B., Díaz-Ortíz, J. A., Alarcón-Tacuba, M., Chávez-Almazán, L. A., Alarcón-Romero, M. A., et al. (2012). Toxicity and paralytic toxin profile in *Pyrodinium bahamense* var. compressum and violet oyster in Bahía de Acapulco. *Harmful Algae News* 45, 2–3.

Gárate-Lizárraga, I., Pérez-Cruz, B., Díaz-Ortiz, J. A., Alarcón-Tacuba, M. A., Alarcón-Romero, M. A., Chávez-Almazán, L. A., et al. (2013). Blooms of *Pyrodinium bahamense* var. compressum and rock oyster toxicity in Costa Chica, Guerrero, Mexico. *CICIMAR Océanides* 28, 37–42.

García-Mendoza, E., Quijano-Scheggia, S. I., Olivos-Ortiz, A., Núñez-Vázquez, E. J., and Pérez-Morales, A. (2016). "Introducción general," in *Florecimientos Algales Nocivos en México*, eds E. García-Mendoza, S. I. Quijano-Scheggia, A. Olivos-Ortiz, and E. J. Núñez-Vázquez (Ensenada: CICESE), 10–20.

Gómez-Aguirre, S. (1998a). First record of *Pyrodinium bahamense* (Dinoflagellata) in brackish waters of the Mexican Caribbean coast. *An. Inst. Biol. Univ. Nac. Autón. Méx. Ser. Zool.* 69, 121–123.

Gómez-Aguirre, S. (1998b). Red tide ocurrences recorded in Mexico from 1980 to 1992. *An. Inst. Biol. Univ. Nac. Autón. Méx. Ser. Zool.* 69, 13–22.

Guillard, R. R. L. (1978). "Counting slides," in *Phytoplankton Manual Monographs on Oceanographic Methodology*, Vol. 6, ed. A. Sournia (Paris: UNESCO), 184.

Hallegraeff, G. M. (2010). Ocean climate change, phytoplankton community responses, and harmful algal blooms: a formidable predictive challenge. *J. Phycol.* 46, 220–235. doi: 10.1111/j.1529-8817.2010.00815.x

Hasle, G. R. (1978). "Using the inverted microscope," in *Phytoplankton Manual Monographs on Oceanographic Methodology*, Vol. 6, ed. A. Sournia (Paris: UNESCO), 191–196.

Heisler, J., Glibert, P. M., Burkholder, J. M., Anderson, D. M., Cochlan, W., Dennison, W. C., et al. (2008). Eutrophication and harmful algal blooms: a scientific consensus. *Harmful Algae* 8, 3–13. doi: 10.1016/j.hal.2008.08.006

Hernández-Becerril, D. U., Alonso-Rodríguez, R., Álvarez-Góngora, C., Barón-Campis, S. A., Ceballos-Corona, G., Herrera-Silveira, J., et al. (2007). Toxic and harmful marine phytoplankton and microalgae (HABs) in Mexican Coasts. *J. Environ. Sci. Health A Tox. Hazard Subst. Environ. Eng.* 42, 1349–1363. doi: 10.1080/10934520701480219

Lawrence, J. F., Niedzwiadek, B., and Menard, C. (2005). Quantitative determination of paralytic shellfish using prechromatographic oxidation and liquid chromatography with fluorescence detection: collaboratory study. *J. AOAC Int.* 88, 1714–1732.

Licea, S., Zamudio, M. E., Luna, R., and Soto, J. (2004). Free-living dinoflagellates in the southern Gulf of Mexico: report of data (1979–2002). *Phycol. Res.* 52, 419–428. doi: 10.1111/j.1440-183.2004.00364.x

Limoges, A., Kielt, J.-F., Radi, T., Ruíz-Fernandez, A. C., and de Vernal, A. (2010). Dinoflagellate cyst distribution in surface sediments along the south-western Mexican coast (14.76°N to 24.75°N). *Mar. Micropaleontol.* 76, 104–123. doi: 10.1016/j.marmicro.2010.06.003

Limoges, A., Londeix, L., and de Vernal, A. (2013). Organic-walled dinoflagellate cyst distribution in the Gulf of Mexico. *Mar. Micropaleontol.* 102, 51–68. doi: 10.1016/j.marmicro.2013.06.002

Lluch-Cota, S. E., Álvarez-Borrego, S., Santamaria-del Ángel, E. M., Muller-Karger, F. E., and Hernandez-Vazquez, S. (1997). The Gulf of tehuantepec and adjacent areas: spatial and temporal variation of satellite-derived photosynthetic pigments. *Cienc. Mar.* 23, 329–340. doi: 10.7773/cm.v23i3.809

Maciel-Baltazar, E. (2015). Dinoflagelados (Dinoflagellata) tóxicos de la costa de Chiapas, México, Pacífico centro oriental. *Cuadernos de Investigación UNED* 7, 39–48.

Maclean, J. L. (1989). "An overview of *Pyrodinium* red tides in the western Pacific," in *Biology, Epidemiology and Management of Pyrodinium Red Tides*, eds G. M. Hallegraeff and J. L. Maclean (Manila: International Center for Living Aquatic Resources Management), 1–8.

Martínez-Hernández, E., and Hernández-Campos, H. (1991). Distribución de quistes de dinoflagelados y acritarcas en sedimentos holocénicos del Golfo de California. *Paleontol. Mex.* 57, 1–133.

Martínez-López, A., Ulloa-Pérez, E., and Escobedo-Urias, D. (2007). First record of vegetative cells of *Pyrodinium bahamense* (Gonyalucales: Goniodomataceae) in the Gulf of California. *Pacific Sci.* 61, 289–293. doi: 10.2984/1534-6188(2007)61[289:FROVCO]2.0.CO;2

Matsuoka, K., and Fukuyo, Y. (2000). *Guía Técnica Para el Estudio de Quistes de Dinoflagelados Actuales*. Tokyo: WESTPAC-HAB/WESTPAC/ IOC.

McClain, C. R., Christian, J. R., Signorini, S. R., Lewis, M. R., Asanuma, I., Turk, D., et al. (2002). Satellite ocean-color observations of the tropical Pacific Ocean. *Deep Sea Res. II* 49, 2533–2560. doi: 10.1016/S0967-0645(02)00047-4

Meave-del Castillo, M. E., Zamudio-Reséndiz, M. E., and Castillo-Rivera, M. (2012). Riqueza fitoplanctónica de la bahía de Acapulco y zona costera aledaña, Guerrero, México. *Acta Bot. Mex.* 100, 405–487. doi: 10.21829/abm100.2012.41

Mee, L. D., Espinosa, M., and Díaz, G. (1986). Paralytic shellfish poisoning with a *Gymnodinium catenatum* red tide on the Pacific Coast of Mexico. *Mar. Environ. Res.* 19, 77–92. doi: 10.1016/0141-1136(86)90040-1

Merino-Virgilio, F. C., Okolodkov, Y. B., Aguilar-Trujillo, A. C., Osorio-Moreno, I., and Herrera-Silveira, J. A. (2014). "Florecimientos algales nocivos en las aguas costeras del norte de Yucatán (2001–2013)," in *Golfo de México, Contaminación e Impacto Ambiental: Diagnóstico y Tendencias*, eds A. V. Botello, J. Rendón von Osten, J. A. Benítez, and G. Gold-Bouchot (Mérida: UAC), 161–180.

Mertens, K. N., Wolny, J., Carbonell-Moore, C., Bogus, K., Ellegaard, M., Limoges, A., et al. (2015). Taxonomic re-examination of the toxic armored dinoflagellate *Pyrodinium bahamense* Plate 1906: can morphology or LSU sequencing separate *P. bahamense* var. compressum from var. bahamense? *Harmful Algae* 41, 1–24. doi: 10.1016/j.hal.2014.09.010

Morquecho, L. (2008). Morphology of *Pyrodinium bahamense* plate (Dinoflagellata) near Isla San José, Gulf of California, Mexico. *Harmful Algae* 7, 664–670. doi: 10.1016/j.hal.2008.02.003

Morquecho, L., Alonso-Rodríguez, R., Arreola-Lizárraga, J. A., and Reyes-Salinas, A. (2012). Factors associated with moderate blooms of *Pyrodinium bahamense* in shallow and restricted subtropical lagoons in the Gulf of California. *Bot. Mar.* 55, 611–623. doi: 10.1515/bot-2012-0171

Morquecho, L., Alonso-Rodríguez, R., and Martínez-Tecuapacho, G. A. (2014). Cyst morphology, germination characteristics, and potential toxicity of *Pyrodinium bahamense* in the Gulf of California. *Bot. Mar.* 57, 303–314. doi: 10.1515/bot-2013-0121

Núñez-Vázquez, E. J., Band-Schmidt, C. J., Hernández-Sandoval, F. E., Bustillos-Guzmán, J. J., López-Cortés, D. J., et al. (2016). "Impactos de los FAN en la salud pública y animal (silvestres y de cultivo) en el Golfo de California," in *Florecimientos Algales Nocivos en México*, eds E. García-Mendoza, S. I. Quijano-Scheggia, A. Olivos-Ortiz, and E. J. Núñez-Vázquez (Ensenada: CICESE), 196–212.

Osorio-Tafall, B. F. (1942). Notas sobre algunos dinoflagelados planctónicos marinos de México con descripción de nuevas especies. *An. Esc. Nac. Cienc. Biol.* 2, 435–447.

Peña-Manjarrez, J. L., Gaxiola-Castro, G., Helenes-Escamilla, J., and Orellana-Cepeda, E. (2001). Cysts of *Lingulodinium polyedrum*, red tide producing organism in the Todos Santos Bay (Winter–Spring, 2000). *Cienc. Mar.* 27, 543–558. doi: 10.7773/cm.v27i4.501

Peña-Manjarrez, J. L., Helenes, J., Gaxiola-Castro, G., and Orellana-Cepeda, E. (2005). Dinoflagellate cysts and bloom events at Todos Santos Bay, Baja California, México, 1999–2000. *Cont. Shelf. Res.* 25, 1375–1393. doi: 10.1016/j.csr.2005.02.002

Pennington, J. T., Mahoney, K. L., Kuwahara, V. S., Kolber, D. D., Calienes, R., and Chavez, F. P. (2006). Primary production in the eastern tropical Pacific: a review. *Prog. Oceanogr.* 69, 285–317. doi: 10.1016/j.pocean.2006.03.012

Phlips, E. J., Badylak, S., Bledsoe, E., and Cichra, M. (2006). Factors affecting the distribution of *Pyrodinium bahamense* var. bahamense in coastal waters of Florida. *Mar. Ecol. Prog. Ser.* 322, 99–115. doi: 10.3354/meps322099

Phlips, E. J., Badylak, S., Youn, S., and Kelley, K. (2004). The occurrence of potentially toxic dinoflagellates and diatoms in a subtropical lagoon, the Indian River Lagoon, Florida, USA. *Harmful Algae* 3, 39–49. doi: 10.1016/j.hal.2003.08.003

Poot-Delgado, C. A. (2016). Florecimientos algales nocivos en las costas de Campeche, Golfo de México. *Investigación y Ciencia de la Universidad Autónoma de Aguascalientes* 68, 91–96.

Poot-Delgado, C. A., Rosado-García, P. I., and Guzmán-Noz, Y. A. (2014). "Fitoplancton marino potencialmente nocivo en las aguas costeras de Campeche," in *Golfo de México, Contaminación e Impacto Ambiental: Diagnóstico y Tendencias*, eds A. V. Botello, J. Rendón von Osten, J. A. Benítez, and G. Gold-Bouchot (Mérida: UAC), 117–132.

Ronsón-Paulin, J. A. (1999). Análisis retrospectivo y posibles causas de las mareas rojas tóxicas en el litoral del sureste mexicano (Guerrero, Oaxaca, Chiapas). *Ciencia y Mar.* 3, 49–55.

Saldate-Castañeda, O., Vázquez-Castellanos, J. L., Galván, J., Sánchez-Anguiano, A., and Nazar, A. (1991). Intoxicaciones por toxina paralizante de molusco en Oaxaca. *Salud. Publica. Mex.* 33, 240–247.

Seliger, H. H., Carpenter, J. H., Loftus, M., Biggley, W. H., and McElroy, W. D. (1971). Bioluminescence and phytoplankton successions in Bahia Fosforescente, Puerto Rico. *Limnol. Oceanogr.* 16, 608–622. doi: 10.4319/lo.1971.16.4.0608

Steidinger, K. A., Tester, L. S., and Taylor, F. J. R. (1980). A redescription of *Pyrodinium bahamense* var. compressa (Böhm) stat. nov. from Pacific red tides. *Phycologia* 19, 329–337. doi: 10.2216/i0031-8884-19-4-329.1

Strickland, J. D. H., and Parsons, T. R. (1972). *A Practical Handbook of Sea-water Analysis*. Ottawa: Canadian Science Publishing.

Terán-Suárez, J. M., Castro, G. V., Mayor-Nucamendi, H. F., and Brito-López, J. A. (2006). Florecimientos algales en tabasco. *Salud en Tabasco* 12, 414–422.

Throndsen, J. (1978). "Preservation and storage," in *Phytoplankton Manual Monographs on Oceanographic Methodology*, Vol. 6, ed. A. Sournia (Paris: UNESCO), 69–74.

Usup, G., Ahmad, A., Matsuoka, K., Lim, T., and Leaw, C. P. (2012). Biology, ecology and bloom dynamics of the toxic marine dinoflagellate *Pyrodinium bahamense*. *Harmful Algae* 14, 301–312. doi: 10.1016/j.hal.2011.10.026

Usup, G., and Azanza, R. V. (1998). "Physiology and bloom dynamics of the tropical dinoflagellate *Pyrodinium bahamense*," in *Physiological Ecology of Harmful Algal Blooms*, eds D. M. Anderson, A. D. Cembella, and G. M. Hallegraeff (Berlin: Springer Verlag), 81–94.

Vargas-Montero, M., Freer-Bustamante, E., Guzmán, J. C., and Vargas, J. C. (2008). Florecimientos de dinoflagelados nocivos en la costa Pacífica de Costa Rica. *Hidrobiológica* 18, 15–23.

Vásquez-Bedoya, L. F., Radi, T., Ruiz-Fernández, A. C., de Vernal, A., Machain-Castillo, M. L., Kielt, J. F., et al. (2008). Organic-walled dinoflagellate cysts and benthic foraminifera in coastal sediments of the last century from the Gulf of Tehuantepec, South Pacific Coast of Mexico. *Mar. Micropaleontol.* 68, 49–65. doi: 10.1016/j.marmicro.2008.03.002

Wall, D., and Dale, B. (1969). The "hystrichosphaerid" resting spore of the dinoflagellate *Pyrodinium bahamense* plate 1906. *J. Phycol.* 5, 140–149. doi: 10.1111/j.1529-8817.1969.tb02595.x

Walsh, J. J., Tomas, C. R., Steidinger, K. A., Lenes, J. M., Chen, F. R., Weisberg, R. H., et al. (2011). Imprudent fishing harvests and consequent trophic cascades on the West Florida shelf over the last half century: a harbinger of increased human deaths from paralytic shellfish poisoning along the southeastern United States, in response to oligotrophication? *Cont. Shelf Res.* 31, 891–911. doi: 10.1016/j.csr.2011.02.007

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Morquecho. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Marine Science

ORIGINAL RESEARCH
published: 05 February 2019
doi: 10.3389/fmars.2019.00023

Microplankton Community Composition Associated With Toxic *Trichodesmium* Aggregations in the Southwest Atlantic Ocean

Mariana Bernardi Bif[1]*, *Márcio Silva de Souza*[2], *Luiza Dy Fonseca Costa*[2] and *João Sarkis Yunes*[2]

[1] Department of Ocean Sciences, Rosenstiel School of Marine and Atmospheric Sciences, University of Miami, Miami, FL, United States, [2] Instituto de Oceanografia, Universidade Federal do Rio Grande (FURG), Rio Grande, Brazil

The spatial distribution and species identification of *Trichodesmium* was assessed during two fall cruises along the Southwest Atlantic Ocean shelf break. Organisms from the microplankton >50 μm were collected using a vertical plankton net for quantification and identification of the microplanktonic community associated with the genus. Additional sub-samples were filtered and prepared for quantification and discrimination of phycotoxins from the particulate matter using High Performance Liquid Chromatography. Physical parameters such as temperature, salinity, wind speed, and mixed layer depth were used in order to evaluate the environmental conditions at the time of sampling and correlate with *Trichodesmium* occurrence. Overall, *Trichodesmium* abundances were higher in the northernmost stations under wind speeds of <8 knots and shallow mixed layer depths <40 m. Besides frequent reports on the occurrence of *T. erythraeum* and *T. thiebautii* for this region, we identified three species as *T. clevei*, *T. hildebrandtii*, and *T. radians*. In the majority of stations where *Trichodesmium* was not the dominant organism, other microplanktonic groups were present such as centric diatoms and dinoflagellates. The toxin analysis was positive for saxitoxins predominantly when *Trichodesmium* was at high numbers of trichomes per liter in the stations; however, there was an inverse relationship between abundance of trichomes and toxin concentrations. Using information from the environmental variables and *Trichodesmium* abundance, we suggest that the toxin production might take place during the aggregation phase of trichomes at surface, and that saxitoxins could be inhibiting the growth of other microplanktonic organisms.

Keywords: marine diazotroph, microbial community composition, phycotoxin, saxitoxins, STX

OPEN ACCESS

Edited by:
Angel Borja,
Centro tecnológico experto en innovación marina y alimentaria (AZTI), Spain

Reviewed by:
Judith Marie O'Neil,
University of Maryland Center for Environmental Science (UMCES), United States
Reut Sorek-abramovich,
Dead Sea and Arava Science Center, Israel

***Correspondence:**
Mariana Bernardi Bif
marianabif@gmail.com

Specialty section:
This article was submitted to Marine Ecosystem Ecology, a section of the journal Frontiers in Marine Science

Received: *28 May 2018*
Accepted: *18 January 2019*
Published: *05 February 2019*

Citation:
Bif MB, de Souza MS, Costa LDF and Yunes JS (2019) Microplankton Community Composition Associated With Toxic Trichodesmium Aggregations in the Southwest Atlantic Ocean. Front. Mar. Sci. 6:23. doi: 10.3389/fmars.2019.00023

INTRODUCTION

The marine cyanobacteria *Trichodesmium* (Ehrenberg, 1830) form colonies that aggregate in the water surface as visible patches in tropical and subtropical oceans. The high abundance of trichomes in the upper water layer is often associated with environmental factors such as hot and calm weather and the presence of a shallow mixed layer (Karl et al., 2002; Agawin et al., 2013). The global

occurrence of *Trichodesmium* is of undoubted importance to biogeochemical cycles, especially nitrogen and carbon (Capone et al., 1997; Westberry and Siegel, 2006); however, the majority of studies involving *Trichodesmium* are restricted to the North Atlantic and North Pacific Subtropical Gyres (Villareal and Carpenter, 2003; LaRoche and Breithbarth, 2005), and the genus is often under-studied in other oceans. More recent reports of *Trichodesmium* in the Andaman Sea (Arun Kumar et al., 2012), coast of India (Srinivas and Sarin, 2013) and Southwest Atlantic Ocean (Silva, 2005; Silva et al., 2008; Detoni et al., 2016a,b; Bif and Yunes, 2017) suggest they are important members of the microbial community composition in those regions.

In terms of ecological role, *Trichodesmium* is often reported in co-occurrence with other members of phytoplankton and zooplankton. The symbionts *Rhizosolenia hebetata* and *Richelia intracellularis*, for example, were present with dense aggregations of *Trichodesmium* (Madhu et al., 2013). The association between the harpacticoid *Macrosetella gracilis* and *Trichodesmium* colonies are thought to benefit the zooplankton, since trichomes were reported containing eggs and larvae attached, and served as food and substrate (Björnberg, 1965; O'Neil et al., 1996). Understanding the microbial community structure surrounding *Trichodesmium* aggregations is therefore an important key to understand its ecology and species interactions, especially in understudied regions such as the South Atlantic Ocean where the genus is often reported (Gianesella-Galvão, 1995; Rörig et al., 1998; Carvalho et al., 2008; Monteiro et al., 2010, 2012). Recent works from our group have identified colonies in the Southwest Atlantic Ocean associated with iron-rich dust particles (Bif and Yunes, 2017) and producing toxins (Detoni et al., 2016a); however, the association of these potentially toxic aggregations with other microbial communities was never assessed. Previous works associated the occurrence of large aggregations of *Trichodesmium* (*T. erythraeum* and *T. thiebautii*) with anoxia-related mortality of fish and crabs in India (D'Silva et al., 2012 and references therein) and bryozoans in the South Brazilian coast (Silva, 2005); however, no toxin analysis was performed at that time (Silva et al., 2008). Given the reports of *Trichodesmium* aggregations along the Brazilian coast (Silva et al., 2008 and references therein), our work aims to investigate their distribution, morphological taxonomy, association with microplanktonic groups and the potential toxicity to these organisms.

MATERIALS AND METHODS

Study Area and Analysis of Physical Parameters

This study was carried out in the area encompassing the Brazilian shelf slope on board the R/V Atlântico Sul with TALUDE project (**Table 1**). Samples were collected between 24° and 35°S (**Figure 1**) during two consecutive fall cruises, the first between June 4th and 11th of 2013 and the second between May 10th and 20th of 2014. Vertical profiles of temperature and salinity were obtained using a CTD sensor (911 SeaBird®). The surface water masses were classified based on the thermohaline intervals described by Möller et al. (2008). The mixed layer depth (MLD) was determined from vertical density profiles ($\partial\rho/\partial z$), excluding the first 5 meters of depth due to noise data. Wind speed and direction were obtained from an anemometer mounted on the ship's foremast ∼18 m above the sea surface. Both sea surface temperature (SST) and salinity (SSS) were then averaged for the upper 30 m of water depth in order to correlate with other environmental parameters, especially community composition.

Analysis of Biological Samples

Samples were collected during two different conditions: areas of high density aggregations of *Trichodesmium*; and in the absence of visible surface aggregations. The sampling efforts were carried out during calm seas and clear skies. During the 2013 cruise we sampled the northernmost section (stations 1 to 4; **Figure 1**) and focused solely in high density of trichomes. The 2014 cruise was carried out in the southernmost stations and sampling was performed during both presence and absence of trichomes.

The organisms were collected by vertical hauls using conical plankton net with 30 cm mouth diameter and 50 μm mesh size, down to 30 m depth of the water column. These samples were preserved in 4% buffered formalin. *Trichodesmium* species were identified following Komárek and Anagnostidis (2005) and quantified as number of trichomes per liter. For the description of each species of *Trichodesmium*, it was necessary to distinguish among cell, trichome (cells filaments) and colony (clusters of trichomes organized as puffs or tufts) (Anagnostidis and Komárek, 1988). As far as possible, co-occurring microplankton (>50 μm) were also identified and counted (minimum 100 cells per group) using an inverted microscope (ZeissAxiovert A1) coupled with a camera (Axio Cam MRc) using the sedimentation chamber technique (Utermöhl, 1958). Samples were counted at ×200, ×400, and ×1000 of magnification and the species were identified according to specific literature for each group i.e., diatoms (Hasle and Syvertsen, 1996) and dinoflagellates (Steindinger, 1996).

Since *Trichodesmium* spp. trichomes were very concentrated in many samples, it was not possible to estimate their abundance per species; instead, the total abundance was estimated by the sum of free trichomes plus number of colonies × 200, as the average of trichomes on each colony (Carpenter, 1983). Total abundance of *Trichodesmium* and other taxa were, then, divided by the 30-m of water column × 0.03-m of mouth diameter of the plankton net, so the final abundance is shown as trichomes (*Trichodesmium* spp.) or cells (other microplankton, excluding *Trichodesmium* spp.) per liter.

Analysis of Toxins

Samples from 6 different stations were collected from visible aggregations of *Trichodesmium* during December-2014, at the same region where biological samples took place. Using a GF/F glass fiber filter of 0.7 μm pore size, the water was filtered until the filter was clogged (60–470 mL of sample), washed with distilled water and maintained at −80°C until chemical analysis. We analyzed two kinds of toxins using High Performance Liquid Chromatography (HPLC UV-DAD): Domoic Acid (DA) (Miguez et al., 1996; Costa et al., 2003)

TABLE 1 | Major parameters of water and weather conditions at the stations.

Stations	Lat (S)	Long (W)	Sampling dates	Local depth (m)	SST**	T (°C)	SSS***	Salinity	MLD* (m)	Surface watermass	Wind speed (knots)	Wind direction
#1	24°16.6′	43°22.2′	06/11/13	692	24.0	23.7	36.8	36.9	45	TW	6	SE
#2	25°07.6′	44°51.4′	06/08/13	190	23.6	24.6	36.2	37.3	17	TW	4.5	NO
#3	25°48.8′	45°04.2′	06/07/13	1287	23.2	22.9	35.8	35.8	40	TW	8	SE
#4	27°6.4′	46°24.7′	06/04/13	830	23.2	23.1	36.8	36.8	80	TW	4.6	SE
#5	28°6.6′	48°00.0′	05/20/14	138	21.8	22.9	36.0	36.7	14	TW	5	NW
#6	29°22.9′	47°08.8′	05/19/14	1876	22.8	22.8	36.8	36.8	35	TW	11.6	SW
#7	29°26.0′	48°24.8′	05/18/14	140	22.7	22.8	36.7	36.8	35	TW	10.4	SW
#8	30°45.6′	48°24′	05/17/14	1505	20.6	20.7	34.3	34.5	80	STSW	12.2	W
#9	31°39.3′	49°29.8′	05/15/14	1816	21.8	22.9	36.0	36.7	43	TW	15.7	NW
#10	31°56.5′	49°57.2′	05/14/14	680	20.6	20.7	34.3	34.5	18	STSW	4	SE
#11	32°12.9′	49°41.1′	05/14/14	1870	21.8	22.9	36.0	36.7	40	TW	5.8	SW
#12	32°18.2′	50°06.6′	05/14/14	806	21.8	22.9	36.0	36.7	40	TW	11	SW
#13	32°33.6′	50°16.9′	05/13/14	575	20.6	20.9	31.1	31.4	47	PPW	18	W
#14	32°55.3′	50°17.6′	05/11/14	676	20.8	22.3	35.0	36.1	15	TW	6	NW
#15	33°04.0′	50°05.0′	05/12/14	1166	23.5	23.5	36.9	36.9	20	TW	6.7	W
#16	33°40.4′	51°23.5′	05/10/14	127	20.6	20.8	31.1	31.3	18	PPW	24	SE

*Temperature (°C) and salinity values are averaged for 30 m of water column. *Mixed Layer Depth, **Sea Surface Temperature, ***Sea Surface Salinity.*

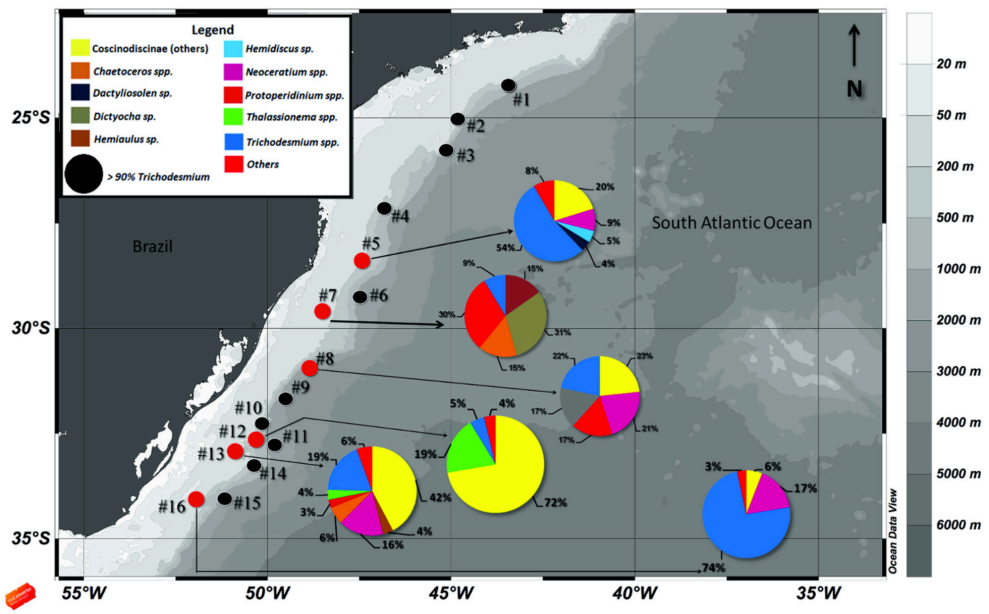


FIGURE 1 | Map of the study area showing the sampling stations along the South Atlantic shelf slope. Colored legend shows the main microplankton species matching with pie charts sections. Stations shown as black circles denote sites where *Trichodesmium* spp. contributed >90% to the total microplankton cell densities, and small red circles represent stations where *Trichodesmium* spp. contributed <90% to the total density.

and saxitoxins (STX), (Rourke et al., 2008). The collection of samples and analysis of STX were reported in detail (Detoni et al., 2016b), and the same samples were used for the analysis of domoic acid. The abundance of *Trichodesmium* was correlated with STX concentrations at the stations. We then calculated the STX:trichome ratio, and reported STX as equivalent saxitoxin concentrations (STX-eq) μg L^{-1} that is, the sum of all the variants present in the analyzed sample.

Statistics

A series of multivariate analyses were conducted in order to verify any spatial pattern in the species distribution, and to verify the relationship between environmental factors (i.e., temperature,

water masses, MLD) and the presence of the different taxonomic groups of microphytoplankton.

A non-metric multidimensional scaling (nMDS) was applied together with a dissimilarity matrix based on the non-metric Bray-Curtis index (Bray and Curtis, 1957) in order to group the taxa using an ordination diagram (Wickelmaier, 2003). An analysis of similarities (ANOSIM) was then applied in order to verify possible differences among the stations (Clarke, 1993). The analyses were carried out using the free software Past (v.1.81) (Hammer et al., 2008).

The Canonical Correspondence Analysis (CCA) was performed in order to identify patterns and variabilities of microplankton species with respect to environmental variables (TerBraak and Prentice, 1988). Biotic variables were represented by total abundances of *Trichodesmium* and other microplankton taxa. Environmental variables included: sea surface temperature; salinity; MLD; wind speed; latitude; and water mass. That latter variable was ascribed as follows: Tropical Water (TW) = 1, Subtropical Shelf Water (STSW) = 2, and Plata Plume Water (PPW) = 3, using latitude as a covariate. All variables were log-transformed before analysis in order to normalize the sets of variables. To test for the CCA significance, we run Monte-Carlo tests based on 499 permutations under a reduced model ($p < 0.05$). Three stations (#1, #15, and #16) were excluded from the final CCA analysis because they represented very extreme conditions in comparison to others.

RESULTS

Physical Conditions

Considering the physical conditions during the two fall cruises (**Table 1**), the Tropical Water (TW, Sal. $\geq$ 36 and Temp. $>$22.3°C) mainly influenced the region. The Sub-Tropical Shelf Water (STSW, 33.5 $<$ Sal. $<$35.5 and Temp. $\sim$20.7°C) was the dominant surface water mass at stations #8 and #10, while the Plata Plume Water (PPW, Sal $<$ 33.5 and Temp $\leq$ 20.9°C) was noticeable at stations #13 and #16 (Möller et al., 2008). The wind direction varied among stations, but was dominated by Southerly or Westerly directions. Stations under the influence of relatively high wind speed $>$10 knots were always related to a non-dominant *Trichodesmium* spp. in the total abundance of trichomes (**Figure 1**; **Table 1**). MLD was also variable among stations, from 14 m down to 80 m of depth, and was not correlated to the local wind speed (**Table 2**).

Microplankton Identification and Spatial Distribution

Figure 1 compiles the map of the study area with the relative contribution of specific microplanktonic groups to the total abundance of organisms at each station. The results show that *Trichodesmium* spp. were the greatest contributors for both fall cruises, comprising more than $>$90% of total abundance in many stations. In a few southernmost stations, the diatoms from Coscinodiscineae group and *Thalassionema* spp., as well as the dinoflagellates *Neoceratium* spp. and *Protoperidinium* spp. co-occurred with the cyanobacterial trichomes at elevated cell numbers (**Figure 1**). Stations #2 and #3 had the most elevated concentrations of trichomes at surface: 2.1×10^6 and 4.4×10^6 trichomes L^{-1}, respectively. These two stations and other four (#1, #4, #10, and #11) had trichome abundances $>8 \times 10^3$ trichomes L^{-1}. At these stations, the accumulation of *Trichodesmium* at surface was visible to the naked eye. In contrast, aggregations were not visible in the remaining stations, where abundances ranged from only $\sim$4 trichomes L^{-1} to 2.5×10^3 trichomes L^{-1} (**Table 2**).

Our statistical analysis separated the stations where *Trichodesmium* was more abundant from those where their abundance was fairly comparable to the abundance of other organisms. The ordination diagram derived from the nMDS analysis defined the group A as composed by stations with $>$ 8,000 trichomes L^{-1} and a group B with those with densities $<$2,500 trichomes L^{-1} (**Figure 2**). These two groups were significantly different (ANOSIM, $r^2 = 0.89$, $p < 0.0001$) and, interestingly, separate visible aggregations from those undetected to the naked eye.

In the stations where *Trichodesmium* spp. contributed $<$90% for the total abundance of organisms, the species composition varied between different groups of microplankton (**Figure 1**; **Table 2**). Stations #14 (18 taxa), #10 (17 taxa) #1, and #5 (13 taxa each) had a relatively high diversity and densities, while #6, #8, #12, and #15 had concentrations of $<$500 cells L^{-1} (**Table 2**). In considering the main microplanktonic organisms present in the area, the centric diatoms from the sub-order Coscinodiscineae, dinoflagellates *Neoceratium* spp. and silicoflagellates (Class Dictyochophyceae) were fairly common across the region and in relatively high abundance (**Table 2**). Interestingly, the harpacticoid copepod *Macrosetella gracilis* was present along most of the stations with eggs and larvae attached to the *Trichodesmium* trichomes (**Figure 3a**). In the Southernmost stations, an unidentified pteropod mollusk appeared in high abundances (data not shown). Symbioses between microplanktonic organisms and N_2- fixers cyanobacteria were pretty common along the stations such as with Foraminifera members and the diatoms *Rhizosolenia* sp. and *Chaetoceros* sp. (**Figures 3b–d**).

The CCA analysis coupled with a Monte-Carlo test of the F-ratio associated the presence of microplankton groups correlated with environmental variables (**Figure 4**). Results indicated that five environmental variables (temperature, salinity, MLD, wind speed and water mass types) and the covariate latitude influenced significantly the spatial distribution of the different groups of microplankton ($p < 0.01$). In fact, environmental variability explained 44% of the community composition, and the first two significant canonical roots cumulatively explained 71.5% of the observed variance in taxa.

Taxonomy of *Trichodesmium* Based on Morphological Features

We identified 5 different species of *Trichodesmium* (Anagnostidis and Komárek, 1988; Komárek and Anagnostidis, 2005), which were mainly based on biometric parameters (length and width of cells within trichomes), as well as phenotypic features (presence/absence of calyptra and shape of apical cell). The

TABLE 2 | Abundance of microplankton groups (trichomes L^{-1} for Trichodesmium spp. and cells L^{-1} for others).

Taxa	Stations															
	#01	#02	#03	#04	#05	#06	#07	#08	#09	#10	#11	#12	#13	#14	#15	#16
CYANOBACTERIA																
Trichodesmium spp.	9×10^5	4×10^6	2×10^6	1×10^5	1180	263	4	13	936	8185	2×10^4	26	1138	2500	461	1504
Other oscillatoriales	268	108	90	30						17						9
DIATOMS																
Chaetoceros spp.	670			36			7			22				834		
Climacodiumfrauenfeldianum					35			10		65		5		194		
Coscinodiscineae	804				444	29	14	14	21	117	10	381	26	5592	9	120
Dactyliosolen sp.					91					4				139	5	
Hemiaulus sp.	1116		45		18		7		11	104			13	500	5	
Hemidiscus sp.					104									46		
Planktoniella sol	45				5					4						
Pleurosigma sp.	42		23	6						9				19		
Rhizosolenia spp.	357		67	30	36					26		10		56		9
Thalassionema spp.										9		100	13	472		86
DINOFLAGELLATES																
Amphisoleniabidentata	90			24												
Ceratocoryshorrida														9		
Dinophysis spp.														9		
Neoceratium spp.*	2411	54	402	269	194			13	11	294		30	10	2167	13	334
Ornithocercus spp.	268		67	6	9				16	65		5		65		9
Protoperidinium spp.	581	54	45	12										408		9
Pyrophacus sp.					22			5	11	43						
Schuetiella mitra																9
OTHERS																
Dictyochophyceae	45		23		9		14	5		22	5			83		
Ciliate										4				9		
Radiolarian					5					30	5			65		26
Foraminiferan	328		23	96	9					39				56		
Total abundance	9×10^5	4×10^6	2×10^6	1×10^5	2161	292	46	60	1006	9059	2×10^4	557	1200	1×10^4	493	2115

**N. concilians, N. contortum var. karstenii, N. extensum, N. fusus, N. horridum, N. pentagonum, N. praelongum, N. symmetricum, N. teres, N. trichoceros, N. vultur var. sumatranum.*

morphologically different forms generally co-occurred at the different stations; however, we did not differentiate the species composition for each station (**Figures 5**, **6**).

T. clevei (J. Schmidt) (Anagnostidis and Komárek, 1988) was found as single trichomes or in colonies containing 20–30 trichomes, with cylindrical and slightly coiled cells. Most of the trichomes were blue-green, but were also found as dark reddish. The cell diameter ranged from 5.9 to 7 μm and were longer than wide, with apical cell rounded and without calyptra. Their occurrence was already registered in Gulf of Siam and Australia, but this is the first time this species is described in South Atlantic waters. *T. erythraeum* (Ehrenberg ex Gomont) was found as both free-floating or in colonies as purple-red trichomes, constricted at the cross-walls that were gradually attenuated at the ends. Cells were 6–11 μm long, with the apical cell containing a distinctive calyptra. The species is documented as forming dense aggregations in tropical and subtropical oceans, including the Atlantic, and was already described in the present region (Rörig et al., 1998; Siqueira et al., 2006; Carvalho et al., 2008). *T. hildebrandtii* (Gomont) was easily distinguished among the other species because of the diameter ranging from 13 to 22 μm with significantly shorter cells. It was found as single trichomes or in colonies with a dark-reddish color, had attenuated cells at the ends and apical cells containing a calyptra. The species can be found in warm seas all over the world, but has not been previously described in South Atlantic Ocean. *T. radians* (Wille) (Golubié) had the most distinctive morphology, characterized by wavy trichomes that were either free or aggregated as star-shaped colonies (**Figure 6**). The cells were always blue-green with large gas vesicles and a cell diameter range of 6–9 μm, longer than wide. Apical cells were rounded and without calyptra. This specie is largely distributed among the oceans, and this is the first report for the South Atlantic waters. *T. thiebautii* (Gomont ex Gomont) was found as single trichomes or colonies that were partly rope-like contorted or radially-arranged. Cells were nearly isodiametric, not constricted at the cell walls, with rounded

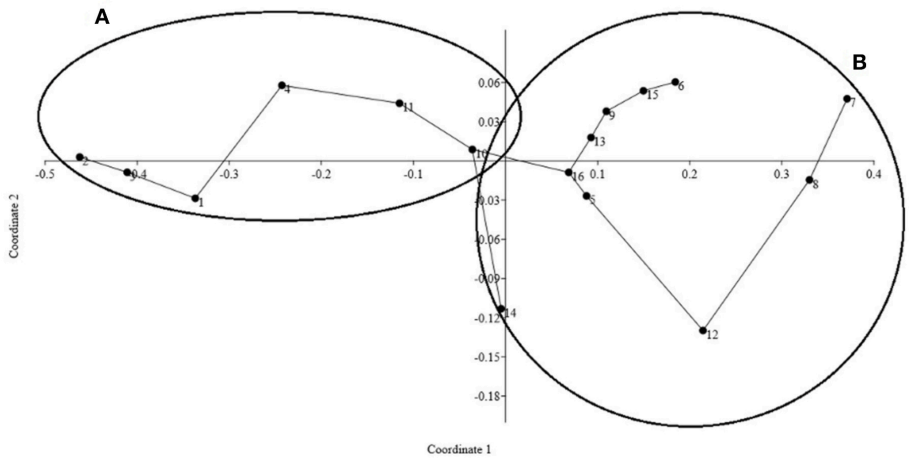


FIGURE 2 | nMDS ordination diagram dividing the stations into two distinctive groups according to *Trichodesmium* abundance of trichomes per liter. Group **(A)** where the stations presented > 8,000 trichomes L^{-1} and Group **(B)** contains stations with <2,500 trichomes L^{-1}. The ANOSIM test revealed statistical differences between the two groups: $p < 0.01$, $R^2 = 0.58$ and Stress = 0.04 (<0.5).

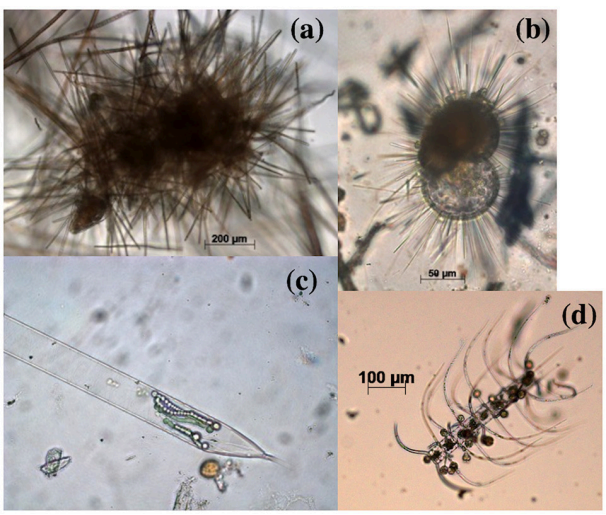


FIGURE 3 | Members of microplankton in association with other organisms found in the stations. Colonies of *Trichodesmium* with juveniles of *Macrosetella gracilis* **(a)**; foraminifera in association with picocyanobacteria **(b)**; symbiosis between *Rhyzosolenia sp.* and *Richelia sp.* **(c)**; and associations between a chain of *Chaetoceros sp.* and picocyanobacteria **(d)**.

apical cells. This species has a wide distribution range and was previously found offshore the Brazilian coast (Björnberg, 1965).

Toxicity of *Trichodesmium*

Our toxin analysis were negative for domoic acid and positive for STX concentrations in all samples analyzed (**Figure 7**). There was an inverse association between density of trichomes and STX concentrations. At the same time, *Trichodesmium* was dominant in terms of abundance for these stations (**Figure 1**). In relation to STX concentration, concentrations ranged from 0.45 to ∼4 (STX-eq) µg L^{-1}, with the qualified and quantified variants as gonyautoxins (GTX), specifically GTX-4 and GTX-2. The GTX-4 was the main variant contributing for total STX (STX-eq) toxicity.

DISCUSSION

The aggregations of *Trichodesmium* were observed during clear skies, calm seas and when wind speed was relatively low <10 knots; such conditions were reported elsewhere as suitable for trichome aggregations (Villareal and Carpenter, 2003; LaRoche and Breithbarth, 2005; Agawin et al., 2013), and this relationship is confirmed by statistical analysis (**Figure 4**). A noticeable feature observed at station 2 is that the aggregation was accompanied by floating objects such as feathers, wood and plastic. This suggests the objects and organisms were concentrating at the convergence region of a Langmuir cells, in agreement with previous observations elsewhere (Evans and Taylor, 1980). A shallow MLD of 17 m depth kept the trichome aggregations at surface, facilitated by gas vesicles inside the cells that give a positive buoyance to the trichomes (Capone et al., 1997; Villareal and Carpenter, 2003). Moreover, Since the genus is well-known to have very low growth rates ∼0.14 d^{-1} (LaRoche and Breithbarth, 2005), it was unlikely that trichomes formed blooms, such as harmful algal blooms (HABs).

Intermediate densities of *Trichodesmium* ∼8×10^3 trichomes L^{-1} (some stations from group B, **Figure 2**) could represent a transient condition before the mixed layer depth (MLD) stabilization and associated with wind speeds <8 knots, suitable for *Trichodesmium* aggregation and colony formation. On the other hand, the deepening of MLD might dilute *Trichodesmium* aggregations; in this case, our vertical net hauls of 30 m deep would not account for these organisms. The importance of MLD on the community structure was confirmed in the CCA's ordination diagram (**Figure 4**).

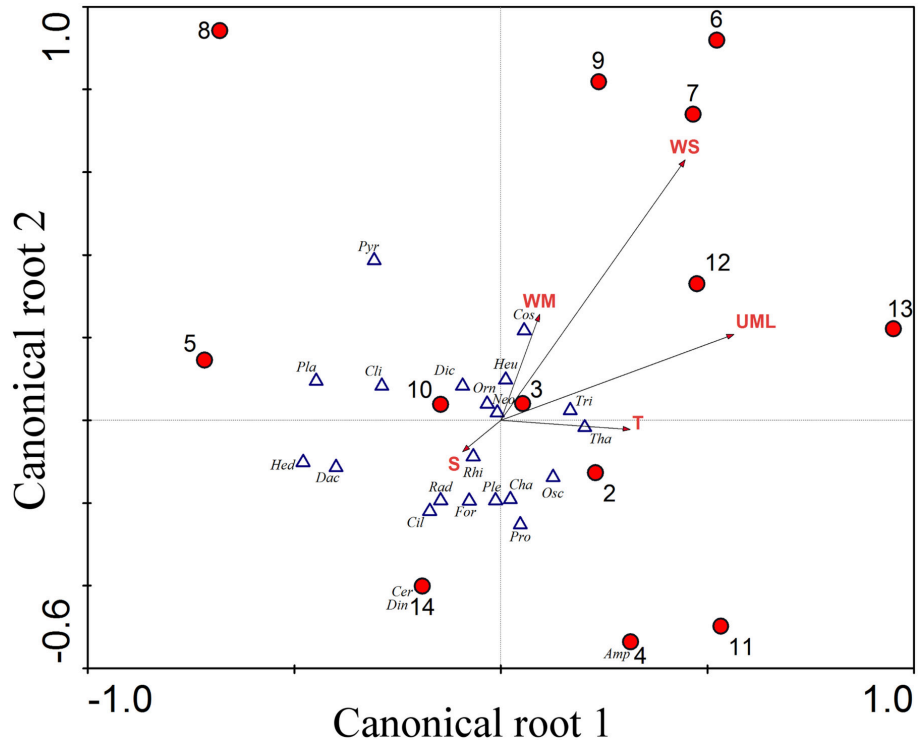


FIGURE 4 | Canonical Correspondence Analysis showing the relationship between the abiotic variables and density of organisms. The first two canonical roots represent 71.5% of microplankton groups vs. environment relationships (1st root explains 40.3% and 2nd root explains 31.2%). The red circles represent the stations, numbered from 1 to 16. For the abiotic variables, represented by arrows: MLD, Upper Mixed Layer; WS, Wind Speed; WM, Water Mass; S, Salinity; T, Temperature. For the taxa, represented by triangles: Tri, *Trichodesmium spp*, Osc, Other Oscillatoriales; Dia, *Diatoms*; Cha, *Chaetocerosspp*; Cli, *Climacodiumfrauenfeldianum*; Cos, *Coscinodiscineae*; Dac, *Dactyliosolen sp;* Heu, *Hemiaulus sp;* Hed, *Hemidiscus sp;* Pla, *Planktoniella sol*; Ple, *Pleurosigma sp;* Rhi, *Rhizosolenia spp;* Tha, *Thalassionema spp;* Amp, *Amphisoleniabidentata;* Cer, *Ceratocoryshorrida*; Din, *Dinophysis spp;* Neo, *Neoceratium spp;* Orn, *Ornithocercus spp;* Pro, *Protoperidinium spp;* Pyr, *Pyrophacus sp;* Sch, *Schuetiellamitra*; Dic, *Dictyochophyceae*; Cil, *Ciliate*; Rad, *Radiolarian;* For, *Foraminiferan*.

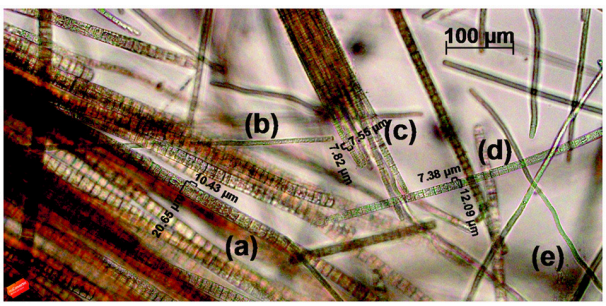


FIGURE 5 | Five species of *Trichodesmium* co-occurring in a sample (×200 of magnification): *T. hildebrandtii* **(a)**, *T. clevei* **(b)**, *T. thiebautii* **(c)**, *T. erythraeum* **(d)**, and *T. radians* **(e)**.

In stations where *Trichodesmium* was present in high densities, we found low densities of microphytoplankton co-occurring (**Table 2**; **Figure 1**). Inorganic nutrient concentrations through our study area, however, were present at high concentrations (Bif and Yunes, 2017) and do not support such low densities of microphytoplankton. From these organisms, typical representatives of open oceans were found at the stations, such as the dinoflagellates *Neoceratium* spp., *Dinophysis* spp. and *Ornithocercus* spp., and the heterotroph *Protoperidinium* spp. These genera have been classified as the major consumers of phytoplankton biomass, where *Protoperidinium* spp. is especially important in open oceans (Sherr and Sherr, 2007). Two genera were found in symbiosis with picocyanobacteria, as the case of *Ornithocercus* spp. and *Rhizosolenia* spp.; such associations were previously seen in other oceans (Madhu et al., 2013). The co-occurrence of *T. hildebrandtii* with other microplankton organisms was already registered in the southern Brazilian coast (Guimarães and Rörig, 1997), but were never detailed.

The presence of STX, thus, is another relevant factor that should possibly control the distribution of microplankton groups across stations. Based on the relationship between trichome densities and toxin production, we found STX (STX-eq) associated with the presence of *Trichodesmium* (**Figure 7**). Higher STX (STX-eq) concentrations were correlated with lower trichomes per liter; thus, we suggest that *Trichodesmium* produces the toxin during the early stage of trichome aggregation. STX are allelochemical compounds produced as secondary metabolites which are not required for metabolism (i.e., growth, development and reproduction), but are considered an important defense against herbivory (Fraenkel, 1959; Stamp, 2003). Our work suggests that STX could be play a central

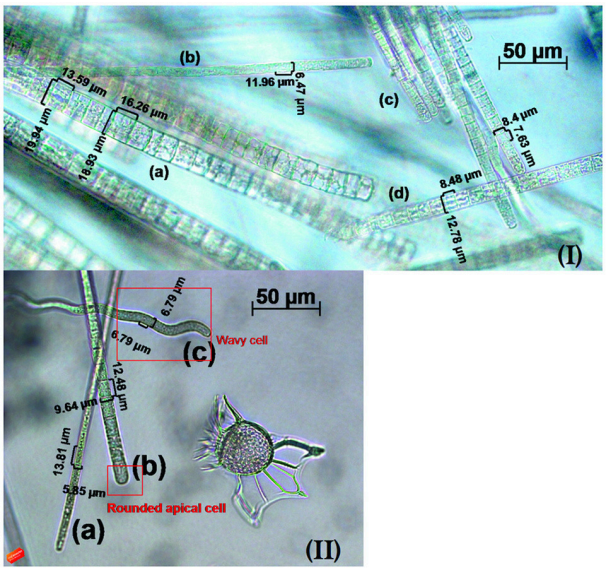


FIGURE 6 | Details of *Trichodesmium* trichomes co-occuring in the samples (×400 of magnification). In the first image **(I)** *T. hildebrandtii* **(a)**, *T. clevei* **(b)**, *T. thiebautii* **(c)**, and *T. erythraeum* **(d)**; in the second image **(II)** *T. clevei* **(a)**, a rounded apical cell in *T. thiebautii* **(b)** and the wavy trichome of *T. radians* **(c)**.

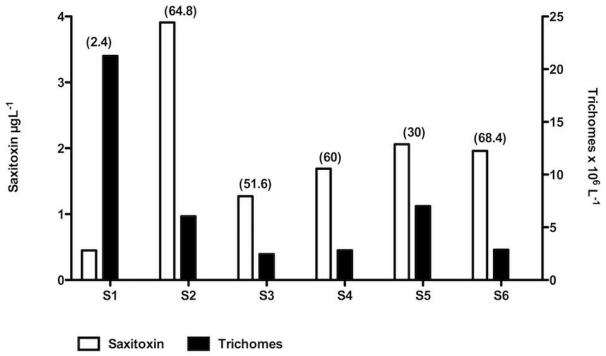


FIGURE 7 | Relationship between aggregations shown as density of trichomes per liter, and Saxitoxin concentrations in µg L^{-1}. The concentration of STX per trichome is shown in parenthesis as µg SXT per $trichome^{-1}$ × 10^{-8}. Aggregations are in order of stations; to a better comparison per density of trichomes: S1 > S5 > S2 > S4 > S6 > S3.

role during initial aggregations of *Trichodesmium* in order to minimize bottom-up (i.e., competition for nutrients) and top-down (i.e., herbivory) controls (Smayda, 1997). This is corroborated by the fact that other organisms were less abundant in the presence of *Trichodesmium*, but were dominant in regions where trichomes were not accumulating or forming colonies (**Figure 1**). Although we identified other potential toxin producers (i.e., STX and domoic acid) in the stations such as *Gonyaulax spinifera*, *Protoceratium reticulatum*, *Chaetoceros* spp., and *Pseudo-nitzschia seriata* (**Table 3**), they were not abundant enough to be responsible for the high toxin concentrations.

Despite the solely morphological/traditional identification of *Trichodesmium* performed in our work, previous studies already linked the traditional taxonomy with molecular approaches and found high agreement between techniques. Some approaches include 16S rRNA gene sequencing (Komárek, 2010), and genetic and biochemical informations that add to the development of a functional classification scheme (Hynes et al., 2012). A detailed description of *Trichodesmium* spp. species was never assessed for the Southwest Atlantic Ocean (Silva et al., 2008 and references therein), so the identification of three new species for the region (*T. clevei*, *T. hildebrandtii*, and *T. radians*) adds to the previous reports of *T. erythraeum* (e.g., Barbosa, 1944; Gianesella-Galvão, 1995; Rörig et al., 1998) and *T. thiebautii* (Björnberg, 1965). Nevertheless, we suggest that further local studies based on the polyphasic approach (i.e., using morphological, ultra-structural, biochemical and genetic features) are still necessary (Komárek et al., 2014).

From the five species of *Trichodesmium* found in the region, *T. erythraeum* and *T. thiebautii* are already known to be toxic to the environment. In ecological tests performed with both species collected and isolated from Caribbean waters, *T. thiebautii* was highly toxic for *Artemia salina* and copepods, and was neurotoxic to mice and some non-grazing copepods (Hawser and Codd, 1992; Hawser et al., 1992). *T. erythraeum* toxicity was assessed in populations from the Northeast of Brazil through HPLC, Elisa and ecotoxicological tests, and was positive for STX and microcystins (Proença et al., 2009). In this same region, the specie was found in co-occurrence with toxic dinoflagellates; but analagous to our study, the organisms were present in small abundances. The toxigenic potential of *T. erythraeum* was recently validated through brine shrimp toxicity assay, showing 100% of mortality after 48 h of incubations using aggregations from the Gulf of Mannar (Shunmugam et al., 2017). The study also demonstrated that *T. erythraeum* is able to synthesize other potent multi-class neurotoxins such as anatoxin-a and gonyautoxin, and microcystins. The toxicity of other *Trichodesmium* species, however, requires further investigation, and the Southwest Atlantic seems to be a good candidate to perform similar studies, given the diversity of *Trichodesmium* species and high abundance along the shelf break.

SUMMARY

The Southwest Atlantic Ocean shows suitable conditions for growth and colony formation of *Trichodesmium* spp. Five species of *Trichodesmium* (*T. clevei*, *T. erythraeum*, *T. hildebrandtii*, *T. radians,* and *T. thiebautii*) were identified, and three out five reported for the first time at the region. During non-*Trichodesmium*-dominated assemblages in the water surface, dinoflagellates and relatively large diatoms were the dominant groups. Our results on microplankton densities in the stations combined with toxin analysis revealed that STX was likely produced by *Trichodesmium* aggregations. Higher concentrations of toxins correlated with moderate numbers of trichomes per liter; lower concentrations were

TABLE 3 | Checklist of organisms found with *Trichodesmium* aggregations.

Taxa	Patch 2, 0 m #45	Patch 2, 15 m #46	Patch2, 0 m #24	Patch (net sample 6)
CYANOBACTERIA				
Trichodesmium spp. (abundance of trichomes and tufts)				
High	X		X	
Low		X		X
COCCOLITHOPHORES				
Umbilicosphaera sibogae				X
DIATOMS				
Bacteriastrum delicatulum				X
Bacteriastrum furcatum				X
Chaetoceros aequatorialis				X
Chaetoceros coarctatus				X
Chaetoceros compressus				X
Chaetoceros dadayi				X
Chaetoceros messanensis				X
Chaetoceros peruvianus				X
Chaetoceros tetrastichon				X
Climacodium frauenfeldianum				X
Corethron pennatum				X
Coscinodiscus sp.	X			
Cylindrotheca closterium	X			
Dactyliosolen spp.				X
Hemiaulus membranaceus	X			X
Hemiaulus sinensis				X
Leptocylindrus mediterraneus				X
Lioloma sp.				X
Naviculaceae <20 μm		X		
Nitzschia longissima			X	
Pleurosigma/ Gyrosigma				X
Proboscia alata	X	X	X	X
Pseudoguinardia recta				X
Pseudo-nitzschia complex *seriata* (t.a. >5 μm)				X
Rhizosolenia styliformis		X		
Rhizosolenia sp.	X	X	X	
Thalassionema bacillare				X
Thalassionema frauenfeldii		X		X
Thalassiosira sp.				X

(Continued)

TABLE 3 | Continued

Taxa	Patch 2, 0 m #45	Patch 2, 15 m #46	Patch2, 0 m #24	Patch (net sample 6)
DINOFLAGELLATES				
Gonyaulax spinifera				X
Gymnodiniales <20 μm		X		
Oxytoxum sp.	X			
Prorocentrum compressum		X		
*Protoperidinium conicum**				X
*Protoperidinium curtipes**				X
*Protoperidinium divergens**				X
*Protoperidinium leonis**				X
*Protoperidinium oblongum**				X
*Protoperidiniumovum**		X	X	X
*Protoperidinium pellucidum**				X
Protoperidinium sp.*	X			
Protoceratiumreticulatum	X			X
Ptychodiscus noctiluca				X
Pyrophacus sp.	X		X	X
Tripos concilians				X
Tripos furca				X
Tripos fusus				X
Tripos horridus				X
Tripos muelleri				X
Tripos teres				X
Tripos trichoceros				X
Cyst	X		X	
Association/symbioses				
*Rhizomonas setigera** + *Leptocylindrus* sp.			X	
Foraminifera*				X
CILIOPHORA*				
*Amphorella*sp.				X
Climacocylis cf. *scalaria*				X
Dadayiella ganymedes				X
Eutintinnus sp.				X
Favella sp.				X
Salpingella spp.				X
Tintinnopsis sp.				X
Undella sp.				X
Xystonella treforti				X
Xystonellopsis spp.				X
Zoothamnium sp.				X
Harpacticoida*				X
Nauplius and copepodites of *Macrosetella gracilis**	X		X	X

*Heterotrophic species.

present during high densities of the genus. The lack of microplankton diversity during *Trichodesmium* dominance might be associated with the toxin production, which seems to be especially important during the early aggregation phase at the surface.

AUTHOR CONTRIBUTIONS

MB developed the research, collected, and analyzed the biological and wrote the article as a result of her Master's Thesis. MdS helped with plankton identification and calculations. LC analyzed the toxins. JY helped with data interpretation. All authors revised and made contributions to methodology, results and discussion in this manuscript.

FUNDING

MB was funded by a Masters scholarship granted from CAPES. MdS and LC were funded by Post-Doc fellowship grants from CAPES. JY was funded by a productivity grant from CNPq. This study was partially funded by CNPq (Grant # 610012/2011-8) through the Brazilian National Institute of Science and Technology (INCT-Ma r-COI).

ACKNOWLEDGMENTS

The authors want to thank Talude project, granted by Chevron®, for the opportunity to collect samples during their cruise.

REFERENCES

Agawin, N. S., Tovar-Sánchez, A., de Zarruk, K. K., Duarte, C. M., and Agustí, S. (2013). Variability in the abundance of *Trichodesmium* and nitrogen fixation activities in the subtropical NE Atlantic. *J. Plankton Res.* 35, 1126–1140. doi: 10.1093/plankt/fbt059

Anagnostidis, K., and Komárek, J. (1988). Modern approach to the classification system of cyanophytes. 3. Oscillatoriales. *Arch. Hydrobiol. Suppl.* 80, 327–472.

Arun Kumar, M., Karthik, R., Sai Elangovan, S., and Padmavati, G. (2012). Occurrence of *Trichodesmium erythraeum* bloom in the coastal waters of South Andaman. *Int. J. Curr. Res.* 4, 281–284.

Barbosa, F. A. S. (1944). O "Tingui—febre de Tamandaré—uma doença desconhecida. *Rev. Bras. Med.* 1, 663–665.

Bif, M. B., and Yunes, J. S. (2017). Distribution of the marine cyanobacteria *Trichodesmium* and association with iron-rich particles in the Southwest Atlantic Ocean. *Aqua Microbial Ecol.* 78, 107–119. doi: 10.3354/ame01810

Björnberg, T. K. S. (1965). Observations on the development and the biology of the Miraciidae Dana (Copepoda: Crustacea). *Bull. Mar. Sci.* 15, 512–520.

Bray, J. R., and Curtis, J. T. (1957). An ordination of the upland forest communities of Southern Wisconsin. *Ecol. Monograph.* 27, 325–349. doi: 10.2307/1942268

Capone, D. G., Zehr, J. P., Paerl, H. W., Bergman, B., and Carpenter, E. J. (1997). Trichodesmium, a globally significant marine cyanobacterium. *Science* 276, 1221–1229. doi: 10.1126/science.276.5316.1221

Carpenter, E. J. (1983). "Nitrogen fixation by marine oscillatoria (*Trichodesmium*) in the world's oceans," in *Nitrogen in the Marine Environment*, eds E. J. Carpenter and D. G. Capone (New York, NY: Elsevier), 65–103.

Carvalho, M., Gianesella, S. M., and Saldanha-Corrêa, F. M. (2008). *Trichodesmium erythraeum* bloom on the continental shelf off Santos, Southeast Brazil. *Braz. J. Oceanogr.* 56, 307–311. doi: 10.1590/S1679-87592008000400006

Clarke, K. R. (1993). Non-parametric multivariate analyses of changes in community structure. *Austr. J. Ecol.* 18, 117–143. doi: 10.1111/j.1442-9993.1993.tb00438.x

Costa, P. R., Rodrigues, S. M., Botelho, M. J., and de M Sampayo, M. A. (2003). A potential vector of domoic acid: the swimming crab *Polybius henslowii* Leach (*Decapoda brachyura*). *Toxicon* 42, 135–141. doi: 10.1016/S0041-0101(03)00107-7

Detoni, A. M. S., Ciotti, A. M., Calil, P. H. R., Tavano, V. M., and Yunes, J. S. (2016a). *Trichodesmium* latitudinal distribution on the shelf break in the southwestern Atlantic Ocean during spring and autumn. *Global Biogeochem. Cycl.* 30, 1738–1753. doi: 10.1002/2016GB005431

Detoni, A. S., Pacheco, L. A., Costa, L. D. F., and Yunes, J. S. (2016b). Toxic *Trichodesmium* bloom occurrence in the southwestern South Atlantic Ocean. *Toxicon* 110, 51–55. doi: 10.1016/j.toxicon.2015.12.003

D'Silva, M. S., Anil, A. C., Naik, R. K., and D'Costa, P. M. (2012). Algal blooms: a perspective from the coasts of India. *Nat. Hazard* 63, 1225–1253. doi: 10.1007/s11069-012-0190-9

Ehrenberg, C. G. (1830). "Organisation, systematik, und geographisches verhältnis der infusionstierchen," in *Druckerei dei Koningliche Akademie der Wissenschaften*, ed F. Dümmler (Berlin) 108.

Evans, G. T., and Taylor, F. J. R. (1980). Phytoplankton accumulation in Langmuir cells. *Limnol. Oceanogr.* 25, 840–845. doi: 10.4319/lo.1980.25.5.0840

Fraenkel, G. S. (1959). The raison d'Etre of secondary plant substances. *Science* 129, 1466–1470. doi: 10.1126/science.129.3361.1466

Gianesella-Galvão, S. M. F, Costa, M. P. F and Kutner, M. B. B. (1995). "Bloom of Oscillatoria (*Trichodesmium*) erythreae (Ehr.) Kutz," in *coastal waters of the Southwest Atlantic.* (São Paulo: Publicações Especiais do Instituto Oceanográfico, USP-SP) Vol. 11, 133–140.

Guimarães, S. C. P., and Rörig, L. R. (1997). Microrganismos associados a florações da cianobactéria *Trichodesmium hildebrandtii* na costa de Santa Catarina. *Notas Técnicas FACIMAR* 1, 43–47.

Hammer, O., Harper, D. A. T., and Ryan, P. D. (2008). *PAST—Palaeontological STatistics, ver. 1.81. Brief Notes*. Available online at: http://folk.uio.no/ohammer/past

Hasle, G., and Syvertsen, E. (1996). "Marine diatoms," in *Identifying Marine Diatoms and Dinoflagellates*, ed C. Tomas (San Diego: Academic Press), 5–385.

Hawser, S. P., and Codd, G. A. (1992). "The toxicity of *Trichodesmium* blooms from caribbean waters," in *Marine Pelagic Cyanobacteria: Trichodesmium and other Diazotrophs. NATO ASI Series (Series C: Mathematical and Physical Sciences)*, Vol. 362, 319–329 eds E. J. Carpenter, D. G. Capone, and J. G. Rueter (Dordrecht: Springer).

Hawser, S. P., O'Neil, J. M., Roman, M. R., and Codd, G. A. (1992). Toxicity of blooms of the cyanobacterium *Trichodesmium* to zooplankton. *J. Appl. Phycol.* 4, 79–86. doi: 10.1007/BF00003963

Hynes, A. M., Webb, E. A., Doney, S. C., and Waterbury, J. C. (2012). Comparison of cultured *Trichodesmium* (Cyanophyceae) with species characterized from the field. *J. Phycol.* 48, 196–210. doi: 10.1111/j.1529-8817.2011.01096.x

Karl, D., Michaels, A., Bergman, B., and Capone, D., et al. (2002). Dinitrogen fixation in the world's oceans. *Biogeochemistry* 57, 47–98. doi: 10.1023/A:1015798105851

Komárek, J. (2010). Modern taxonomic revision of planktic nostocacean cyanobacteria: a short review of genera. *Hydrobiologia* 639, 231–243. doi: 10.1007/s10750-009-0030-4

Komárek, J., and Anagnostidis, K. (2005). "*Cyanoprokaryota II*. Teil Oscillatoriales,"in *SüBwasserflora von Mitteleuropa*, eds B. Büdel, L. Krienitz, G. Gärtner, and M. Schagerl (Munchen: Elsevier Gmbh) 19:750.

Komárek, J., Kaštovský, J., Mareš, J., and Johansen, J. R. (2014). Taxonomic classification of cyanoprokaryotes (*Cyanobacterial genera*), using a polyphasic approach. *Preslia* 86, 295–335.

LaRoche, J., and Breithbarth, E. (2005). Importance of the diazotrophs as a source of new nitrogen in the ocean. *J Sea Res.* 53, 67–91. doi: 10.1016/j.seares.2004.05.005

Madhu, N. V., Meenu, P., Ullas, N., Ashwini, R., and Rehitha, T. V. (2013). Occurrence of cyanobacteria (*Richelia intracellularis*)—diatom (*Rhizosolenia hebetata*) consortium in the Palk Bay, southeast coast of India. *Indian J. Mar. Sci.* 42, 453–457.

Miguez, A., Fernandez,M. L., and Fraga, S. (1996). "First detection of domoic acid in Galicia (NW of Spain)," in *Harmful and Toxic Agal Blooms,* eds T. Yasumoto, Y. Oshima, and Y. Fukuyo (Paris: IOC-UNESCO), 143–145.

Möller, O. O. Jr., Piola, A. R., Freitas, A. C., and Campos, E. J. D. (2008). The effects of river discharge and seasonal winds on the shelf off southeastern South America. *Continent. Shelf Res.* 28, 1607–1624. doi: 10.1016/j.csr.2008.03.012

Monteiro, J. J. F., Leça, E. E., Koening, M. L., and Macedo, S. J. (2010). New record of *Trichodesmium thiebautii* Gomont ex Gomont (Oscillatoriales-Cyanophyta) for the continental shelf of northeast Brazil. *Acta Bot. Bras.* 24, 1104–1106. doi: 10.1590/S0102-33062010000400027

Monteiro, J. J. F., Leça, E. E., Koening, M. L., and Macedo, S. J. (2012). Distribution and annual variation of *Trichodesmium thiebautii* Gomont Ex Gomont (Oscillatoriales - cyanophyta) in tropical waters of northeastern Brazil (Western Atlantic). *Trop. Oceanogr.* 40, 319–326. doi: 10.5914/tropocean.v40i2.5409

O'Neil, J. M., Metzler, P. M., and Glibert, P. M. (1996). Ingestion of $^{15}N_2$-1abelled *Trichodesmium* spp. and ammonium regeneration by the harpacticoid copepod *Macrosetella gracilis*. *Mar. Biol.* 125, 89–96.

Proença, L. A. O., Tamanaha, M. S., and Fonseca, R. S. (2009). Screening the toxicity and toxin content of blooms of the cyanobacterium Trichodesmium erythraeum (Ehrenberg) in northeast Brasil. *J. Venomous Anim. Toxins Trop. Dis.* 15, 204–215. doi: 10.1590/S1678-91992009000200004

Rörig, L. R., Yunes, J. S., Kurohsima, K. N., Schetinni, C. A. F., Pezzuto, P. R., and Proença, L. A. O. (1998). "Studies on the ecology and toxicity of *Trichodesmium* spp. blooms in southern Brazilian coastal waters," in *Harmful Algae. Proceedings of the VII International Conference on Harmful Alga*, eds B. Reguera, J. Blanco, M. L. Fernández, and T. Wyatt (Vigo).

Rourke, W. A., Murphy, C. J., and Pitcher, G., van de Riet, et al. (2008). Rapid postcolumn methodology for determination of paralytic shellfish toxins in shellfish tissue. *J. AOAC Int.* 91, 589–597.

Sherr, E. B., and Sherr, B. F. (2007). Heterotrophic dinoflagellates: a significant component of microzooplankton biomass and major grazers of diatoms in the sea. *Mar. Ecol. Prog. Ser.* 352, 187–197. doi: 10.3354/meps07161

Shunmugam, S., Gayathri, M., Prasannabalaji, N., Thajuddin, N., and Muralitharan, G. (2017). Unraveling the presence of multi-class toxins from Trichodesmium bloom in the Gulf of Mannar region of the Bay of Bengal. *Toxicon* 135, 43–50. doi: 10.1016/j.toxicon.2017.06.003

Silva, L. M. (2005). *Ocorrência de Cianobactérias No Estuário e Costa Adjacente à Lagoa dos Patos, Rio Grande, RS: Avaliação Preliminar dos Riscos a Balneabilidade*. Master Dissertation. Programa de Pós-Graduação em Oceanografia Biológica (Rio Grande, TX: FURG), 144.

Silva, L. M., Matthiensen, A., and Yunes, J. S. (2008). "Florações de *Trichodesmium* na costa brasileira," in *Poluição Marinha*, eds J. A. Baptista Neto, M. Wallner-Kersanach, S. M. Patchineelam (Rio Grande, TX: Editora Interciência), 3–19.

Siqueira, A., Kolm, H., and E; Brandini, F. P. (2006). Offshore distribution patterns of the cyanobacterium *Trichodesmium erythraeum* ehrenberg and associated phyto and bacterioplankton in the southern atlantic coast (Paraná-Brazil). *Braz. Arch. Biol. Technol.* 49, 323–337. doi: 10.1590/S1516-89132006000300018

Smayda, T. J. (1997). Harmful algal blooms: their ecophysiology and general relevance to phytoplankton blooms in the sea. *Limnol. Oceanogr.* 42, 157–1153. doi: 10.4319/lo.1997.42.5_part_2.1137

Srinivas, B., and Sarin, M. M. (2013). Atmospheric deposition of N, P and Fe to the Northern indian ocean: implications to C-and N-fixation. *Sci. Total Environ.* 456, 104–114. doi: 10.1016/j.scitotenv.2013.03.068

Stamp, N. (2003). Out of the quagmire of plant defense hypotheses. *Q. Rev. Biol.* 78, 23–55. doi: 10.1086/367580

Steindinger, T. (1996). "Dinoflagellates," in *Identifying Marine Phytoplankton*, ed C. R. Tomas (San Diego, CA: Academic Press), 387–584.

TerBraak, C. J. F., and Prentice, I. C. (1988). A theory of gradient analysis. *Adv. Ecol. Res.* 18, 271–317. doi: 10.1016/S0065-2504(08)60183-X

Utermöhl, H. (1958). Zurvervollkommnung der quantitativen phytoplankton-methodik. *Mitt Int. Vertheorangew. Limnol.* 9, 1–38.

Villareal, T. A., and Carpenter, E. J. (2003). Buoyancy regulation and the potential for vertical migration in the oceanic cyanobacterium *Trichodesmium*. *Microbial Ecol.* 45, 1–10. doi: 10.1007/s00248-002-1012-5

Westberry, T. K., and Siegel, D. A. (2006). Spatial and temporal distribution of *Trichodesmium* blooms in the world's oceans. *Global Biogeochem. Cycl.* 20. doi: 10.1029/2005GB002673

Wickelmaier, F. (2003). *An Introduction to MDS*. Alaborg: Sound Quality Research Unit at Alaborg University.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Bif, de Souza, Costa and Yunes. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Marine Science

ORIGINAL RESEARCH
published: 07 February 2019
doi: 10.3389/fmars.2019.00024

Salinity-Growth Response and Ichthyotoxic Potency of the Chilean *Pseudochattonella verruculosa*

Jorge I. Mardones[1], Gonzalo Fuenzalida[1], Katherine Zenteno[2], Catharina Alves-de-Souza[3], Allisson Astuya[4] and Juan José Dorantes-Aranda[5]*

[1] Centro de Estudios de Algas Nocivas, Instituto de Fomento Pesquero, Puerto Montt, Chile, [2] Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile, [3] Algal Resources Collection, MARBIONC, Center for Marine Sciences, University of North Carolina Wilmington, Wilmington, NC, United States, [4] Laboratorio de Cultivo Celular y Genómica Marina, Departamento de Oceanografía, Facultad de Ciencias Naturales y Oceanográficas y Centro de Investigación Oceanográfica COPAS Sur-Austral de la Universidad de Concepción, Concepción, Chile, [5] Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, TAS, Australia

Despite salmon farmers suffering the worst damage from a harmful algal bloom in Chile's history (US$800M) due to a massive outbreak of the dictyochophyte *Pseudochattonella verruculosa* in 2016 (~7000–20,000 cells ml^{-1}), the effect of environmental drivers and the potency of lytic toxins produced by the local clones of this species remain still largely unexplored. Based on the drastic oceanographic anomalies observed in the Chilean fjords during the 2016-El Niño "Godzilla" event, the role of salinity (15 to 35 psu) on *Pseudochattonella* cell growth and cytotoxicity was studied by culturing, scanning electron microscopy (SEM) and using a rainbow trout cell line RTgill-W1 assay to define: (1) vegetative growth rates, (2) cell taxonomy, (3) ichthyotoxicity of monoclonal cultures at 25 and 35 psu in salinity, (4) differences in toxicity of lysed cells and supernatant at different cell concentrations (from 10 to 100,000 cells ml^{-1}), and (5) temporal stability of lytic compounds. This study formally confirms the presence of *P. verruculosa* in Chilean waters using the large subunit (LSU) of the nuclear ribosomal RNA gene. The Chilean *P. verruculosa* ARC498 strain showed maximum cell densities at 30 psu (max. 84,333 ± 4,833 cells ml^{-1}) and maximum growth rates (μ_{max}) at 20 psu (1.44 cells d^{-1}). Cultures at 15 psu showed suppressed maximum cell density (max. 269 ± 71 cell ml^{-1}) but high μ_{max} were recorded at the beginning of the exponential growth (1.31 cells d^{-1}). No significant differences were observed between lysed cells and supernatant treatments in the two salinity levels, suggesting that the most lytic portion is released into the cell-free media instead of remaining cell bound. Cytotoxicity was correlated to cell abundance, reducing gill cell viability down to 80 and 65% compared to controls at 10,000 and 100,000 cells ml^{-1}, respectively. Unexpectedly, lytic compounds from *P. verruculosa* ARC498 at 35 psu showed to be less toxic than cultures at 25, where a noticeable presence of peripheral mucocysts were observed by SEM. Lytic compounds from *in vitro* experiments are weakly toxic even at high cell concentrations, highly unstable and rapidly degraded in the light after 5 days of storage at 15°C. Our results point to the important effect of salinity on growth and ichthyotoxic potency of *Pseudochattonella* species and highlight the need for a deeper insight into the role of mucocysts in fish gill damage, which would provide a greater understanding as to the harmful modes of action of this species.

Keywords: molecular identification, fish-kills, fish cell line assay, ichthyotoxicity, Chilean fjords

OPEN ACCESS

Edited by:
Stelios Katsanevakis, University of the Aegean, Greece

Reviewed by:
Jose Luis Iriarte, Universidad Austral de Chile, Chile
Sandra Lage, Swedish University of Agricultural Sciences, Sweden

****Correspondence:***
Jorge I. Mardones
jorge.mardones@ifop.cl

Specialty section:
This article was submitted to Marine Ecosystem Ecology, a section of the journal Frontiers in Marine Science

Received: *02 August 2018*
Accepted: *18 January 2019*
Published: *07 February 2019*

Citation:
Mardones JI, Fuenzalida G, Zenteno K, Alves-de-Souza C, Astuya A and Dorantes-Aranda JJ (2019) Salinity-Growth Response and Ichthyotoxic Potency of the Chilean Pseudochattonella verruculosa. Front. Mar. Sci. 6:24. doi: 10.3389/fmars.2019.00024

INTRODUCTION

Harmful Algal Blooms (HABs) have become an important threat for coastal aquaculture in the last decades (Hallegraeff et al., 2017). The more severe harmful effects occur when some microalgae produce toxic secondary metabolites that display potent biological activity against a wide variety of marine organisms or in humans upon consumption of contaminated seafood (Skjelbred et al., 2011). Among the ∼80 species described as having the capacity to produce toxins, flagellates are recognized as the most harmful group (Wright and Cembella, 1998; Hallegraeff, 2003).

The heterokont genus *Pseudochattonella* encompass marine ichthyotoxic phytoflagellates recently added to the list of fish killing microalgae (Eckford-Soper and Daugbjerg, 2016b). Originally described as *Chattonella verruculosa* (Raphidophyceae) by Hara et al. (1994), molecular genetic analysis later on erected a new genus and *Pseudochattonella verruculosa* (Y. Hara and Chihara) Hosoi-Tanabe, Honda, Fukaya, Otake, Inagaki and Sako, was transferred to the class Dictyochophyceae (Hosoi-Tanabe et al., 2007). Subsequently a second species, *Pseudochattonella farcimen* (Eikrem, Edvardsen, and Throndsen), was assigned to this genus (Edvardsen et al., 2007; Eikrem et al., 2009). Blooms of *Pseudochattonella* species have caused several fish kills in Northern Europe (Lu and Goebel, 2000; Naustvoll et al., 2002; Edvardsen et al., 2007; Riisberg and Edvardsen, 2008), Japan (Yamamoto and Tanaka, 1990; Baba et al., 1995; Imai et al., 1998), New Zealand (MacKenzie et al., 2011; Chang et al., 2014) and Chile (Mardones et al., 2012; Clément et al., 2016; León-Muñoz et al., 2018).

The first reported bloom of *Pseudochattonella* sp. in Chilean waters occurred on January of 2004 in the locality of Cholgo – Los Lagos region (Mardones et al., 2012). *Pseudochattonella* sp. has since then been held responsible for several salmon mortality events in January and February of 2005, 2009, 2011, and in March 2016 (Mardones et al., 2012; Clément et al., 2016; León-Muñoz et al., 2018). The last bloom was the more massive one, extending several Km^2 in the inner zone of fjords and channels and causing the worst damage from HABs on Chilean salmon industry so far. Losses for the salmon industry in this occasion were calculated in 100,000 metric tons of Atlantic and Coho salmon and trout, equivalent to an export loss of US$800 million (15% of Chile's yearly production) (Clément et al., 2016). This massive salmon mortality event evidenced the high fish-killing potency of the Chilean *Pseudochattonella* species, however, its ichthyotoxic mechanism in not yet understood.

Research on *Pseudochattonella* spp. toxicity has proven to be difficult and has led to different opinions. Some studies have shown that cell culture extracts can produce a toxic effect on planktonic organisms and cell lines (Skjelbred et al., 2011; Chang et al., 2014), whereas studies conducted by Andersen et al. (2015) suggested that live cells are required to induce a toxic effect on fish. The latter hypothesis relates the toxicity of *Pseudochattonella* spp. to the presence of peripheral saccular extrusomes called "mucocysts" that give these algae their characteristic warty appearance. The ecological function of the mucocysts is not clear but it has been suggested their implication as a mechanism to capture bacteria (Jeong et al., 2010) or as grazer deterrents (Tillmann and Reckermann, 2002).

The Chilean fjords are characterized by freshwater inputs from precipitation and melt water from Patagonian ice fields, which results in strong haline vertical gradients creating a unique highly productive area (Rignot et al., 2003). These stratified systems act as a barrier against the vertical propagation of turbulence from adjacent high mixing layers allowing for vertically heterogeneous phytoplankton distributions (Stacey et al., 2007; Yamazaki et al., 2010). Horizontally extensive subsurface patches of phytoplankton, the so-called "thin layers," have been observed to persist from hours to weeks and can contain more than 75% of the total microalgae biomass in the water column (Holliday et al., 2003; Sullivan et al., 2010). Interestingly, key target HAB species in the northern Patagonian waters, such as *Dinophysis* spp. and the ichthyotoxic *Pseudochattonella* sp., have been observed in subsurface 'thin layers' (Alves-de-Souza et al., 2014; Clément et al., 2016). This fact points to the crucial role of salinity, as well as, nutrient availability and fluctuations in pCO_2/pH for HAB formation in these highly heterogeneous estuarine systems.

The 2016 bloom seemed to be related to exceptional ocean and inshore water conditions in late 2015 due to a strong El Niño event and the positive phase of the Southern Annular Mode that altered the atmospheric circulation in the adjacent Pacific Ocean (León-Muñoz et al., 2018). The resultant increase in surface water temperature (>15°C) and reduced freshwater input allowed the advection of more saline (∼33–35 in salinity) and nutrient-rich offshore waters into the fjords. These changes toward relatively high salinity in the Reloncaví Sound (25–27 psu) were observed prior to the bloom in February. By mid-March there was an increase in salinity (>30 psu) and a gradual drop in temperature, probably associated to lower river streamflow, which could have stimulated the massive *Pseudochattonella* bloom (León-Muñoz et al., 2018). The maximum cell concentrations during the event were estimated to be between 7,000 and 20,000 cells ml^{-1} (Clément et al., 2016; León-Muñoz et al., 2018), several orders of magnitude higher of those recorded in previous *Pseudochattonella* sp. blooms in the area (Mardones et al., 2012). It has been suggested that high salinity variations in estuarine systems can strongly alter microalgae composition (Kirst, 1990), therefore this variable could have played key role in the proliferation, as well as, ichthyotoxic potency of *Pseudochattonella* sp. in the 2016 bloom event. However, ecophysiological studies on Chilean strains have not been yet undertaken since cultures of this species were only established after 2016 (Paredes et al., 2016; this study).

The aim of this study was to officially confirm the taxonomic identification of the Chilean *Pseudochattonella* populations, as well as, to provide the first assessment of growth parameters of species of this genus under different salinity levels. The potential lytic activity of *Pseudochattonella* species was also tested in intra- and extracellular metabolites using a fish gill cell line assay, as this has important implications for their mechanistic effects.

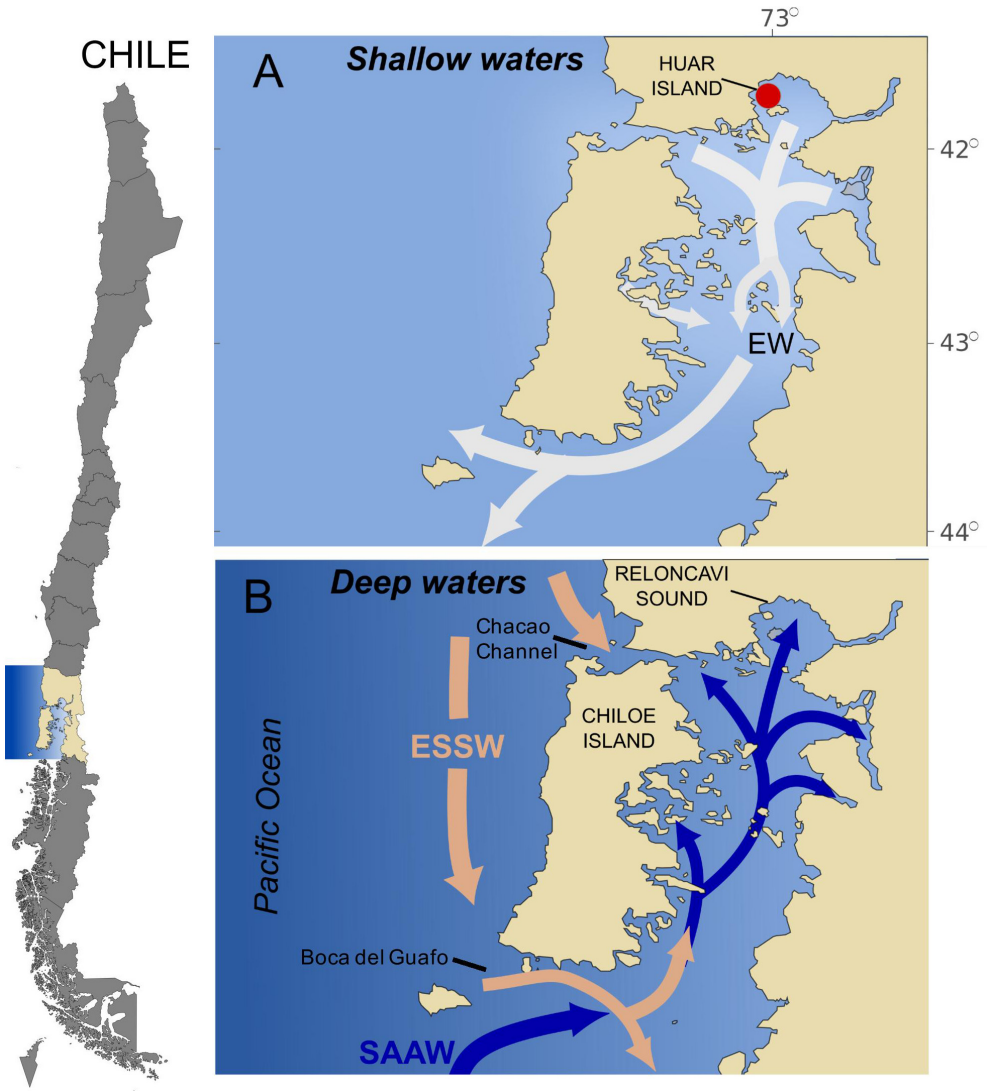


FIGURE 1 | Study area showing a schematic horizontal circulation model by layers (adapted from Silva et al., 1998). **(A)** Surface Estuarine Water EW (0–∼30 m); **(B)** intermediate Subantarctic Water (SAAW) (∼30–∼150 m) and deep Equatorial Subsurface Water (ESSW) (∼150 m to the bottom). The red circle shows the origin of the *Pseudochattonella* ARC 498 strain.

MATERIALS AND METHODS

Study Area

In the northern Patagonian fjords (42–44°S), where *Pseudochattonella* sp. bloom events have been registered, the vertical distribution of water masses presents a three-layer structure. A surface layer of Estuarine Waters (EW) (EW-Marine 21 to 31 psu; EW-Brackish 11 to 21 psu and EW-Freshwater 2 to 11 psu) between 0 and 20–30 m moves offshore increasing its salinity compared to fresh water sources (**Figure 1A**). An intermediate layer of Sub-Antarctic Waters (SAAW ∼33 psu) between 30 and 150 m enters the inner part of the fjords from the south. The mix between EW and SAAW in the inner part of the fjords is called Modified Sub-Antarctic Waters (MSAAW). A deep layer of remnants of Equatorial Subsurface Waters (ESSW > 33) from 150 m to the ocean bottom enters the inland sea of Chiloé through the Chacao channel and Boca del Guafo (**Figure 1B**; Silva et al., 1998). According to Castillo et al. (2016), the waters in the Reloncaví fjord are dominated along the seasons by EW in the upper layer and MSAAW in the deep layer.

Culture Origin and Maintenance

Algal Culturing

A monoclonal culture of the toxic dictyochophyte *Pseudochattonella verruculosa* ARC498 strain was isolated from the Reloncaví sound during the 2016 bloom, kept in culture in the Algal Resources Collection at the University of North Carolina Wilmington and later transferred to the CREAN-IFOP laboratory to perform this study. Non-axenic cultures were grown in F/2 medium (Guillard and Ryther, 1962) at 15°C

in sterile filtered (0.22 μm) seawater (25 psu) at 20 μmol photon m^{-2} s^{-1} (cool white fluorescent lamps) under a 12:12 h light:dark cycle.

Gill Cell Line

The cell line RTgill-W1, originally cultured from gill filaments of rainbow trout *Oncorhynchus mykiss* (Bols et al., 1994), was acquired from the American Type Culture Collection (CRL-2523, ATCC). Cells were cultured at 17°C in the dark in Leibovitz's L-15 medium (L1518 Sigma), supplemented with 10% (v/v) fetal bovine serum (FBS, 12003C, Sigma), and an antibiotic–antimycotic solution (A5955, Sigma) containing amphotericin B (25 mg ml^{-1}), streptomycin (10 mg ml^{-1}) and penicillin (10,000 units ml^{-1}) in 25-cm^2 culture-treated flasks (3100–025, Iwaki). 0.25% trypsin–0.02% EDTA in Hank's balanced salt solution (59428C, Sigma) was used to detach cells that grew as an adherent monolayer at the bottom of the flask. Subcultures were normally established twice per week at a ratio of 1:2 with L-15 medium renewal.

Molecular Identification of *P. verruculosa*

Cells from monoclonal cultures of the ARC498 strain (GenBank accession number MK106355.1) were pelleted by centrifugation at 1500 *g* for 10 min at 4°C, the supernatant discarded and the cell pellets incubated at 65°C for 3 h in 1 ml CTB buffer and 10 μl Proteinase K (10 mg/ml). The LSU (D1-D3) region of the rDNA gene was amplified using the primers D1R and D2C (Scholin et al., 1994; Edvardsen et al., 2003) in a SimpliAmp Thermo-Cycler (Applied Biosystems). The PCR protocol was as follow: initial denaturation step of 95°C for 1 min, followed by 35 cycles of 95°C for 30 s, 56°C for 30 s, 72°C for 30 s, followed by a 7 min extension at 72°C. PCR products were visualized on a 1.5% agarose gel and the product was purified using the Illustra GFX PCR DNA and gel band purification kit (GE Healthcare), and sequenced in both directions in Macrogen Korea. LSU rDNA sequences were aligned along with sequences available in GenBank using MUSCLE (Edgar, 2004), implemented in Geneious (Biomatters Ltd.). Unalienable regions were excluded from the LSU alignment. jModelTest (Posada, 2008) was used to identify appropriate models of sequence evolution (LSU: GTR + I), and maximum likelihood analyses were conducted using PhyML (Guindon et al., 2010). Support values were estimated using Likelihood Ratio Test (aLRT, Anisimova and Gascuel, 2006) and bootstrap analysis (1000 replicates). Bayesian analyses were conducted using MrBayes V3.1.2 (Ronquist and Huelsenbeck, 2003) using the appropriate model (GTR + I).

Morphological Characteristics and Growth of *P. verruculosa* Cultures

Scanning Electron Microscopy (SEM) of *P. verruculosa*

Scanning electron microscopy analysis was performed following Botes et al. (2002). Briefly, a *P. verruculosa* culture was fixed 1:1 with 2% osmium tetroxide (OsO_4) made up with filtered seawater. The 1% solution of fixed cells and OsO_4 was applied on coverslips coated with poly-L-lysine (P-4832, Sigma) and left for ∼30 min for *P. verruculosa* cells attachment. Attached cells were washed by submerging the coverslips for 10 min in a 1:1 solution of distilled water and filtered seawater, followed for a 10-min wash in distilled water. The coverslips were then taken through a graded ethanol series (30, 50, 70, 80, 90, 95, and 100%, 10 min each step). Following removal from the 100% ethanol, few drops of hexamethyldisilazane (HMDS) were immediately added onto the coverslips to substitute critical point drying (CPD). Samples were coated with gold-palladium (60:40) and viewed with a JEOL JSM 6380LV SEM.

Pseudochattonella verruculosa Growth

Exponentially growing cultures of *P. verruculosa* ARC498 were acclimated to the target salinity treatments of 15, 20, 25, 30, and 35 psu for 3 weeks prior to the experiments (corresponding to 10 generations). Pre-acclimated cultures were inoculated into sterile 60 ml polystyrene culture flasks containing 50 ml of F/2 media adjusted to the target salinity conditions. All treatments were performed in three replicates with an initial cell density of 500 cells ml^{-1}. The light intensity of 20 μmol photon m^{-2} s^{-1} was chosen as it was considered the optimum saturation irradiance for the Chilean ARC498 *P. verruculosa* growth from pre-experimental measurements (data not shown). Thus, the growth rates obtained at the five salinity treatments correspond to maximum rates. The experiment was carried out for 27 days with samples taken every 3–7 days.

Cells concentration was determined immediately after sampling, based on buffered-Lugol's fixed cells quantified under an inverted light microscope (Olympus, CKX41) using a Sedgewick-Rafter chamber. In every sampling date, the mean cell number obtained from the three replicates was used to calculate the growth rate μ (d^{-1}):

$$\mu = \frac{ln\,(c_1/c_0)}{t_1 - t_0} \quad (1)$$

were c_o and c_1 are the cell concentrations (cell ml^{-1}) at the beginning (t_0) and end (t_1) of the incubation period (days), respectively. At the end of the experiment, all cultures were left for 15 days under the original experimental conditions to observe the behavior of multinucleated cell aggregations as described by Tomas et al. (2004). These cell aggregations are believed to serve as resting stages and can potentially release a huge number of daughter cells that could alter the *in vitro* estimation of growth rates due to a sudden cell increase.

Pseudochattonella Cytotoxicity Against Fish Gill Cells

The cytotoxicity assay with *P. verruculosa* ARC498 was carried out using conventional multiwell microplates according to Dorantes-Aranda et al. (2011). Cultures with confluent gill cells were trypsinized (59428C, Sigma) for detachment, counted using a hemocytometer and adjusted to a concentration of 2×10^5 cell ml^{-1} in L-15 medium. Subsequently, gill cells were seeded in quadruplicate in 96-well flat bottom microplates (3860-096, Iwaki, Japan), using a volume of 100 μl per well. After 48 h at 17°C in the dark for gill cell attachment, L-15 medium was discarded, the cells rinsed with PBS and exposed to 100 μl

Pseudochattonella lysed cells and supernatant medium from cultures at 25 and 35 psu at 17°C in the dark. A salinity of 25 corresponds to a value measured above the pycnocline during the 2016 El Niño event in the Reloncaví Sound (Clément et al., 2016), and 35 psu is consistent with salinity values measured in the offshore Equatorial Subsurface Water (ESSW) (Silva et al., 1998). Supernatant and lysed cells treatments were prepared from *Pseudochattonella* cultures in the exponential growth phase at five different concentrations (10, 100, 1000, 10,000, and 100,000 cells ml^{-1}). The supernatant suspension was prepared by centrifugation of cultures for 5 min at 3500 rpm and then diluted as needed. The lysed cell suspension was prepared by sonication of diluted samples for 10 min at an amplitude of 10 μm peak to peak at 17°C and filtered using a syringe with a nylon filter (0.22 μm). After 1 h exposure, the viability of the gill cells was determined using L-15/ex medium (Schirmer et al., 1997), a modified version of the L-15 medium, containing 5% of the indicator dye alamarBlue (DAL1025, Invitrogen) (Pagé et al., 1993). The medium containing the indicator dye was added to all cell-seeded wells and incubated for 2 h in the dark (Dayeh et al., 2005). Using a microplate reader (FLUOstar OMEGA, BMG Labtech), the fluorescence signal of alamarBlue was detected using excitation and emission filters of 540 and 570 nm, respectively. The viability of the gill cells was expressed as response percentage of the treatments relative to the controls (% of control).

Data Analysis

To explore the effect of salinity on growth and the ichthyotoxic potency of *P. verruculosa*, analysis of variance (ANOVA) from simple linear regression models on maximum cell density, maximum specific growth rate (μ_{max}) and gill cell viability against *Pseudochattonella* cell density and intra- and extracellular compounds was performed. Normality and homogeneity of variances were assessed by the Kolmogorov-Smirnov method and Levene's test. A *post hoc* analysis using a Tukey HSD test was performed to determine differences among treatments. The null hypothesis (no difference in responses) was rejected in all statistical analyses if the respective p-value was <0.05. These analyses were performed using the software R v. 3.0.1 (Ihaka and Gentleman, 1996[1]).

RESULTS

Molecular Identification of *Pseudochattonella*

The 250 bp partial LSU rDNA sequence obtained from *P. verruculosa* ARC498 was identical to six sequences attributed to *P. verruculosa* (AB217643.1, AM850226.1, AM850225.1, AM850224.1, AB217642.1, and AM040504.1), and differed from sequences of *Pseudochattonella farcimen* by 4–5 substitutions. Phylogenetic trees obtained from these sequences and outgroup rooted with relatives of *Pseudochattonella* species demonstrate this pattern and give a relative scale of variation between the Chilean strain and other *P. verruculosa* sequences as well as *P. farcimen*. The LSU tree shows the Isla Huar isolate resolved in the *P. verruculosa* clade, although not as a monophyletic group (**Figure 2**).

Morphological Characteristics and Growth of *P. verruculosa* Cultures

Cells showed a variable morphology with form and size changing in response to growth phase and salinity treatments. All treatments showed cell shapes ranging from small spherical/oval (∼4–10 μm) to conical-elongated (∼8–19 μm). Two heterokont flagella were present. The shortest flagellum faces backward and a longer anteriorly directed flagellum (∼12–22 μm) pulls the cell forward (**Figure 3A**). Mucocysts were mainly present in larger than smaller cells. SEM analysis micrographs of the *P. verruculosa* ARC498 cultures (used for the gill cell line assay) showed a more recurrent presence of mucocysts in *P. verruculosa* cells at 25 psu than cells at 35 psu (**Figures 3A,B**). Mucus secretion was observed to form cell aggregations (**Figure 3C**).

The response of *P. verruculosa* ARC498 to changes in salinity was significantly different in terms of maximum cell density and maximum specific growth rate (μ_{max}) (ANOVA, df = 4, $p < 0.001$, **Figure 4**). The highest maximum cell density among all treatments was obtained at salinity 30 (84333 ± 4833 cells ml^{-1}), and the lowest at salinity 15 (269 ± 71 cells ml^{-1}) (**Table 1**). The highest μmax of 1.44 $days^{-1}$ was reached at 20 psu and the lowest at 30 psu with a mean value of 0.88 $days^{-1}$. Multinucleated cell aggregations were observed at the bottom of the flasks throughout the experimental period. After ∼15 days, some cultures exhibited vegetative cell growth that reached modest concentrations under depleted-nutrient conditions (data not shown).

P. verruculosa Cytotoxicity Against Fish Gill Cells

Viability of gill cells after 1 h exposure to *P. verruculosa* did not show significant differences between the supernatant and ruptured cell treatments (ANOVA, $p > 0.05$, **Figure 5**), and lytic activity showed to slightly increase together with *Pseudochattonella* cell density at both salinity treatments. Cytotoxicity of intra- and extracellular compounds of *P. verruculosa* did not show significant differences (ANOVA, $p < 0.05$; except treatment at 100,000 *Pseudochattonella* cells ml^{-1} at 25 psu-day1; **Figure 5A**) and treatments at salinity 25 showed to be more potent than toxic compounds obtained at salinity 35, reducing gill cell viability down to 52% of controls at 100,000 cells ml^{-1}. Lytic compounds of both salinity treatments rapidly degraded in the light conditions, reducing gill cell viability to only less than 20% after 5-days storage at 15°C (**Figure 5**).

DISCUSSION

As *P. verruculosa* is a newly reported species for Chilean waters, almost nothing is known about its ecology. This is the first study to formally confirm the taxonomic identification of Chilean

[1]http://www.r-project.org

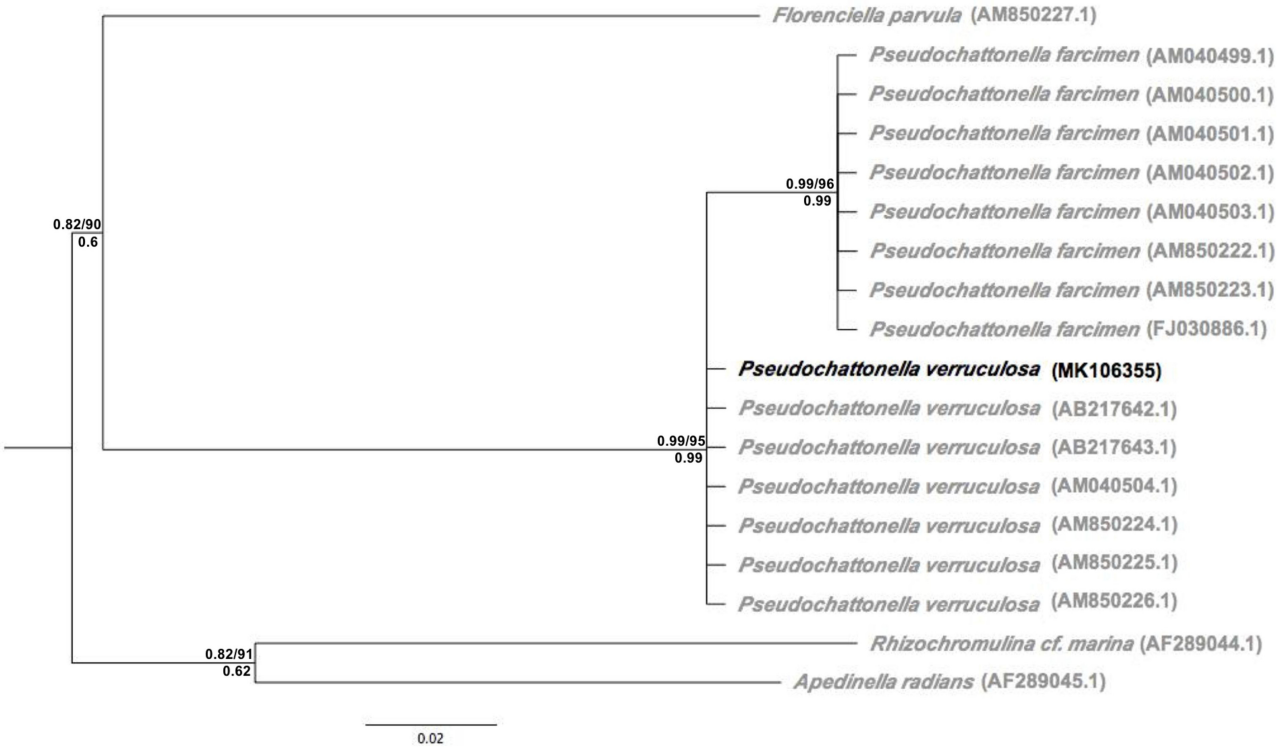


FIGURE 2 | Maximum likelihood phylogeny derived from partial nuclear LSU sequences (D1–D2 region) including the Chilean *P. verruculosa* strain (ARC498) (depicted in black). Support values are shown on each branch: aLRT/ML bootstrap (above) and Bayesian posterior probability (below).

Pseudochattonella populations as *P. verruculosa* using a genetic approach and the first assessment of *in vitro* cell growth and cytotoxicity.

Molecular Characterization

To the best of our knowledge, this is the first formal molecular characterization of a *Pseudochattonella* strain isolated from the massive 2016 bloom in the Reloncaví sound (southern Chile), using a sequence obtained from the LSU rDNA region (**Figure 1**). These results resolved the Chilean *P. verruculosa* strain (ARC498) within a clade with others *P. verruculosa* strains from Japan and New Zealand in agreement with Chang et al. (2014) for the same gene. However, other markers (18S, Rbcl, COI, and ITS) should be used to confirm our Chilean *Pseudochattonella* strain within the *P. verruculosa* clade. This is mainly due to the fact that different gene markers can differ in results. For instance, a *Pseudochattonella* strain Wellington Harbour (New Zealand) was found to be similar to *P. verruculosa* using the large subunit of ribulose bisphosphate carboxylase (rbcL) and the partial sequences of the nuclear encoded LSU rDNA, whereas sequences of the mitochondrial cytochrome c oxidase subunit (COI) gene grouped this strain as *P. farcimen* (Chang et al., 2014). Moreover, unpublished sequence data from field samples collected during the 2016 bloom have indicated the potential co-existence of *P. farcimen* blooming together with *P. verruculosa* in the Patagonian fjord area (ADL Diagnostic Chile- personal communication). Under this scenario and given the high morphological variability already described for *Pseudochattonella* species (Eckford-Soper and Daugbjerg, 2016b), the description of molecular markers becomes a key tool for precise species detection in the Chilean waters. The *P. verruculosa* ARC498 sequence described in this study has the potential for the design of primers for quantitative PCR (qPCR) aiming a rapid and low-cost identification and quantification tool as used in field samples from *Pseudochattonella* sp. blooms in Danish waters (Eckford-Soper and Daugbjerg, 2016a). In future studies, HPLC pigment analysis could also help for a better *Pseudochattonella* species discrimination since violaxanthin, lutein, and anteroxanthin are only detected in *P. verruculosa* (Tomas et al., 2004).

Effect of Salinity on *P. verruculosa* Cultures

High salinity variations observed in estuarine systems due to water mix or stratification can produce alterations in marine microalgae composition. These effects can include (i) changes in the cellular ionic ratios due to the selective ion permeability of the membrane, (ii) osmotic stress with direct impact on the cellular water potential and (iii) ionic stress caused by the unavoidable uptake or loss of ions (Kirst, 1990). The Reloncaví Sound (41°35′S, 72°20′W), which is recognized as the 'hot spot' for *Pseudochattonella* outbreaks in the south of Chile, has a broad range of salinity that can vary from <10 psu at the surface to >30 psu in deep waters (Castillo et al., 2016). In our study, the *P. verruculosa* ARC498 strain

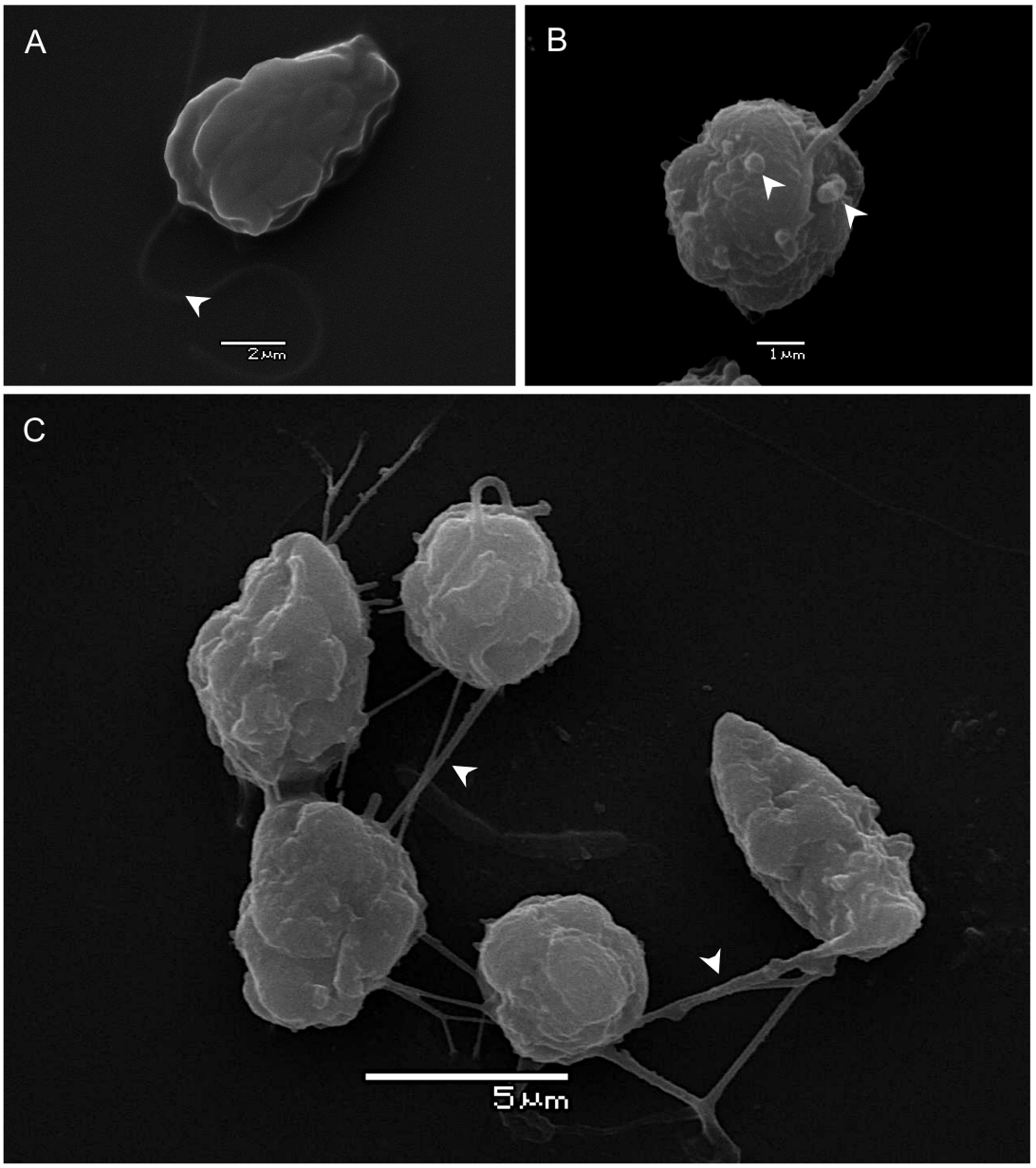


FIGURE 3 | Scanning electron microscopy of *P. verruculosa* ARC498 strain. **(A)** Ovoid cell with a long forwardly directed flagellum (arrowhead) from culture at 35 in salinity, **(B)** spherical cell with mucocysts at the periphery of the cell (arrowheads) from culture at 25 in salinity, and **(C)** group of cells interconnected by thin mucus-filaments (arrowheads).

isolated from the Reloncaví sound reached high cell densities between 20 and 35 psu. At salinity 15, exponential cell growth was 8 days delayed compared to the other salinity treatments and cell densities were several orders of magnitude lower, although positive net growth lasted for several days. These results suggest that freshwater inputs in the upper layer of the fjord may act as an environmental control for *P. verruculosa* cell growth.

The capacity to maintain high and positive net growth at a broad range of salinity values may have several competitive advantages in a dynamic estuarine system when turbulence breaks the halocline and vertically disperse cell through the water column. This pattern of broad salinity optima has also been observed in the distantly related *Dictyocha speculum* Ehrenberg (Henriksen et al., 1993). However, it has been suggested that microalgal cell growth primarily depends on temperature and light, with the tolerated salinity range becoming broader as these parameters approach optimality (Kirst, 1990). An increasing tolerance to a high salinity range by our *P. verruculosa* strain could be then a result of *in vitro* optimal conditions (20 µmol

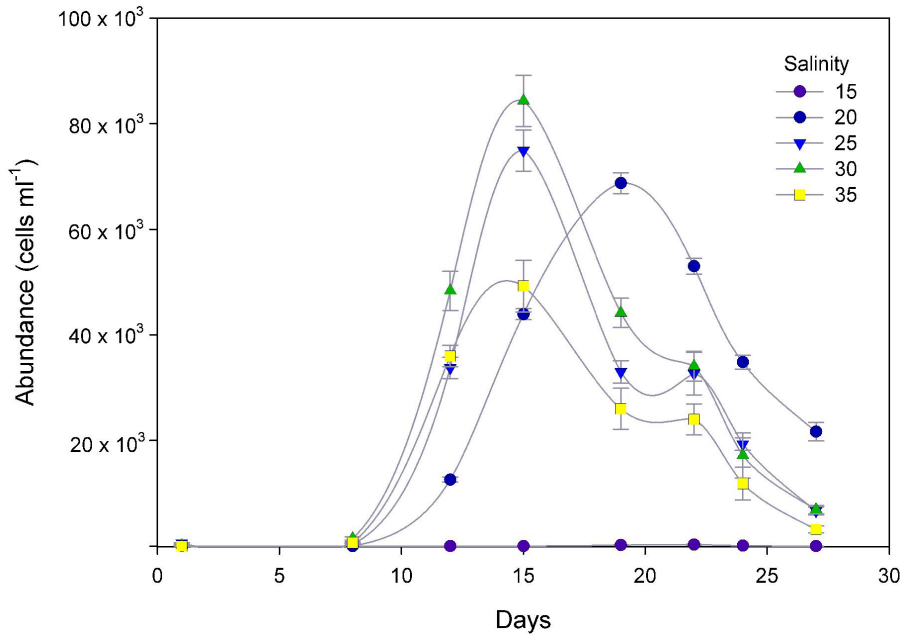


FIGURE 4 | Temporal cell growth curves of *P. verruculosa* cultures under five salinity treatments. Symbols represent the mean and error bars the standard deviation of cell counts from triplicate measurements.

photon m^{-2} s^{-1} and 15°C) and it is likely to change in the highly dynamic fjords. Other aspect to take into account is that the experiments were performed using strains previously acclimated for several generations. Further studies should assess the effect of drastic salinity changes on *P. verruculosa* growth.

In this study, we documented for the first time the cell growth rates for a Chilean *P. verruculosa* strain. Maximum growth rates that ranged between 0.88 to 1.44 $days^{-1}$ were higher compared to those observed by Skjelbred et al. (2013) for Danish *P. verruculosa* strains (0.61 $days^{-1}$) and lower than 1.74 $days^{-1}$, which was recorded for a Japanese strain (Yamaguchi et al., 1997). The relative high μmax observed in our experiment could be attributed to a high cell division rate but also to the presence of multinucleated cell aggregations noted at the bottom of our treatment flasks throughout and ∼15 days after the end of the experiment. Similar *in vitro* cell aggregations have also been observed in other *P. verruculosa* strains from the North Sea and Japan (Tomas et al., 2004). It has been suggested that these massive aggregations could act as resting stages when conditions become unfavorable (Jakobsen et al., 2012; Chang et al., 2014). Thus, *in vitro* growth rate values could be enhanced by germling cell input from resting stages and also might point to homothallism in our monoclonal ARC498 strain.

TABLE 1 | Growth parameters of *Pseudochattonella verruculosa* cultures (mean ± SD) maximum cell density and μ_{max} mean maximum specific growth rate and under five different salinity treatments.

Treatment (salinity)	Maximum cell density (cells ml^{-1})	μ_{max} (d^{-1})
15	269 ± 71	1.31
20	68750 ± 1963	1.44
25	74916 ± 3892	1.12
30	84333 ± 4833	0.88
35	49250 ± 4793	1.01

Lytic Activity of *P. verruculosa*

Species of the genus *Pseudochattonella* has been reported to be highly ichthyotoxic with important economic losses for the Chilean salmon industry since 2004 (Mardones et al., 2012). Most examinations of affected salmon during natural fish-kill events in Chile have shown noticeable edema, hyperplasia and necrosis of secondary gill lamellae (C. Sandoval – VEHICE, personal communication). Despite this, research on the toxic compounds produced by this species is incipient and has proven to be difficult.

The fish cell line RTgill-W1 assay has been widely used to assess cell viability in ichthyotoxic-microalgal studies (Dorantes-Aranda et al., 2015). In this work, we experimentally demonstrated lytic activity of the Chilean *P. verruculosa* ARC498 strain toward the RTgill-W1 cells, although ecologically realistic *P. verruculosa* bloom concentrations (10,000–100,000 cells ml^{-1}) exhibited only very limited loss of viability (down to max. 45% of controls). These results differ from field observations in the Chilean bloom, where *P.* cf. *verruculosa* showed to be extremely toxic at cell concentrations as low as 5 cells ml^{-1} (Mardones et al., 2012). Similarly, Montes et al. (2018), based on Chilean phytoplankton data bases, estimated that <1 cell ml^{-1} can be associated with anomalous salmon behavior in the Patagonian fjords. Comparable to these observations in the Chilean waters, only 10 cells ml^{-1} of *P. verruculosa*

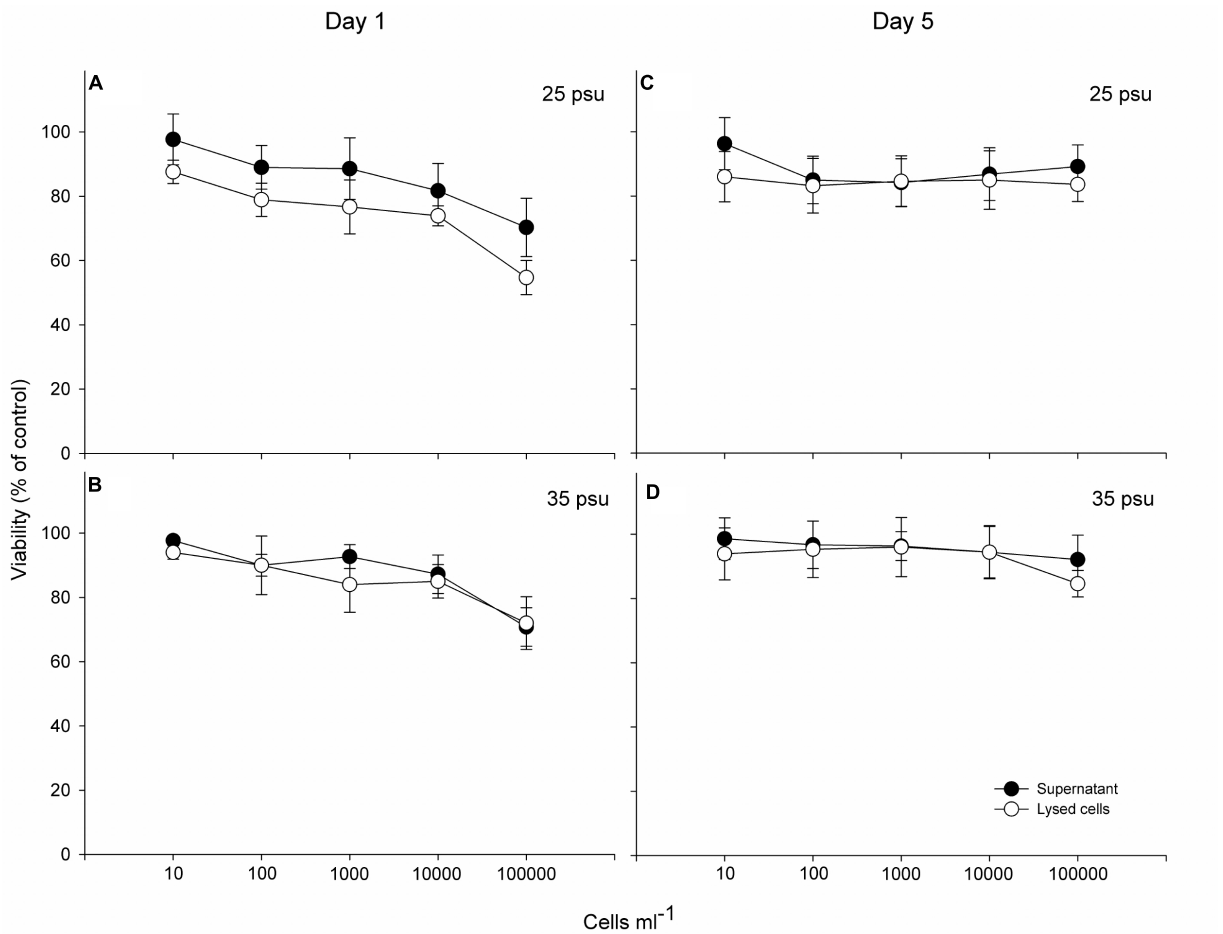


FIGURE 5 | Concentration-dependent toxicity (supernatant and lysed cells treatments) of the *Pseudochattonella* ARC498 strain at 1 **(A,B)** and 5 days of culture harvest **(C,D)** (at 25 and 35 psu) to the cell line RTgill-W1. Symbols represent the mean and error bars the SD of cell viability from quadruplicate measurements.

were enough to cause mortality of salmon in New Zealand (MacKenzie et al., 2011). There might be a few experimental conditions that can explain our findings: (1) gill cells were shortly exposed to *P. verruculosa* toxic compounds (1 h) compared to other studies (Dorantes-Aranda et al., 2015; Mardones et al., 2015). Mardones et al. (2015) showed that some strains of the toxic dinoflagellate *Alexandrium catenella* were able to reduce gill cell viability down to 20% of controls at 4,000 cells ml^{-1} after 2 h exposure due to net K^+ efflux from fish gill cells (Mardones et al., 2018). Thus, an increment in the time of exposure to *P. verruculosa* toxic compounds is likely to produce a more severe gill tissue damage; (2) in this study lytic compounds from *P. verruculosa* treatments were tested against the gill cells at 1 and 5 days after their extraction. This storage procedure showed that cytotoxic potency rapidly vanished over a 5-day period. It is likely therefore that *P. verruculosa* exudates could be more ichthyotoxic as soon as they are released into the surrounding marine environment. This early toxic effect was previously observed in *in vitro* cytotoxic assays using algal extracts of *Pseudochattonella* species (Skjelbred et al., 2011); (3) live cells are required to induce a toxic effect on fish as suggested by Andersen et al. (2015); and/or (4) the reduced cytotoxicity observed on gill cells could be merely due to the fact that the ARC498 strain is not highly toxic.

The ichthyotoxic mechanism in *Pseudochattonella* species is elusive with the mode of action yet to be completely determined (Eckford-Soper and Daugbjerg, 2016b). In this study, cytotoxicity of lysed *P. verruculosa* cells did not show significant differences to that of the supernatant. This suggests that the most lytic portion is released into the cell-free media instead of remaining cell bound. These results might lead to a future explanation of the nature of the toxins produced by *Pseudochattonella* species and their role in chemical ecology. Initial ideas focused on the concept that phycotoxins acted as defensive chemicals against competitors and predators (Smetacek, 2001). Modern studies suggest that cell bound phycotoxins might play a different ecological function compared to secondary toxic metabolites that are release from the cell to the surrounding environment. Excretion of a toxic compound by individual cells may aim to cease grazing by a motile predator or produce an antibiotic effect to reduce the colonization of microalgal cells by viruses

or bacteria (Cembella, 2003). For instance, allelochemical activity by the dinoflagellate *Alexandrium tamarense* against the predatory dinoflagellate *Oxyrrhis marina* was shown to be unrelated to paralytic shellfish toxins (PST) (Alpermann et al., 2010). Similarly, Mardones et al. (2015, 2018) showed that PST are not involved in fish gill damage, but rather an alternative icthyotoxic mechanism was suggested based on lipid peroxidation products due to a synergistic interaction between Reactive Oxygen Species (ROS) and Polyunsaturated Fatty Acids (PUFA). Interestingly, *Pseudochattonella* species have shown high proportions of a rare PUFA C1s8:5n-3 and C16:1n5 and high ratios of docosahexaenoic acid (DHA) to eicosapentaenoic acid (EPA) (Giner et al., 2008; Dittami and Edvardsen, 2012). This rises a big question about the potential involvement of a PUFA/ROS synergism in the potent ichthyotoxicity observed for the Chilean *P. verruculosa* ARC498. Key research on PUFAs cell content, as well as, ROS and other active secondary metabolites production for Chilean strains remain to be conducted.

Salinity had a significant effect on *P. verruculosa* ichthyotoxic potency. Cultures at salinity 25 were more toxic than cultures at 35 psu. It was also observed that *Pseudochattonella* cells at salinity 25 were bigger and showed a higher presence of mucocysts than cells grown at salinity 35. These mucocysts are present in several species of raphidophytes that have been related to fish-killing events, e.g., *Heterosigma akashiwo* (Twiner et al., 2005) and *Fibrocapsa japonica* (Pezzolesi et al., 2010). This suggests the putative involvement of mucocysts to induce a toxic effect on fish gill tissue as suggested by Andersen et al. (2015). However, no experiments to test cytotoxicity with whole cells of Chilean *Pseudochattonella* have been performed yet.

Ecological Implications

In this study, changes in salinity were an important driver for *in vitro Pseudochattonella* cell growth and ichthyotoxic potency. The enhanced cell growth under moderate-high salinity conditions (∼30) is in line with field observations during the massive 2016 *Pseudochattonella* sp. outbreak. The strong El Niño 2015–2016 resulted in an extremely dry summer in the southeast Pacific coast with a record low streamflow and higher than normal solar radiation (León-Muñoz et al., 2018). These extreme meteorological conditions allowed vertical advection of saline and nutrient-rich waters (∼30 psu) that ultimately resulted in an enhanced *Pseudochattonella* sp. bloom that reached peak cell densities of ∼7,700 to 20,000 cells ml^{-1} (Clément et al., 2016; León-Muñoz et al., 2018). Thus, the lower *Pseudochattonella* cell concentrations recorded in the 2005, 2009, and 2011 outbreaks could have been the result of highly stratified water columns with higher freshwater inputs in the surface. However, those low-cell density outbreaks produced substantial fish gill damage with less than 20 cells ml^{-1}, suggesting the important effect of moderate-lower salinity on the enhanced ichthyotoxic potency (∼20–25 psu).

Although the macro-scale El Niño 2015–2016 event produced evident changes in the haline structure of the water column in the Reloncaví Sound, it is important to highlight that pCO_2/pH and nutrient composition/availability, among other variables, should also have been affected. Mardones et al. (2016) showed that spatio-temporal pCO_2/pH fluctuations in Chilean fjords can strongly affect physiological responses in *A. catenella*. Physiological changes such as reduced cell size and enhanced chain formation at high pH/low pCO_2 (i.e., phytoplankton bloom conditions) were observed. On the other hand, the entry of offshore waters rich in inorganic nutrients (i.e., NO_3^-) vs. inland waters, highly influenced by organic nutrients (i.e., NH_4^+) due to intense aquaculture, might have contributed to changes in phytoplankton community composition by differences in NH_4^+ and NO_3^- taxon-specific metabolism. The role of these key chemical variables in the bloom dynamics of *Pseudochattonella* species should be pursued in future experiments.

Finally, the extremely high cell densities obtained in our *in vitro* experiments (>80,000 cells ml^{-1}) compared to field counts, may reflect the difficulty in the *Pseudochattonella* sp. cell identification using light microscopy. The complex life cycle of *Pseudochattonella* species, that also includes very small flagellate and large multinucleated stages (Chang et al., 2014; this study), likely lead to misidentification and cell count sub-estimations of the 2016 *Pseudochattonella* sp. outbreak. Therefore, the advent of molecular approaches becomes an important tool for improving the effectiveness of monitoring programs.

In conclusion, the present work has formally confirmed the presence of *P. verruculosa* in Chilean waters, as well as, showed the effect of salinity on cell growth and ichthyotoxic potency. Overall, high growth rates observed at moderate-high salinity might help to explain the massive outbreak recorded in 2016. The increasing cytotoxicity by *P. verruculosa* under low-salinity conditions may be important to understand fish-killing properties of the Chilean strains since blooms of this dictyochophyte occur in highly variable oceanographic conditions, such as those occurring in the southern Chilean coast. The role of mucocysts, as well as, production of PUFAs, ROS and/or secondary metabolites that could explain ichthyotoxicity by Chilean strains of *P. verruculosa* remain to be investigated.

AUTHOR CONTRIBUTIONS

All authors conceived, designed, and performed the experiments and analysis. JM analyzed the data and wrote the manuscript with contributions from all authors.

FUNDING

Funding was provided by the "Instituto de Fomento Pesquero-IFOP" (Grants: MR 656-103 and CORFO 480-018) and UNCW's Marine Biotechnology Program (MARBIONC) funded by the State of North Carolina (United States).

ACKNOWLEDGMENTS

We thank Pamela Carbonell, Bianca Olivares, and Marco Pinto for technical assistance.

REFERENCES

Alpermann, T. J., Tillmann, U., Beszteri, B., Cembella, A. D., and John, U. (2010). Phenotypic variation and genotypic diversity in a planktonic population of the toxigenic marine dinoflagellate *Alexandrium tamarense* (Dinophyceae). *J. Phycol.* 46, 18–32. doi: 10.1111/j.1529-8817.2009.00767.x

Alves-de-Souza, C., Varela, D., Contreras, C., de La Iglesia, P., Fernández, P., Hipp, B., et al. (2014). Seasonal variability of *Dinophysis* spp. and *Protoceratium reticulatum* associated to lipophilic shellfish toxins in a strongly stratified Chilean fjord. *Deep Sea Res. II* 101, 152–162. doi: 10.1016/j.dsr2.2013.01.014

Andersen, N. G., Hansen, P. J., Engell-Sørensen, K., Nørremark, L. H., Andersen, P., Lorenzen, E., et al. (2015). Ichthyotoxicity of the microalga *Pseudochattonella farcimen* under laboratory and field conditions in Danish waters. *Dis. Aquat. Organ.* 116, 165–172. doi: 10.3354/dao02916

Anisimova, M., and Gascuel, O. (2006). Approximate likelihood-ratio test for branches: a fast, accurate, and powerful alternative. *Syst. Biol.* 55, 539–552. doi: 10.1080/10635150600755453

Baba, T., Momoyama, K., and Hiraoka, M. (1995). A harmful flagellate plankton increased in Tokuyama Bay. *Bull. Yamaguchi Prefect. Naikai Fish. Exp. Stn.* 24, 121–122.

Bols, N. C., Barlian, A., Chirino-Trejo, M., Caldwell, S. J., Goegan, P., and Lee, L. E. J. (1994). Development of a cell line from primary cultures of rainbow trout, *Oncorhynchus mykiss* (Walbaum), gills. *J. Fish. Dis.* 17, 601–611. doi: 10.1111/j.1365-2761.1994.tb00258.x

Botes, L., Price, B., Waldron, M., and Pitcher, G. C. (2002). A simple and rapid scanning electron microscope preparative technique for delicate "Gymnodiniod" dinoflagellates. *Microsc. Res. Tech.* 59, 128–130. doi: 10.1002/jemt.10184

Castillo, M. I., Cifuentes, U., Pizarro, O., Djurfeldt, L., and Cáceres, M. (2016). Seasonal hydrography and surface outflow in a fjord with a deep sill: the Reloncaví fjord. *Chile Ocean Sci.* 12, 533–544. doi: 10.5194/os-12-533-2016

Cembella, A. (2003). Chemical ecology of eukaryotic microalgae in marine ecosystems. *Phycologia* 42, 420–447. doi: 10.2216/i0031-8884-42-4-420.1

Chang, F. H., Sutherland, J. E., McVeagh, M., and Gall, M. (2014). Molecular phylogeny, pigment composition, toxicology and life history of *Pseudochattonella* cf. *verruculosa* (class Dictyochophyceae) from Wellington harbour, New Zealand. *Harmful Algae* 34, 42–55. doi: 10.1016/j.hal.2014.02.002

Clément, A., Lincoquero, L., Saldivia, M., Brito, C. G., Muñoz, F., Fernández, C., et al. (2016). Exceptional summer conditions and HABs of *Pseudochattonella* in Southern Chile create record impacts on salmon farms. *Harmful Algal News* 53, 1–3.

Dayeh, V. R., Schirmer, K., Lee, L. E. J., and Bols, N. C. (2005). "Rainbow trout gill cell line microplate cytotoxicity test," in *Small-Scale Freshwater Toxicity Investigations*, eds C. Blaise and J. F. Férard (Berlin: Springer), 473–503. doi: 10.1007/1-4020-3120-3_16

Dittami, S. M., and Edvardsen, B. (2012). Culture conditions influence cellular RNA content in ichthyotoxic flagellates of the genus *Pseudochattonella* (Dictyochophyceae). *J. Phycol.* 48, 1050–1055. doi: 10.1111/j.1529-8817.2012.01183.x

Dorantes-Aranda, J. J., Seger, A., Mardones, J. I., Nichols, P. D., and Hallegraeff, G. M. (2015). Progress in understanding algal bloom-mediated fish kills: the role of superoxide radicals, phycotoxins and fatty acids. *PLoS One* 10:e0133549. doi: 10.1371/journal.pone.0133549

Dorantes-Aranda, J. J., Waite, T. D., Godrant, A., Rose, A., Tovar, C. D., Woods, G. M., et al. (2011). Novel application of a fish gill cell line assay to assess ichthyotoxicity of harmful marine microalgae. *Harmful Algae* 10, 366–373. doi: 10.1016/j.hal.2011.01.002

Eckford-Soper, L. K., and Daugbjerg, N. (2016a). A quantitative real-time PCR assay for identification and enumeration of the occasionally co-occurring ichthyotoxic *Pseudochattonella farcimen* and *P. verruculosa* (Dictyochophyceae) and analysis of variation in gene copy numbers during the growth phase of single and mixed cultures. *J. Phycol.* 52, 174–183. doi: 10.1111/jpy.12389

Eckford-Soper, L. K., and Daugbjerg, N. (2016b). The ichthyotoxic genus *Pseudochattonella* (Dictyochophyceae): distribution, toxicity, enumeration, ecological impact, succession and life history – A review. *Harmful Algae* 58, 51–58. doi: 10.1016/j.hal.2016.08.002

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32, 1792–1797. doi: 10.1093/nar/gkh340

Edvardsen, B., Eikrem, W., Shalchian-Tabrizi, K., Riisberg, I., Johnsen, G., Naustvoll, L., et al. (2007). Verrucophora farcimen gen. et sp. nov. (Dictyochophyceae, Heterokonta) – a bloom-forming ichthyotoxic flagellate from the Skagerrak, Norway. *J. Phycol.* 43, 1054–1070. doi: 10.1111/j.1529-8817.2007.00390.x

Edvardsen, B., Shalchian-Tabrizi, K., Jakobsen, K. S., Medlin, L. K., Dahl, E., Brubak, S., et al. (2003). Genetic variability and molecular phylogeny of *Dinophysis* species (Dinophyceae) from Norwegian waters inferred from single cell analyses of rDNA. *J. Phycol.* 39, 395–408. doi: 10.1046/j.1529-8817.2003.01252.x

Eikrem, W., Edvardsen, B., and Throndsen, J. (2009). Renaming *Verrucophora farcimen* Eikrem, Edvardsen et Throndsen. *Phycol. Res.* 57:170. doi: 10.1111/j.1440-1835.2009.00535.x

Giner, J.-L., Zhao, H., and Tomas, C. (2008). Sterols and fatty acids of three harmful algae previously assigned as *Chattonella*. *Phytochemistry* 69, 2167–2171. doi: 10.1016/j.phytochem.2008.05.013

Guillard, R. R., and Ryther, J. H. (1962). Studies of marine planktonic diatoms. I. Cyclotella nana Hustedt, and Detonula confervacea (cleve) Gran. *Can. J. Microbiol.* 8, 229–239. doi: 10.1139/m62-029

Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W., and Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst. Biol.* 59, 307–321. doi: 10.1093/sysbio/syq010

Hallegraeff, G. M. (2003). "Harmful algal blooms: a global overview," in *Manual on Harmful Marine Algae*, eds G. M. Hallegraeff, D. M. Anderson, and A. D. Cembella (Paris: UNESCO), 25–49.

Hallegraeff, G. M., Dorantes-Aranda, J. J., Mardones, J. I., and Seger, A. (2017). "Review of progress in our understanding of fish-killing microalgae: implications for management and mitigation," in *Marine and Fresh-water Harmful Algae. Proceedings of the 17th International Conference on Harmful Algae*, eds L. A. O. Proença and G. M. Hallegraeff (Paris: International Society for the Study of Harmful Algae and Intergovernmental Oceanographic Commission of UNESCO), 148–153.

Hara, Y., Doi, K., and Chihara, M. (1994). Four new species of *Chattonella* (Raphidophyceae, Chromophyta) from Japan. *Jpn. J. Phycol.* 42, 407–420.

Henriksen, P., Knipschildt, F., Moestrup, Ø., and Thomsen, H. A. (1993). Autecology, life history and toxicology of the silicoflagellate *Dictyocha speculum* (Silicoflagellata, Dictyochophyceae). *Phycologia* 32, 29–39. doi: 10.2216/i0031-8884-32-1-29.1

Holliday, D. V., Donaghay, P. L., Greenlaw, C. F., McGehee, D. E., McManus, M. A., Sullivan, J. M., et al. (2003). Advances in defining fine- and micro-scale pattern in marine plankton. *Aquat. Living Res.* 16, 131–136. doi: 10.1016/S0990-7440(03)00023-8

Hosoi-Tanabe, S., Honda, D., Fukaya, S., Otake, I., Inagaki, Y., and Sako, Y. (2007). Proposal of *Pseudochattonella verruculosa* gen. nov., comb. nov. (Dictyochophyceae) for a formal raphidophycean alga *Chattonella verruculosa,* based on 18S rDNA phy- logeny and ultrastructural characteristics. *Phycol. Res.* 55, 185–192. doi: 10.1111/j.1440-1835.2007.00461.x

Ihaka, R., and Gentleman, R. (1996). A language for data analysis and graphics. *J. Comput. Graph. Stat.* 5, 299–314.

Imai, I., Yamaguchi, M., and Watanabe, M. (1998). "Ecophysiology, life cycle and bloom dynamics of *Chattonella* in the Seto Inland Sea, Japan," in *Physiological Ecology of Harmful Algal Blooms*, eds D. M. Anderson, A. Cembella, and G. Hallegraeff (Berlin: Springer), 95–112.

Jakobsen, R., Hansen, P. J., Daugbjerg, N., and Andersen, N. G., (2012). The fish-killing dictyochophyte *Pseudochattonella farcimen*: adaptations leading to bloom formation during early spring in Scandinavian waters. *Harmful Algae* 18, 84–95. doi: 10.1016/j.hal.2012.04.008

Jeong, H. J., Seong, K. A., Kang, N. S., Yoo, Y. D., Nam, S. W., Park, J. Y., et al. (2010). Feeding by raphidophytes on the cyanobacterium *Synechococcus* sp. *Aquat. Microb. Ecol.* 58, 181–195. doi: 10.3354/ame01354

Kirst, G. O. (1990). Salinity tolerance of eukaryotic marine algae. *Annu. Rev. Plant. Phys.* 41, 21–53. doi: 10.1093/gbe/evw152

León-Muñoz, J., Urbina, M. A., Garreaud, R., and Iriarte, J. L. (2018). Hydroclimatic conditions trigger record harmful algal bloom in western Patagonia (summer 2016). *Sci. Rep.* 8:1330. doi: 10.1038/s41598-018-19461-4

Lu, D., and Goebel, J. (2000). *Chattonella* sp. bloom in North Sea, spring 2000. *Harmful Algae News* 21, 10–11.

MacKenzie, L. A., Smith, K. F., Rhodes, L. L., Brown, A., Langi, V., Edgar, M., et al. (2011). Mortalities of sea-cage salmon (*Oncorhynchus tshawytscha*) due to a bloom of *Pseudochattonella verruculosa* (Dictyochophyceae) in Queen Charlotte Sound, New Zealand. *Harmful Algae* 11, 45–53. doi: 10.1016/j.hal.2011.07.003

Mardones, J., Clément, A., and Rojas, X. (2012). Monitoring potentially ichthyotoxic phytoflagellates in the southern fjords of Chile. *Harmful Algae News* 45, 6–7.

Mardones, J. I., Dorantes-Aranda, J. J., Nichols, P. D., and Hallegraeff, G. M. (2015). Fish gill damage by the dinoflagellate *Alexandrium catenella* from Chilean fjords: synergistic action of ROS and PUFA. *Harmful Algae* 49, 40–49. doi: 10.1016/j.hal.2015.09.001

Mardones, J. I., Müller, M. N., and Hallegraeff, G. M. (2016). Toxic dinoflagellate blooms of *Alexandrium catenella* in Chilean fjords: a resilient winner from climate change. *ICES J. Mar. Sci.* 74, 988–995. doi: 10.1093/icesjms/fsw164

Mardones, J. I., Shabala, L., Shabala, S., Dorantes-Aranda, J. J., Seger, A., and Hallegraeff, G. M. (2018). Fish gill damage by harmful microalgae newly explored by microelectrode ion flux estimation techniques. *Harmful Algae* 80, 55–63. doi: 10.1016/j.hal.2018.09.004

Montes, R. M., Rojas, X., Artacho, P., Tello, A., and Quiñones, R. A. (2018). Quantifying harmful algal bloom thresholds for farmed salmon in southern Chile. *Harmful Algae* 77, 55–65. doi: 10.1016/j.hal.2018.05.004

Naustvoll, L., Dahl, E., Danielssen, D., Aure, J., Skogen, M., and Budgell, P. (2002). Chattonella i Skagerrak – en ny trussel for oppdrettsnæringen? Havets Miljø, Fisk. *Havet Særnummer* 2, 126–129.

Pagé, B., Pagé, M., and Noël, C. (1993). A new fluorometric assay for cytotoxicity measurements in vitro. *Int. J. Oncol.* 3, 473–476. doi: 10.3892/ijo.3.3.473

Paredes, J., Aguilera, A., Olivares, B., Uribe, C., Urrutia, G., Seguel, M., et al. (2016). "Morphological variability and genetic identification of ichthyotoxic species *Pseudochattonella* sp. isolated from severe outbreak in 2016 at the northern Patagonian Fjord, southern Chile," in *Poster Presentation at the 17th International Conference on Harmful Algae*, Florianópolis.

Pezzolesi, L., Cucchiari, E., Guerrini, F., Pasteris, A., Galletti, P., Tagliavini, E., et al. (2010). Toxicity evaluation of *Fibrocapsa japonica* from the Northern Adriatic Sea through a chemical and toxicological approach. *Harmful Algae* 9, 504–514. doi: 10.1016/j.hal.2010.03.006

Posada, D. (2008). jModelTest: phylogenetic model averaging. *Mol. Biol. Evol.* 25, 1253–1256. doi: 10.1093/molbev/msn083

Rignot, E., Rivera, A., and Casassa, G. (2003). Contribution of the Patagonia Icefields of South America to sea level rise. *Science* 302, 434–437. doi: 10.1126/science.1087393

Riisberg, I., and Edvardsen, B. (2008). Genetic variation in bloom-forming ichthyotoxic *Pseudochattonella* species (Dictyochophyceae, Heterokonta) using nuclear, mitochondrial and plastid DNA sequence data. *Eur. J. Phycol.* 43, 413–422. doi: 10.1080/09670260802299602

Ronquist, F., and Huelsenbeck, J. P. (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–1574. doi: 10.1093/bioinformatics/btg180

Schirmer, K., Chan, A. G. J., Greenberg, B. M., Dixon, D. G., and Bols, N. C. (1997). Methodology for demonstrating and measuring the photocytotoxicity of fluoranthene to fish cells in culture. *Toxicol. In Vitro* 11, 107–119. doi: 10.1016/S0887-2333(97)00002-7

Scholin, C. A., Herzog, M., and Anderson, D. M. (1994). Identification of group and strain specific genetic markets for globally distributed *Alexandrium* (Dinophyceae). II. Sequence analysis of a fragment of the LSU rRNA gene. *J. Phycol.* 30, 999–1011. doi: 10.1111/j.0022-3646.1994.00999.x

Silva, N., Calvete, C., and Sievers, H. (1998). Masas de agua y circulación general para algunos canales australes Chilenos entre Puerto Montt y Laguna San Rafael (Crucero CIMAR-Fiordo 1). *Cienc. Tecnol. Mar.* 21, 17–48.

Skjelbred, B., Edvardsen, B., and Andersen, T. (2013). Environmental optima for seven strains of *Pseudochattonella* (Dictyochophyceae, Heterokonta). *J. Phycol.* 49, 54–60. doi: 10.1111/jpy.12008

Skjelbred, B., Horsberg, T. E., Tollefsen, K. E., Andersen, T., and Edvardsen, B. (2011). Toxicity of the ichthyotoxic marine flagellate *Pseudochattonella* (Dictyochophyceae, Heterokonta) assessed by six bioassays. *Harmful Algae* 10, 144–154. doi: 10.1016/j.hal.2010.08.007

Smetacek, V. (2001). A watery arms race. *Nature* 411:745. doi: 10.1038/35081210

Stacey, M. T., McManus, M. M., and Steinbuck, J. V. (2007). Convergences and divergences and thin layer formation and maintenance. *Limnol. Oceanogr.* 52, 1523–1532. doi: 10.4319/lo.2007.52.4.1523

Sullivan, J. M., McManus, M. A., Cheriton, O. M., Benoit-Bird, K. J., Goodman, L., Wang, Z., et al. (2010). Layered organization in the Coastal Ocean: an introduction to thin layers and the LOCO project. *Cont. Shelf Res.* 30, 1–6. doi: 10.1016/j.csr.2009.09.001

Tillmann, U., and Reckermann, M. (2002). Dinoflagellate grazing on the raphidophyte *Fibrocapsa japonica*. *Aquat. Microb. Ecol.* 26, 247–257. doi: 10.3354/ame026247

Tomas, C. R., Ono, C., Yoshimatsu, S., and Go·bel, J. (2004). "*Chattonella verruculosa* and related species from Japan, Europe (North Sea) and U.S. coastal waters: cases of mistaken identity?," in *Harmful Algae*, eds K. A. Steidinger, J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Tallahassee: Florida Fish and Wildlife Conservation Commission), 425–427.

Twiner, M. J., Chidiac, P., Dixon, S. J., and Trick, C. G. (2005). Extracellular organic compounds from the ichthyotoxic red tide alga *Heterosigma akashiwo* elevate cytosolic calcium and induce apoptosis in Sf9 cells. *Harmful Algae* 4, 789–800. doi: 10.1016/j.hal.2004.12.006

Wright, J. L. C., and Cembella, A. D. (1998). "Ecophysiology and biosynthesis of polyether marine biotoxins," in *Physiological Ecology of Harmful Algae Blooms*, eds D. M. Anderson, A. D. Cembella, and G. M. Hallegraeff (Berlin,: Springer), 427–451.

Yamaguchi, M., Itakura, S., Nagasaki, K., Matsuyama, Y., Uchida, T., and Imai, I. (1997). Effects of temperature and salinity on the growth of the red tide flagellates *Heterocapsa circularisquama* (Dinophyceae) and *Chattonella verruculosa* (Raphidophyceae). *J. Plankton Res.* 19, 1167–1174. doi: 10.1093/plankt/19.8.1167

Yamamoto, C., and Tanaka, Y. (1990). Two species of harmful red tide plankton increased in Fukuoka Bay. *Bull. Fukuoka Fish. Exp. Stn.* 16, 43–44.

Yamazaki, H., Honma, H., Nagai, T., Doubell, M., Amakasu, K., and Kumagai, M. (2010). Multilayer biological structure and mixing in the upper water column of Lake Biwa during summer 2008. *Limnology* 11, 63–70. doi: 10.1007/s10201-009-0288-2

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Mardones, Fuenzalida, Zenteno, Alves-de-Souza, Astuya and Dorantes-Aranda. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Marine Science

ORIGINAL RESEARCH
published: 19 February 2019
doi: 10.3389/fmars.2019.00013

Oceanography of Harmful Algal Blooms on the Ecuadorian Coast (1997–2017): Integrating Remote Sensing and Biological Data

Mercy J. Borbor-Cordova[1], Gladys Torres[2], Gabriel Mantilla-Saltos[3], Ashley Casierra-Tomala[1], J. Rafael Bermúdez[1,4], Willington Renteria[2] and Bonny Bayot[1,5]*

[1] Escuela Superior Politécnica del Litoral, ESPOL, Facultad de Ingeniería Marítima y Ciencias del Mar, Guayaquil, Ecuador, [2] Instituto Oceanográfico de la Armada, INOCAR, Dirección de Oceanografía Naval, Guayaquil, Ecuador, [3] Escuela Superior Politécnica del Litoral, ESPOL, Facultad de Ciencias Naturales y Matemáticas, Guayaquil, Ecuador, [4] Galapagos Marine Research and Exploration Program (GMaRE), ESPOL-CDF Joint Research Program, Charles Darwin Research Station, Santa Cruz, Ecuador, [5] Escuela Superior Politécnica del Litoral, ESPOL, Centro Nacional de Acuicultura e Investigaciones Marinas, CENAIM, Guayaquil, Ecuador

OPEN ACCESS

Edited by:
Marius Nils Müller, Federal University of Pernambuco, Brazil

Reviewed by:
Maximo Jorge Frangopulos, University of Magallanes, Chile
Márcio Silva de Souza, Fundação Universidade Federal do Rio Grande, Brazil

***Correspondence:**
Mercy J. Borbor-Cordova
meborbor@espol.edu.ec

Specialty section:
This article was submitted to Marine Ecosystem Ecology, a section of the journal Frontiers in Marine Science

Received: *01 August 2018*
Accepted: *14 January 2019*
Published: *19 February 2019*

Citation:
Borbor-Cordova MJ, Torres G, Mantilla-Saltos G, Casierra-Tomala A, Bermúdez JR, Renteria W and Bayot B (2019) Oceanography of Harmful Algal Blooms on the Ecuadorian Coast (1997–2017): Integrating Remote Sensing and Biological Data. Front. Mar. Sci. 6:13. doi: 10.3389/fmars.2019.00013

Ocean climate drivers and phytoplankton life strategies interact in a complex dynamic to produce harmful algal blooms (HABs). This study aims to integrate historical biological data collected during "red tide" events along the Ecuadorian coast between 1997 and 2017 in relation to five ocean variables derived from satellite remote sensing data to explain the seasonal drivers of coastal processes associated with HABs dynamics. Seasonality of the occurrence of HABs was assessed in relation to oceanographic variables by applying multiple correspondence analysis (MCA) to the Ecuadorian central coast (Zone 1) and at the outer and inner Gulf of Guayaquil (Zone 2). Sixty-seven HABs events were registered between 1997 and 2017. From a total of 40 species of phytoplankton identified, 28 were identified as non-toxic and the remaining 12 are well known to produce toxins. Dinoflagellates were the taxonomic group most highly associated with potential HABs events along the entire Ecuadorian coast. HABs appear to be constrained by the Humboldt coastal upwelling, high precipitation, and associated coastal runoff, with higher biomass abundance in the Gulf of Guayaquil than in the central coast. Results from the MCA reveal that in the central Ecuadorian coast (oligotrophic system), toxic HABs occurred with low abundance of dinoflagellates, while in the Gulf of Guayaquil (eutrophic system), toxic HABs corresponded to a high abundance of dinoflagellates. In both cases, high values were found for sea surface temperature, precipitation, and irradiance—characteristic of wet seasons or El Niño years. Non-toxic HABs occurred with a high abundance of dinoflagellates, ciliates, and centric diatoms, corresponding to colder waters and low levels of precipitation and irradiance. These findings confirm that dinoflagellates display several strategies that enhance their productive capacity when ocean conditions are warmer, allowing them to

produce toxins at high or at low concentrations. Considering that the Gulf of Guayaquil is essential to tourism, the shrimp industry, fisheries, and international shipping, these findings strongly suggest the need to establish an ecosystem health research program to monitor HABs and the development of a preventive policy for tourism and public health in Ecuador.

Keywords: remote sensing, phytoplankton taxonomic groups (PTG), Gulf of Guayaquil, dinoflagellates, upwelling, Humboldt Current

INTRODUCTION

For decades, global coastal waters have experienced events known as "red tides" that are related to phytoplankton blooms, micro-algal blooms, and toxic algal or potentially harmful algal blooms (HABs) (Hallegraeff, 1993; Anderson, 2014; Anderson et al., 2017). Blooms are described as occurrences of a high biomass of plankton and benthic species, while HABs have been broadly classified as potent natural toxin producers created either by attaining high biomass levels or even through low numbers of toxic species. HABs may take place when a group of microalgae—photosynthetic cells found in the sea, brackish water, and freshwater—grow to very high numbers, producing toxic or harmful effects on shellfish, fish, marine mammals, birds, and humans (Hallegraeff, 1993; Zingone and Enevoldsen, 2002; Fleming et al., 2006; Jiménez and Gualancañay, 2006; Pitcher et al., 2017).

High-density algal blooms may cause anoxia to marine life, killing it and blocking sunlight on the surface of the water column. However, international research on HABs has recognized that some species have toxic effects even at low cells densities (Reguera et al., 2012; Anderson, 2014). The reasons why an algal bloom becomes toxigenic, either at high or low concentrations, is still not well understood. However, oceanographic and climatic conditions play an important role in HAB occurrence, which may be constrained by upwelling systems and stratification of the water column provoked by low wind stress and marine heat waves (Hallegraeff, 2010; Díaz et al., 2013; Pitcher et al., 2017). HABs may also be exacerbated by climatic variability, extreme events, like El Niño–Southern Oscillation (ENSO) (Sellner et al., 2003), and climate change due to a long-term increase in sea surface temperatures (Wells et al., 2015; Miller et al., 2006; Pitcher et al., 2017). In addition, anthropogenic nutrient loading from agricultural and urban watersheds, ballast water discharges from vessels, and nutrients released from mariculture activities, among others, are factors that drive HABs in coastal waters (Moore et al., 2008; Hallegraeff, 2010; Davidson et al., 2014; Berdalet et al., 2016).

Currently, the development of ocean remote sensing-derived data makes possible the exploration of the synoptic climatology of regions, biogeography of the primary productivity and HABs biomass, spring bloom growth, and even the identification of species at a good scale of resolution in space and time (McClain et al., 2004; Nair et al., 2008; Wei et al., 2008; McKibben et al., 2015). Thus, HAB dynamics can be explained by using *in situ* biological data and remote sensing observations of oceanographic variables (Moisan et al., 2011; Anderson, 2014; Blondeau-Patissier et al., 2014).

South America's Ecuadorian coast of the eastern tropical Pacific can be characterized by two biogeographical regions: the northern coast, influenced by the warm Panama Current, and the Gulf of Guayaquil, influenced by the Humboldt Current (Cucalon, 1989; Longhurst, 2006). Both regions are heavily affected by ENSO (Lee et al., 2014) and the eastern upwelling system, and these oceanographic structures can modify the ocean biogeochemistry periodically. All these oceanographic characteristics may trigger the occurrence of HABs along the Ecuadorian coast (Chavez et al., 2003; Pennington et al., 2006; Trainer et al., 2010). Following the global trend, the coast of Ecuador has experienced the occurrence of "red tides" for decades. Ecuadorian researchers have recorded approximately 132 events since 1968, resulting in reports of fish, juvenile shrimp, and shrimp larvae mortality (Torres, 2000, 2011, 2013, 2015, 2017; Torres and Tapia, 2002). Sixty-seven HABs events were registered between 1997 and 2017.

Despite their negative impacts on coastal activities, attempts to explain the oceanographic and climate drivers for the potential development of HABs are limited (Jiménez and Intriago, 2001; Jiménez and Gualancañay, 2006; Torres et al., 2017; Conde and Prado, 2018; Conde et al., 2018). Unfortunately, at this time there is no official HAB monitoring program for Ecuador that can analyze which toxins are generated by the phytoplankton species. Using a novel approach, this study intends to integrate ocean features, derived from synoptic remote sensing, and biological data, compiled from reports collected during "red tide" events, to identify which oceanographic conditions lead to the distribution and seasonal occurrence of HABs on the coast of Ecuador.

MATERIALS AND METHODS

Study Area

The study area is situated along the center and south of the Ecuadorian coast (South America) and was divided into two zones (**Figure 1**): Zone 1, corresponding to the area situated between the coastal localities of Manta (80.8°W, 0.8°S) and La Libertad (81°W, 2°S); and Zone 2, which includes the coastal area of the Gulf of Guayaquil (outer estuary) and the Guayas River estuary (inner estuary). The Guayas River estuary is connected to the El Morro and Jambelí channels and is comprised of two main bodies of water: Estero Salado and

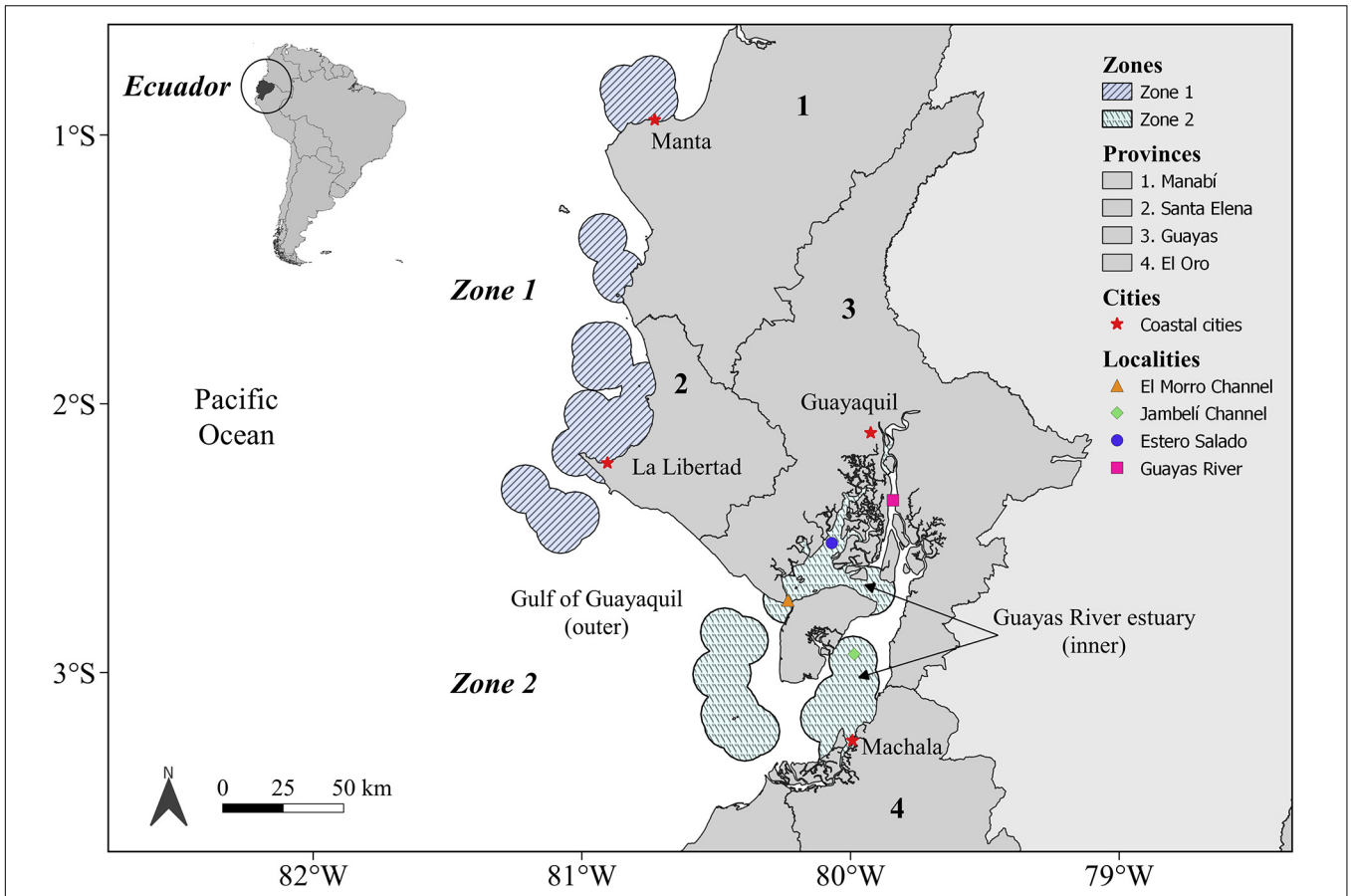


FIGURE 1 | Study area. Zone 1 is situated between the coastal cities of Manta and La Libertad; Zone 2 is located around the outer and inner (Estero Salado and Jambelí Channel) Gulf of Guayaquil estuary.

the Guayas River (**Figure 1**). Zone 1 is characterized mostly by sandy beaches used for tourism, and it is the location of important international cargo ports and fishery activities. Zone 2 includes the largest estuarine system on the South America coast adjacent to the Pacific Ocean and is where the cities of Guayaquil and Machala are located (**Figure 1**). The main economic activities carried out in Zone 2 are connected to shrimp farming, international cargo ports, and artisanal fisheries. Climate seasonality of the Ecuadorian coast is characterized by a wet season (December–May), with a unimodal peak of warm temperatures (March–April), and a dry season (June–November) characterized by the absence of rainfall and lower temperatures than those experienced during the wet season (Cucalon, 1989; Bendix et al., 2011).

Oceanographic and Atmospheric Data

Monthly averages of five variables recorded over the 1997–2017 period were derived from different satellite data compiled at ∼4 km of spatial resolution around Zones 1 and 2 on the Ecuadorian coast. Precipitation (PRE) was chosen as an atmospheric variable associated with climate seasonality (Nair et al., 2008; Shen et al., 2012) and therefore used as a proxy for seasonal atmospheric processes. In addition, seasonality of the region is strongly related to precipitation, which is also disturbed by the occurrence of El Niño and La Niña events in the equatorial eastern tropical Pacific (Pennington et al., 2006). The other four variables were oceanographic, representing select conditions of the ocean at specific times and places. Sea surface temperature (SST) is related to cold nutrient-rich currents and upwelling or with nutrient-poor warm currents (Pennington et al., 2006; Wei et al., 2008; Mélin and Hoepffner, 2011). Chlorophyll *a* (chl-a) was used as a proxy for phytoplankton abundance. Absorption due to phytoplankton at 443 nm (aph) represents the band of chlorophyll associated with the HABs peaks (Shen et al., 2012). Photosynthetically active radiation (PAR) was chosen as an indicator of the amount of light available for photosynthesis (Shi et al., 2014). Previously, SST and PRE have been related to the oceanographic and atmospheric processes of HABs, respectively (Wei et al., 2008). Satellite images were collected in NetCDF format and converted to GeoTiff files for the analyses using R software (version 3.5.0). Several satellites and sensors were used throughout the period of analysis (**Table 1**). Each variable was standardized with the respective global mean and standard deviation.

TABLE 1 | Description of oceanographic (SST, aph, chl-a, PAR) and atmospheric (PRE) variables from remote sensors.

Data (units)	Abbreviation	Satellite (sensor)	Temporal range	Source
Sea surface temperature (°C)	SST	Pathfinder	Monthly mean from September 1997 to December 2000	Casey et al., 2010
		GOES	Monthly mean from January 2001 to December 2016	NOAA NESDIS COASTWATCH and NOAA SWFSC ERD, 2018
Absorption due to phytoplankton at 443 nm (m^{-1})	aph	SeaWiFS	Monthly mean from September 1997 to January 2000	NASA Ocean Biology Processing Group, 2018c
		Terra MODIS	February 2000–December 2016	NASA Ocean Biology Processing Group, 2018a
Precipitation (mm)	PRE	Persiann	January 1997–August 2016	Sorooshian et al., 2014
Chlorophyll a (mg/m^3)	chl-a	Orbview-2 SeaWiFS	September 1997–December 2002	NASA Ocean Biology Processing Group, 2018d
		Aqua MODIS	January 2003–December 2016	NASA Ocean Biology Processing Group, 2017
Photosynthetically active radiation (Einstein/m^2/day)	PAR	SeaWiFS	September 1997–January 2000	NASA Ocean Biology Processing Group, 2018e
		Terra MODIS	February 2000–December 2016	NASA Ocean Biology Processing Group, 2018b

Phytoplankton Data

Phytoplankton data were compiled from 67 algal bloom events reported from 1997 to 2017 in the literature generated by the Ecuadorian Navy Army Oceanographic Institute (INOCAR), the National Institute of Fisheries, and reports from shrimp hatchery producers. Most of these data are included in the periodical research publications of INOCAR[1]. According to these reports, samples were collected from the sea surface. Data included taxa identification, abundance, location, and an observational description. This study also includes 8 unpublished reports from INOCAR and other researchers, increasing the original number of 59 registered events to 67 events in total. More detailed information on each event can be found in the publications and reports referenced in **Table 2**.

Some phytoplankton names have been updated according to the records in the World Register of Marine Species (WoRMS[2]). For the biological analysis performed in INOCAR, samples (a minimal volume of 250 mL of surface water) were collected from the "red tide" events and immediately transported to INOCAR's laboratories. All surface samples were fixed with Lugol solution until they changed to a light brown color (8–10 drops). The quantitative analysis was carried out using the Utermohl method described in Reguera et al. (2012) and adapted by Torres (2017). Samples were briefly homogenized and placed in a sedimentation chamber (25 mL) for 24 h.

Phytoplankton identification and abundance was assembled up to the genus or species level according to Jiménez (1983); Pesantes (1983), Balech (1988); Taylor et al. (1995), and Reguera et al. (2012) using a Leica (DMIL) inverted microscope. Cell abundance estimations were performed in horizontal transects of the chamber at 400× magnification, with a 15% error margin. Data were converted into cells L^{-1}. These data were further assembled in four phytoplankton taxonomic groups (PTG): dinoflagellates (DINO), centric diatoms (CDIAT), pennate diatoms (PDIAT), and ciliates (CILI), based on the GEOHAB criterion (WoRMS[3]; Pitcher et al., 2017). The species were categorized as toxic (TOX) or non-toxic (NOC) using the List of Harmful Microalgae (Moestrup et al., 2009).

Data Analysis

Each of four phytoplankton taxonomic groups, as well as the five remote sensing variables, were transformed into categorical variables (two categories: low and high) using the median as a cut-off. In addition, two categorical variables were also included: type of HAB event and season (identifying whether the event occurred during the wet or dry season) (Vila and Masó, 2005). All categorical variables were used for multi-correspondence analysis (MCA) for Zones 1 and 2. This analysis allowed for an investigation of the PTG patterns associated with the oceanographic and atmospheric variables in each zone.

RESULTS

Oceanographic Characteristics Associated With Potential HABs Using Remote Sensing Data

In Zone 1, chlorophyll *a* showed a unimodal peak at the end of the wet season, in April–May (∼1.5 mg m^{-3}), whereas a peak in April (∼5.0 mg m^{-3}) and a lower sustained value (3.0 mg m^{-3}) in September, during the dry season, were observed in Zone 2 (**Figures 2A,B**). The levels of chlorophyll *a* were higher in Zone 2 (2.5–5.0 mg m^{-3}) when compared to Zone 1 (0.5–1.5 mg m^{-3}).

A similar pattern was observed in the sea surface temperature (SST) monthly average in both zones, with a peak of 25–26°C in February, during the wet season, and lower values of 22.5–23°C in September (**Figures 2C,D**). Absorption due to phytoplankton at 443 nm (aph) showed a unimodal pattern, with high values in Zone 1 (peak in April; 0.05 m^{-1}) and the highest value in Zone 2 (April–May; 0.15 m^{-1}) (**Figures 2E,F**). Photosynthetically active radiation (PAR) presented a bimodal pattern in both zones. Zone 1 saw a maximum value in April (48 Einstein m^{-2} day^{-1}) and a lower peak in September (30 Einstein m^{-2} day^{-1}). In

[1]http://www.inocar.mil.ec

[2]http://www.marinespecies.org/

[3]http://www.marinespecies.org/

TABLE 2 | List of potentially harmful algal blooms (HABs) recorded in events along the coast of Ecuador (1997–2017).

Species	PTG	Type	ZONE 1		ZONE 2				References for toxicity	References for HABs recurrences in Ecuador
			Wet season		Dry season		Wet season			
			N	Max log cells L^{-1}	*N*	Max log cells L^{-1}	*N*	Max log cells L^{-1}		
Diarrhetic Shellfish Poisoning (DSP)										
Dinophysis caudata (Saville-Kent, 1881)	DINO	TOX	1		1				Ignatiades and Gotsis-Skretas, 2010; Nagai et al., 2011; Anderson et al., 2017	Torres, 2000, 2006, 2011
Dinophysis spp. (Ehrenberg, 1839)	DINO	TOX	1						Yasumoto et al., 1985; Hallegraeff, 1993; Lloyd et al., 2013; Reguera et al., 2014	Torres, 2011
Gonyaulax spp. (Diesing, 1866)	DINO	TOX			1				Wang, 2008	Torres and Palacios, 2007a
Prorocentrum mexicanum (Osorio-Tafall, 1942)	DINO	TOX	1	3.37			1		Ignatiades and Gotsis-Skretas, 2010; Muciño-Márquez et al., 2015	Coello, 2010; Torres, unpublished data (u.d)
Prorocentrum micans (Ehrenberg, 1834)	DINO	TOX	1	3.37	2		1		Razali et al., 2015; Lee et al., 2016	Torres, 2000, u.d; Torres and Tapia, 2002; Torres and Palacios, 2007b
Diarrhetic Shellfish Poisoning (DSP) and Venerupin Shellfish Poisoning										
Prorocentrum cordatum (Ostenfeld) (Dodge, 1975)	DINO	TOX			1		3		Heil et al., 2005; Lundholm, 2011	Torres et al., 2004; Torres and Palacios, 2007b; Torres, 2011
Paralytic Shellfish Poisoning (PSP)										
Alexandrium spp. (Halim, 1960)	DINO	TOX			1				Wang, 2008; Anderson et al., 1990, 2017; Food and Agriculture Organization [FAO], 2004; Ignatiades and Gotsis-Skretas, 2010	Torres and Palacios, 2007a
Gymnodinium catenatum (Graham, 1943)	DINO	TOX	1		3	6.05	1		Ignatiades and Gotsis-Skretas, 2010	Jiménez and Intriago, 2001; Torres and Palacios, 2007a; Torres, u.d.
Gymnodinium spp. (Stein, 1878)	DINO	TOX	4	5.22	6	7.05	6	7.45	Wang, 2008	Torres, 2000, 2006, 2011, u.d.; Torres and Palacios, 2007a,b
Amnesic Shellfish Poisoning (ASP)										
Pseudo-nitzschia spp. (Peragallo and Peragallo, 1900)	DINO	TOX			1	6.68			Rhodes et al., 1998; Trainer et al., 2012; Anderson et al., 2017	Torres, 2006, 2011
Neurologic or Neurotoxic Shellfish Poisoning (NSP)										
Gonyaulax spinifera (Claparède and Lachmann) (Diesing, 1866)	DINO	TOX			1				Wang, 2008; Alexander et al., 2009; Anderson et al., 2017	Torres and Palacios, 2007a
Karenia brevis (C. C. Davis) (Daugbjerg et al., 2000)	DINO	TOX					4	7.72	Food and Agriculture Organization [FAO], 2004; Pierce and Henry, 2008; Corcoran et al., 2014	Torres et al., 2004

(Continued)

TABLE 2 | Continued

Species	PTG	Type	ZONE 1		ZONE 2				References for toxicity	References for HABs recurrences in Ecuador
			Wet season		Dry season		Wet season			
			N	Max log cells L^{-1}	*N*	Max log cells L^{-1}	*N*	Max log cells L^{-1}		
Potentially Ichthyotoxic Species										
Gyrodinium spp. (Kofoid and Swezy, 1921)	DINO	NOC	2	4.45	3		2	7.75	Alonso-Rodríguez and Páez-Osuna, 2003; Ignatiades and Gotsis-Skretas, 2010	Torres, 2000, 2011, u.d.; Torres et al., 2004; Torres and Palacios, 2007b
Margalefidinium catenatum (Okamura, 1916; Gómez et al., 2017)	DINO	NOC	2	6.94	1		1		Cortés et al., 2004; Matsuoka et al., 2008; Kudela and Gobler, 2012; Gómez et al., 2017	Torres et al., 2004; Torres, 2011, 2016, u.d.
Potentially Fish-killing and Bloom-forming Species										
Mesodinium rubrum (Lohmann, 1908)	CILI	NOC	9	6.69	4	7.06			Jiménez and Intriago, 2001; Cortés-Lara, 2002; Gárate-Lizárraga et al., 2002; Wang et al., 2008; Torres, 2011	Torres, 2000, 2006, 2011; Jiménez and Intriago, 2001; Coello, 2003; Jiménez and Gualancañay, 2006; Coello and Cajas, 2007
Coscinodiscus spp. (Ehrenberg, 1839)	CDIAT	NOC			1	5.20	1	4.30	Mathew et al., 1988; Torres, 2011	Torres and Palacios, 2007b; Coello et al., 2010
Skeletonema costatum (Greville) (Cleve, 1873)	CDIAT	NOC	1	6.10	1	5.88	4	6.14	Hallegraeff, 1993; Suaréz and Guzmán, 1994; Chen et al., 2007; Anderson et al., 2017	Torres et al., 2004; Torres and Palacios, 2007b; Coello et al., 2010; Torres, 2016
Akashiwo sanguinea (K. Hirasaka) (Daugbjerg et al., 2000)	DINO	NOC	1	7.15					Hallegraeff, 2004; White et al., 2014; Anderson et al., 2017	Coello, 2003; Torres et al., 2004; Torres, 2011
Ceratium tripos (O. F. Müller) (Nitzsch, 1817)	DINO	NOC	1						Suaréz and Guzmán, 1994; Hallegraeff, 2004; Torres et al., 2004; Torres, 2011	Torres, 2000
Cochlodinium spp. (Schütt, 1896)	DINO	NOC			2	6.72	1	6.64	Hallegraeff, 2004; Kudela and Gobler, 2012; Razali et al., 2015	Torres, 2000, 2011; Torres et al., 2004; Torres, 2011
Kryptoperidinium foliaceum (F. Stein) (Lindemann, 1924)	DINO	NOC					1	4.00	Kempton et al., 2002; Saburova et al., 2012; Merino-Virgilio et al., 2014	Jiménez and Gualancañay, 2006; Torres, 2011
Levanderina fissa (Levander) (Moestrup et al., 2014)	DINO	NOC	6	7.00					Alonso-Rodríguez and Páez-Osuna, 2003; Gárate-Lizárraga, 2014	Jiménez and Intriago, 2001; Jiménez and Gualancañay, 2006; Torres, 2011
Noctiluca scintillans (Macartney) (Kofoid and Swezy, 1921)	DINO	NOC	2	3.97	2	6.06	4	6.41	Thangaraja et al., 2007; Wang et al., 2008; Ibrahem and Al-Shawi, 2015; Razali et al., 2015	Torres, 2000, 2011, 2016, u.d.; Coello, 2003; Torres et al., 2004
Prorocentrum gracile (Schütt, 1895)	DINO	NOC	2						Gul and Saifullah, 2011; Muciño-Márquez et al., 2015; Anderson et al., 2017	Jiménez and Intriago, 2001; Torres et al., 2004
Tripos furca (Ehrenberg) (Gómez, 2013)	DINO	NOC	2		3				Edwards et al., 2006; Ibrahem and Al-Shawi, 2015; Razali et al., 2015; Yurimoto et al., 2015	Torres, 2000, 2006, 2011; Torres and Palacios, 2007a

(Continued)

TABLE 2 | Continued

Species	PTG	Type	ZONE 1		ZONE 2				References for toxicity	References for HABs recurrences in Ecuador
			Wet season		Dry season		Wet season			
			N	Max log cells L^{-1}	*N*	Max log cells L^{-1}	*N*	Max log cells L^{-1}		
Potentially Bloom-forming Species										
Chaetoceros affinis (Lauder, 1864)	CDIAT	NOC	1		1		1		Vyas et al., 1986; Konno et al., 2010; Razali et al., 2015; Anderson et al., 2017	Torres, 2000; Torres et al., 2004
Chaetoceros curvisetus (Cleve, 1889)	CDIAT	NOC	1		1				Shen et al., 2012; Begum et al., 2015; Lo et al., 2016	Torres, 2000
Chaetoceros levis (Schütt, 1896)	CDIAT	NOC	1						Razali et al., 2015	Torres, 2000
Thalassiosira spp. (Cleve, 1873; Hasle, 1973)	CDIAT	NOC	1	4.37	2	6.70	1	5.48	Hallegraeff, 2004; Torres, 2011; Anderson et al., 2017	Torres and Palacios, 2007a; Coello et al., 2010; Torres, 2011, u.d.
Navicula spp. (Blainville, 1825)	PDIAT	NOC					1	4.48	Alonso-Rodríguez and Páez-Osuna, 2003; Razali et al., 2015	Coello et al., 2010
Asterionella frauenfeldii (Grunow, 1863)	PDIAT	NOC			1		1		Perumal et al., 1999; Jugnu and Kripa, 2008; Naha et al., 2014	Torres, 2000; Torres et al., 2004
Nitzschia longissima (Brébisson) (Pritchard, 1861)	PDIAT	NOC					1		Merino-Virgilio et al., 2014; Razali et al., 2015; Anderson et al., 2017	Torres et al., 2004
Thalassionema nitzschioides (Grunow) (Mereschkowsky, 1902)	PDIAT	NOC					2	4.78	Perumal et al., 1999; Merino-Virgilio et al., 2014; Begum et al., 2015; Padmakumar et al., 2018	Torres et al., 2004; Coello et al., 2010
Ceratium spp. (Schrank, 1793)	DINO	NOC	1						Thangaraja et al., 2007; Gómez et al., 2008; Padmakumar et al., 2018	Jiménez and Gualancañay, 2006; Torres, 2011
Ceratium trichoceros (Ehrenberg) (Saville-Kent, 1881; Kofoid and Swezy, 1921)	DINO	NOC	1				1		Jiménez and Intriago, 2001; Thangaraja et al., 2007; Torres, 2011; Ibrahem and Al-Shawi, 2015	Jiménez and Intriago, 2001
Protoperidinium quarnerense (B. Schröder) (Balech, 1974)	DINO	NOC			1				Torres et al., 2004; Gómez et al., 2008	Torres, 2000
Pyrophacus steinii	DINO	NOC	2		1				Razali et al., 2015; Wall and Cicily, 2015	Torres, 2000, 2011, u.d.
Triadinium polyedricum (Schiller) (Wall and Dale, 1971)	DINO	NOC			1				Shin et al., 2016	Torres and Palacios, 2007a
Tripos macroceros (Ehrenberg) (Gómez, 2013)	DINO	NOC	2				1		Thangaraja et al., 2007; Torres, 2011; Ibrahem and Al-Shawi, 2015	Torres, 2000
Tripos dens (Ostenfeld and Johannes Schmidt) (Gómez, 2013)	DINO	NOC	1		3	6.54	1		Horner et al., 1997; Pitcher and Calder, 2000; Alonso-Rodríguez and Páez-Osuna, 2003; Torres, 2011	Torres and Palacios, 2007a; Torres, 2011

The table includes potentially toxic (TOX) and non-toxic (NOC) species. The toxic species for humans are represented through syndromes. The non-toxic species are: (i) Potentially ichthyotoxic species—this group causes physical damage or irritation of the gills, are toxigenic, and cause hypoxia from oxygen depletion. It is also capable of causing the massive death of fish. The causal mechanisms are uncertain and the studies carried out are inconclusive (Andersen, 2012); (ii) Potentially fish-killing species—this group differs from the previous one because the death of the fish is caused by the decrease of oxygen as a consequence of the bloom's great biomass; (iii) Potentially bloom-forming species. The species are classified in four phytoplankton taxonomic groups (PTG): dinoflagellates (DINO), centric diatoms (CDIAT), pennate diatoms, and ciliates (CILI). N, number of occurrences. Log C, maximum abundances in log10 (cells/L). The occurrences and concentrations are shown by dry and wet seasonality in the study areas (Zone 1 and Zone 2).

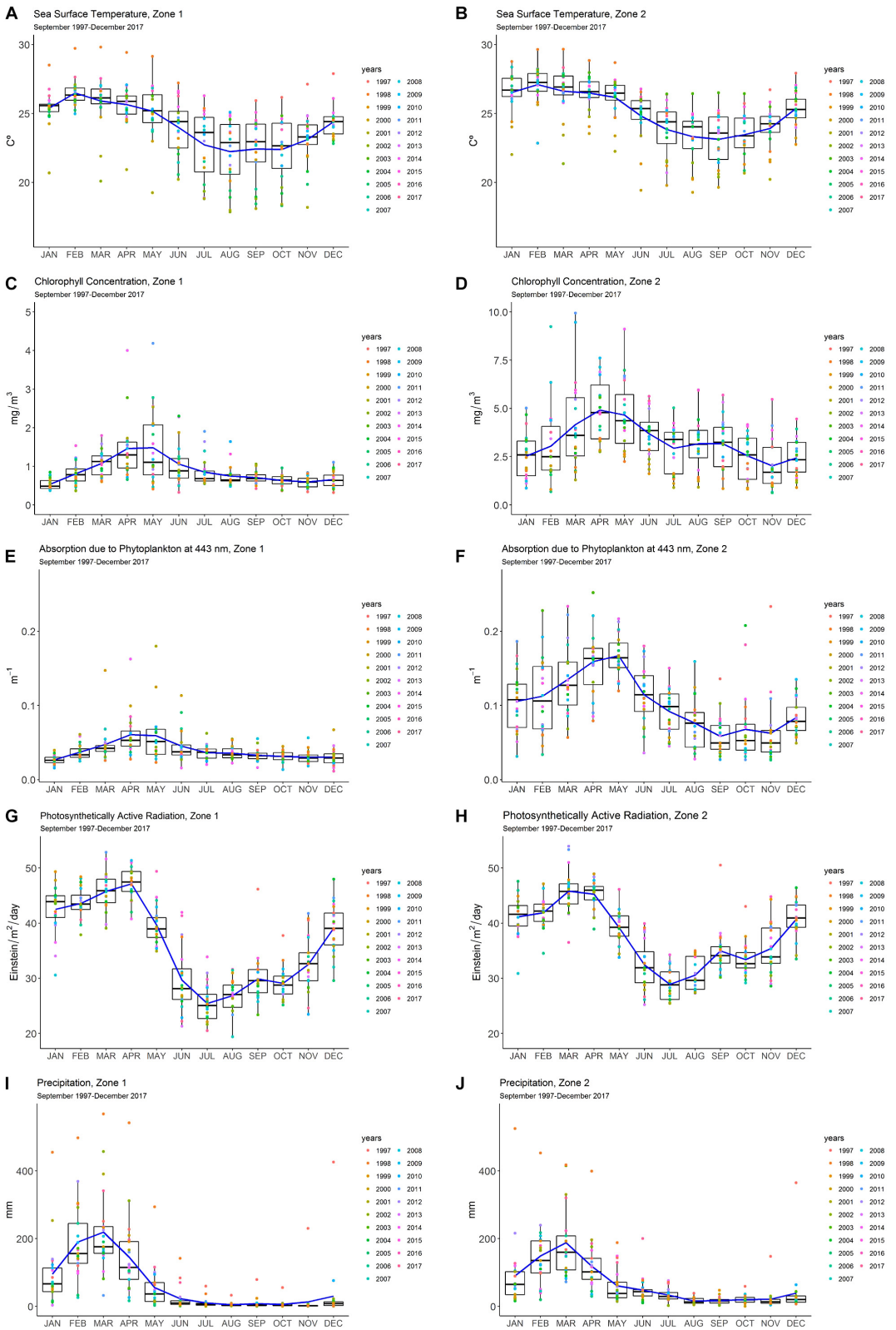


FIGURE 2 | Panel diagram of the monthly means of the remote sensing data of oceanographic variables: **(A,B)** sea surface temperature (SST); **(C,D)** chlorophyll *a* (chl-a); **(E,F)** absorption due to phytoplankton at 443 nm (aph); **(G,H)** photosynthetically active radiation (PAR) and atmospheric variable; **(I,J)** precipitation (PRE) for Zones 1 and 2 on the Ecuadorian coast (1997–2017). Seasonal pattern and inter-annual variability are shown with the blue line and the colored dots, respectively.

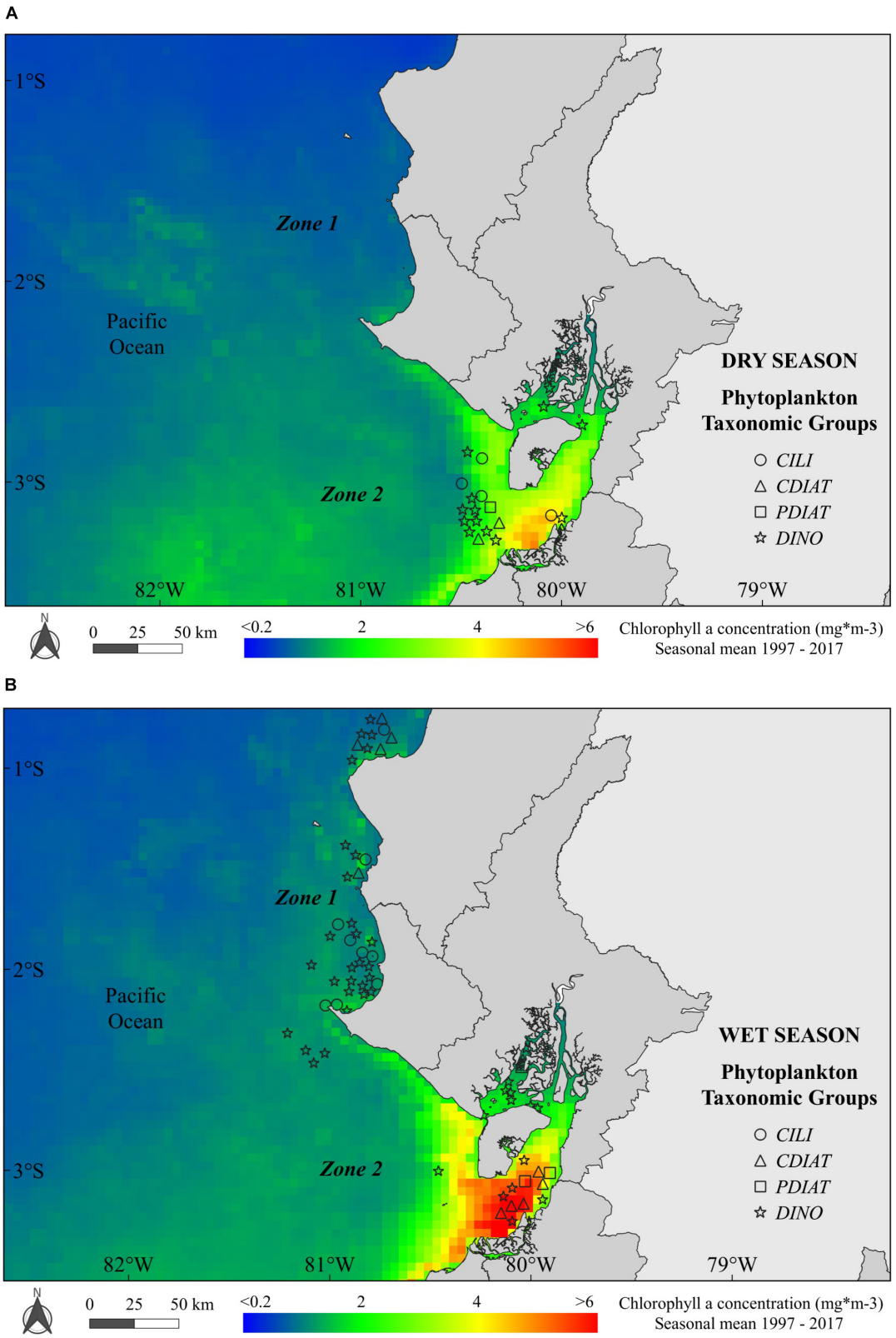


FIGURE 3 | Spatial distribution of occurrences of phytoplankton taxonomic groups (PTGs: ciliates, centric diatoms, pennate diatoms and dinoflagellates) along the Ecuadorian coast during **(A)** the dry season—occurrences of PTGs and **(B)** the wet season—occurrences of PTGs in Zones 1 and 2 of the study area. Background colors show the seasonal mean of chlorophyll *a* for the dry and wet seasons.

Zone 2, the maximum average value was registered in March (48 Einstein m^{-2} day^{-1}) and a second peak in September (32 Einstein m^{-2} day^{-1}) (**Figures 2G,H**). Precipitation showed a similar pattern for both zones, with an average peak in the wet season (March) both for Zone 1 (∼200 mm) and Zone 2 (180 mm) (**Figures 2I,J**).

Distribution and Seasonality of HAB and Phytoplankton Taxonomic Groups

A detailed checklist of the four PTGs is presented in **Table 2** and includes 40 species, 67 HAB occurrences, maximum abundance levels according to category in log (log base 10) of cells L^{-1}, HAB category (NOC or TOX), season (dry and wet), and the respective toxicity and reporting references (See **Suplementary Tables S1, S2**).

Harmful algal blooms showed strong seasonal characteristics: (a) there were 46 registered occurrences of PTG in Zone 2 during the dry season (**Figure 3A**; there were no data for Zone 1; see section "Materials and Methods"); and b) 48 (Zone 1) and 40 (Zone 2) PTG occurrences during the wet season (**Figure 3B**). Regarding the spatial distribution during the dry season, the chl-a ranged from ∼1–1.5 mg m^{-3} in Zone 1 (from Manta to La Libertad) to ∼2–3 mg m^{-3} in Zone 2 at the Gulf of Guayaquil, and it varied along with the clusters of PTG (**Figure 3A**). On the other hand, during the wet season, most of the PTG were DINO, both in Zones 1 and 2. In particular, PTGs coincided with higher chl-a (3–5 mg m^{-3}) in Zone 2 at the Jambelí Channel (**Figure 3B**).

During the dry season, in Zone 2, DINO were the most common PTG (72% of occurrences), followed by CDIAT (13%), PDIAT (4%), and CILI (11%) (**Figure 4**). On the other hand, during the wet season, in both zones DINO reached ∼70%, while CDIAT comprised only 10% in Zone 1 and 18% in Zone 2. PDIAT amounted to 13% in Zone 2 and 19% was CILI, but only in Zone 1 (**Figure 4**).

Regarding the abundance of PTG, dinoflagellates presented great variability in their abundance, ranging from 3.4 to 7.8 log of cells L^{-1} during both the dry and wet seasons, reaching a maximum in Zone 2 of the Gulf of Guayaquil. Centric diatoms were higher during the dry season (5.2–6.7 log of cells L^{-1}) than in the wet season (4.3–6 log of cells L^{-1}), reaching a maximum in Zone 2. Pennate diatoms were reported only 4 times in 20 years of data, with a maximum concentration of 6.7 log of cells L^{-1} (**Figure 5**).

Harmful Algal Bloom Species Assembled Along the Ecuadorian Coast

Harmful algal blooms reports from 1997 to 2017 determined a total of 40 species of phytoplankton: 28 were identified as non-toxic, whereas the other 12 are well known to produce toxins (**Table 2**). The most recurrent genera were *Gymnodinium* (21 occurrences) and *Prorocentrum* (10). Other observed genera

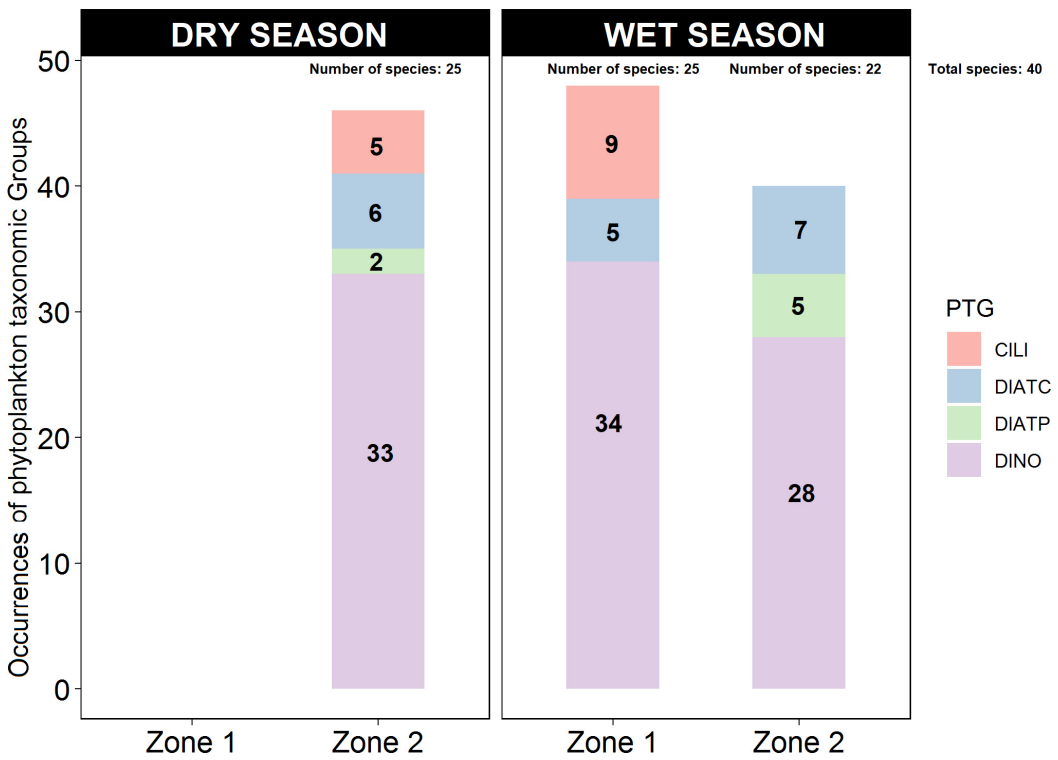


FIGURE 4 | Occurrences of phytoplankton taxonomic groups (PTGs) in Zones 1 and 2 of the study area during the dry and wet seasons. For the period 1997–2017, a total of 46 PTG occurrences with 25 species were reported in the dry season in Zone 2, 48 PTG occurrences with 25 species were reported in Zone 1, and 40 PTG occurrences and 22 species were reported in Zone 2 in the wet season.

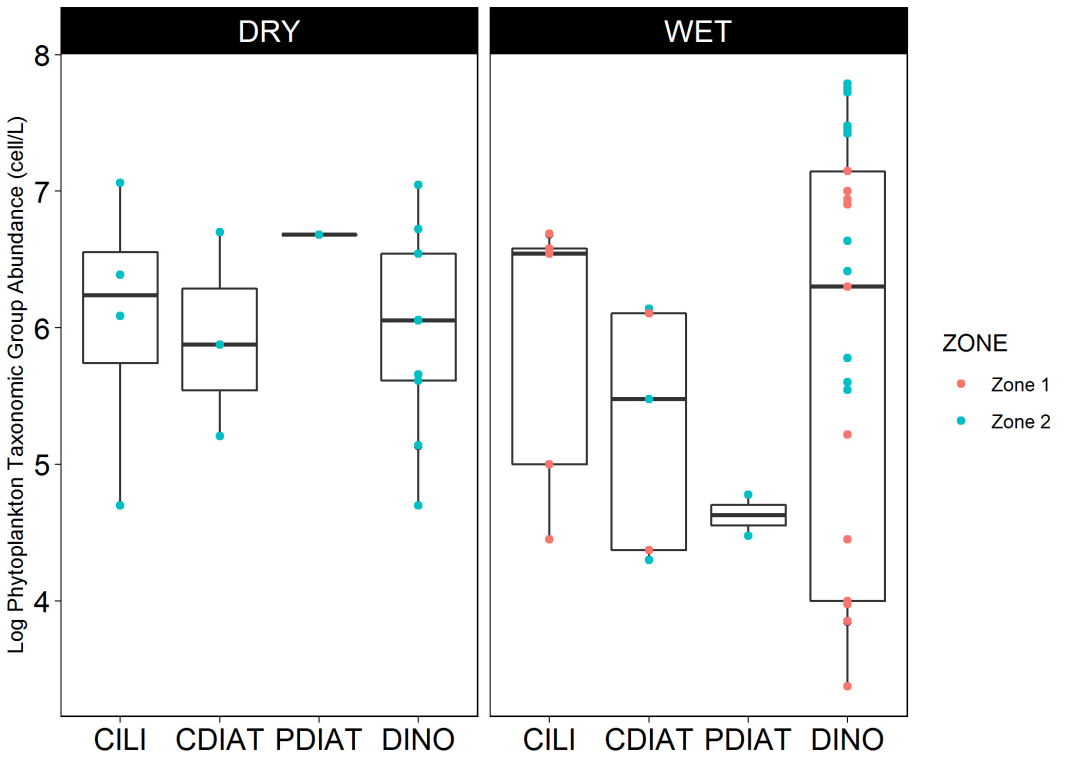


FIGURE 5 | Seasonal abundance of phytoplankton taxonomic groups during HABs events in Zones 1 and 2 of the study area.

were *Karenia* (4), *Dinophysis* (3), and *Pseudo-nitzschia* (1). *Gymnodinium catenatum* (5 occurrences) was observed in the years 2000, 2004, 2008, and 2014 in several locations in Zone 1 and in the Jambeli Channel in Zone 2, with a maximum abundance of 6.05 log of cells L^{-1}. *Prorocentrum cordatum* (4 occurrences) was observed in 2003 and 2004 in the Jambelí Channel and Estero Salado in Zone 2, but unfortunately without abundantly available data. *Prorocentrum mexicanum* (2 occurrences) with a maximum abundance of 3.4 log of cells L^{-1} in 2009 and 2016 was reported in Manta in Zone 1 and in Puerto Bolivar and the Jambelí Channel in Zone 2. *Prorocentrum micans* (4 occurrences), with a maximum abundance of 3.4 log of cells L^{-1}, was reported in Manta in Zone 1 and in Isla Santa Clara, Puerto Bolivar, in Zone 2. Microphotographs of Lugol-preserved colonies of *Gymnodinium catenatum* are shown in **Figures 6A,B**. This sample was found in San Pedro (Zone 1) in March 2018. A potentially harmful, non-toxic *Cochlodinium catenatum*, a synonym of *Margalefidinium catenatum*, is shown in **Figure 6C**. This sample was found in La Libertad in March 2018. However, the HAB-forming dinoflagellate *Cochlodinium catenatum* needs further samplings/taxonomic studies to ensure a precise identification.

Other groups of 28 species were categorized as non-toxic algal blooms in order of occurrence: 16 taxa of dinoflagellates, including *Noctiluca scintillans*, *Gyrodinium* spp., *Levanderina fissa*, *Tripos dens*, and *Tripos furca*, six centric diatoms, including *Skeletonema costatum*, *Thalassiosira* spp., and *Chaetoceros affinis*, four pennate diatoms, including *Asterionella frauenfeldii*, *Thalassionema nitzschioides*, *Nitzschia longissima*, and *Navicula* spp., and just one ciliate—*Mesodinium rubrum* (**Table 2**). The highest NOC species abundance was represented by *Gyrodinium* spp., attaining 7.75 log of cells L^{-1}, found in Estero Salado (Zone 2) during 1999's wet season. The highest abundance of *Mesodinium rubrum* was 7.06 log of cells L^{-1}, found in Jambeli Channel (Zone 2) during the 2007 dry season. A greater variability in abundance was observed during the wet season for most of the PTG.

Multi-Correspondence Analysis of PTG and Remote Sensing Data

In Zone 1 (**Figure 7**), the occurrence of toxic HABs corresponded to low abundances of DINO and CDIAT. These biological features seem to be associated with high values of SST, PRE, and PAR and to low chl-a and aph values (443 nm). All these environmental features characterized the wet season (**Figure 7**). Conversely, non-toxic HABs corresponded to a high abundance of DINO and CILI and to low SST and PRE, but with high aph (**Figure 7**).

During the wet season in Zone 2 (**Figure 8**), TOX events corresponded to high biomass levels of DINO, coupled with high SST, PAR, and PRE. However, during the dry season, low PRE and PAR and colder waters were linked to a high abundance of CDIAT. These features are simultaneously related to NOC species.

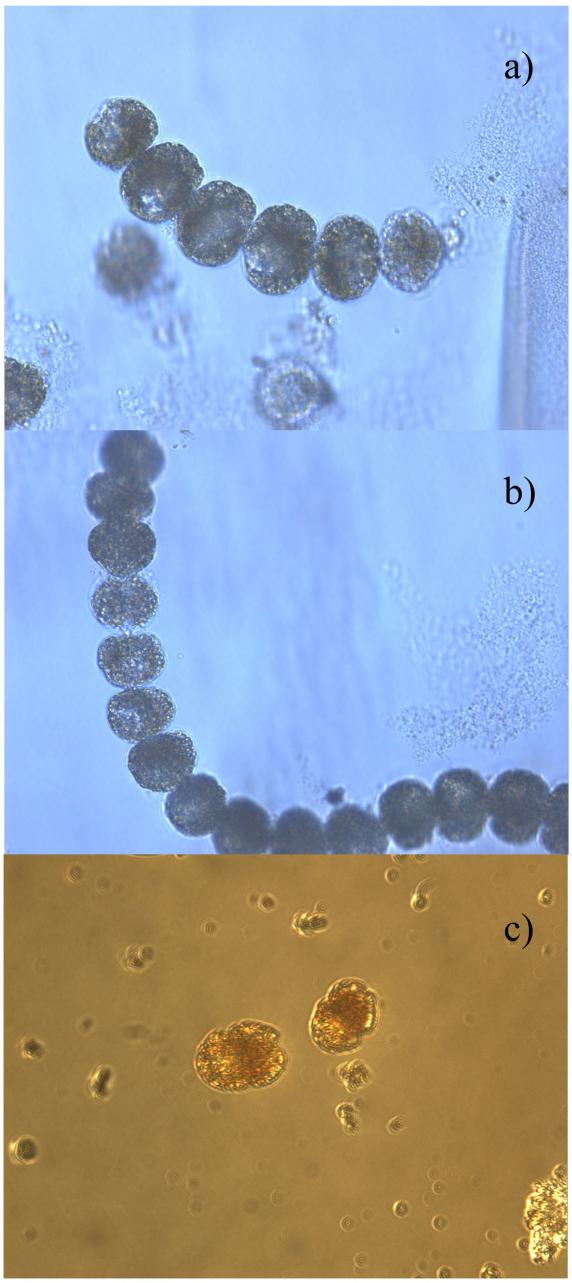


FIGURE 6 | Microphotographs of Lugol-preserved colonies associated with toxic or non-toxic species collected on the Ecuadorian coast: **(A,B)** *Gymnodinium catenatum* (Graham, 1943) found in San Pedro (Zone 1) in 2018. This taxon is associated with a paralytic toxin; **(C)** *Cochlodinium catenatum* (Schütt, 1896), found in La Libertad in Zone 1 in March 2018.

DISCUSSION

Oceanography of HABs and Remote Sensing Data

This study confirms that HAB dynamics in the Ecuadorian coastal zone seem to be constrained by the Humboldt upwelling system, high precipitation, and terrestrial nutrients from the Guayas Basin, aligning with other HAB studies in coastal ecosystems and upwelling zones (Kudela et al., 2005; Vila and Masó, 2005; Borbor-Cordova et al., 2006; Anderson et al., 2017; Conde et al., 2018; Oyarzún and Brierley, 2018). In addition, high sea surface temperature during ENSO and ocean heat waves seem to affect all the aforementioned drivers, along with the adaptive strategies of the phytoplankton species and their community assemblage (Masó and Garcés, 2006; Anderson, 2014; Anderson et al., 2017).

The findings of this study reveal that a higher biomass of HABs was found in Zone 2 than in Zone 1 when considering eutrophic and oligotrophic systems, respectively, based on chl-a concentration. Dinoflagellates were the taxonomic group most highly associated with HABs all along the Ecuadorian coast. An interesting finding is that toxic species of DINO appeared either at low or high levels of abundance in Zone 1 or Zone 2, respectively, mostly during the wet season.

These results agree with the findings presented in previous research, demonstrating that dinoflagellate species that form blooms utilize diverse ecological strategies as colonists and r-strategists in nutrient-rich, disturbed environments, or are nutrient stress tolerant in oligotrophic systems (Smayda and Reynolds, 2003; Smayda and Trainer, 2010). Dinoflagellates use a C-strategy (as colonists) to adapt, reaching high levels of biomass and becoming potentially toxic species in nutrient-enriched, high irradiance, estuarine habitats, such as that found in Zone 2. Whereas in the oligotrophic Zone 1, with its highly stratified and high irradiance conditions, a nutrient stress-tolerant ensemble of dinoflagellate species occurred at very low biomass levels (Smayda and Reynolds, 2003; Suparna, 2005; Smayda and Trainer, 2010; Corcoran et al., 2014). For instance, *Gymnodinium catenatum* and *Karenia brevis*, considered toxic species, were found at high levels of abundance in Zone 2, mostly in the wet season, while *Prorocentrum micans* and *Prorocentrum mexicanum* were reported at low concentrations in the oligotrophic Zone 1, but also in Zone 2, in the wet season.

Considering the bloom behavior of diatoms, previous research found that they occur in coastal and upwelling zones and are annually recurrent and prolonged in duration, with high species diversity (Smayda and Reynolds, 2003). In our work, annual periodicity and high diversity were not evident, with only 7–5 occurrences of CDIAT (6 species) and PDIAT (5 species), respectively, mostly in the Jambeli Channel in Zone 2. Non-toxic HABs occurred with high abundance values of DINO, CILI, and CDIAT and were mostly associated with the cold water from the upwelling system, low precipitation, and irradiance, both in Zones 1 and 2 in environmental conditions more related to the dry season. This work suggests that there is a higher risk of HABs in the Gulf of Guayaquil, at the entrance of the Guayas Estuary (dry season), in the Jambeli Channel and Estero Salado (wet season), and in La Libertad and Manta in the dry season (**Figures 2A,B**).

However, the question remains as to the biological drivers of the presence of potentially toxic or non-toxic HABs on the Ecuadorian coast (in the eastern Pacific Ocean). It is known that species-specific adaptive strategies within the phytoplankton

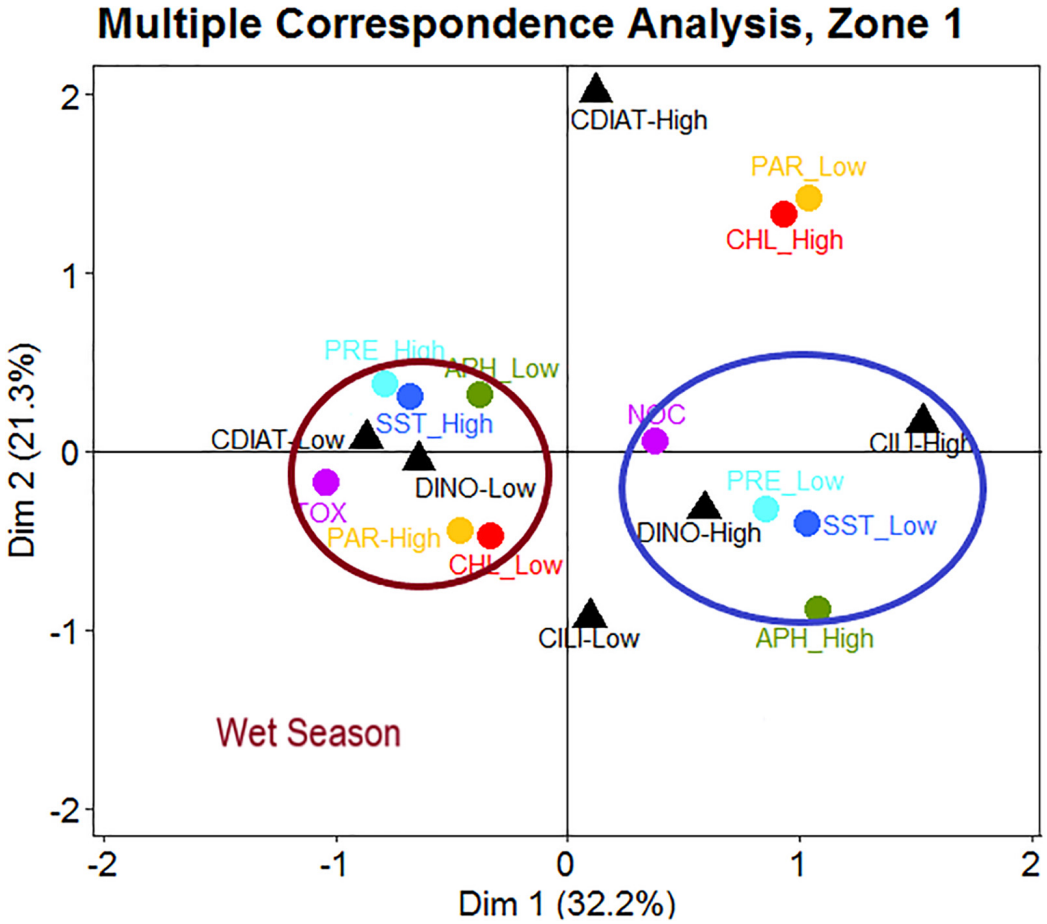


FIGURE 7 | In Zone 1, occurrences of toxic HABs (TOX) linked to low concentrations of dinoflagellates (DINO) and centric diatoms (CDIAT) are associated with high levels of sea surface temperature (SST), precipitation (PRE), and photosynthetic active radiation (PAR) as well as low levels of absorption coefficient of phytoplankton at 443 nm (aph) and chlorophyll *a* (chl-a) appearing during the wet season. Events of non-toxic HABs (NOC) appearing with high concentrations of DINO and ciliates (CILI) are associated with low levels of SST and PRE, accompanied by high values of aph.

community enable adaption to specific habitats and climate conditions (e.g., ENSO) (Prado et al., 2015). Adaptive and behavioral adaptation strategies of HABs may include mobility behavior, such as vertical migration, swimming patterns, phototaxis, and life cycle strategies as resting cysts, and phytoplankton development phases (Masó and Garcés, 2006; Anderson, 2014; Anderson et al., 2017). Future research on these aspects of the adaptive strategies of HABs are necessary for the region.

On the other hand, this study suggests that upwelling systems are related to HABs in the Gulf of Guayaquil. The Humboldt upwelling system is considered to be one of the most productive systems globally (Daneri et al., 2000; Taylor, 2008; Oyarzún and Brierley, 2018). Research groups, such as GEOHAB (Hallegraeff, 2004), have highlighted the potential impacts of HABs on upwelling systems in the context of climate change (Kudela et al., 2005; Edwards et al., 2006; Wei et al., 2008; Trainer et al., 2010; Díaz et al., 2016; Pitcher et al., 2017). In the case of Ecuador and Peru, the Humboldt upwelling system is the foundation of both countries' fishery productivity and the economic benefits of the fishery industry, which can be impacted by emergent HABs (Pennington et al., 2006; Trainer et al., 2010). Considering that there are limited studies on the upwelling systems in the Gulf of Guayaquil region (Oyarzún and Brierley, 2018), understanding these dynamics is critical to assess the future impacts of a changing climate in this very productive ecosystem.

Harmful Algal Blooms and Human Health

Emergent issues concerning upwelling systems and HABs relate to the occurrence of phytoplankton species able to produce phycotoxins and jeopardize human health through paralytic shellfish poisoning, diarrhetic shellfish poisoning, and amnesic shellfish poisoning, as well as other harmful non-toxic blooms resulting in hypoxic and anoxic environments with potentially high negative impacts on fishery resources (Trainer et al., 2010; Pitcher et al., 2017). Phycotoxins can be transferred to human beings through the ingestion of contaminated seafood, thereby posing a potential risk to human health (Fleming et al., 2001; Alonso-Rodríguez and Páez-Osuna, 2003; Backer et al., 2003; Berdalet et al., 2016). Since the Gulf of Guayaquil and Zone 1 are very important for tourism activities, and are particularly known

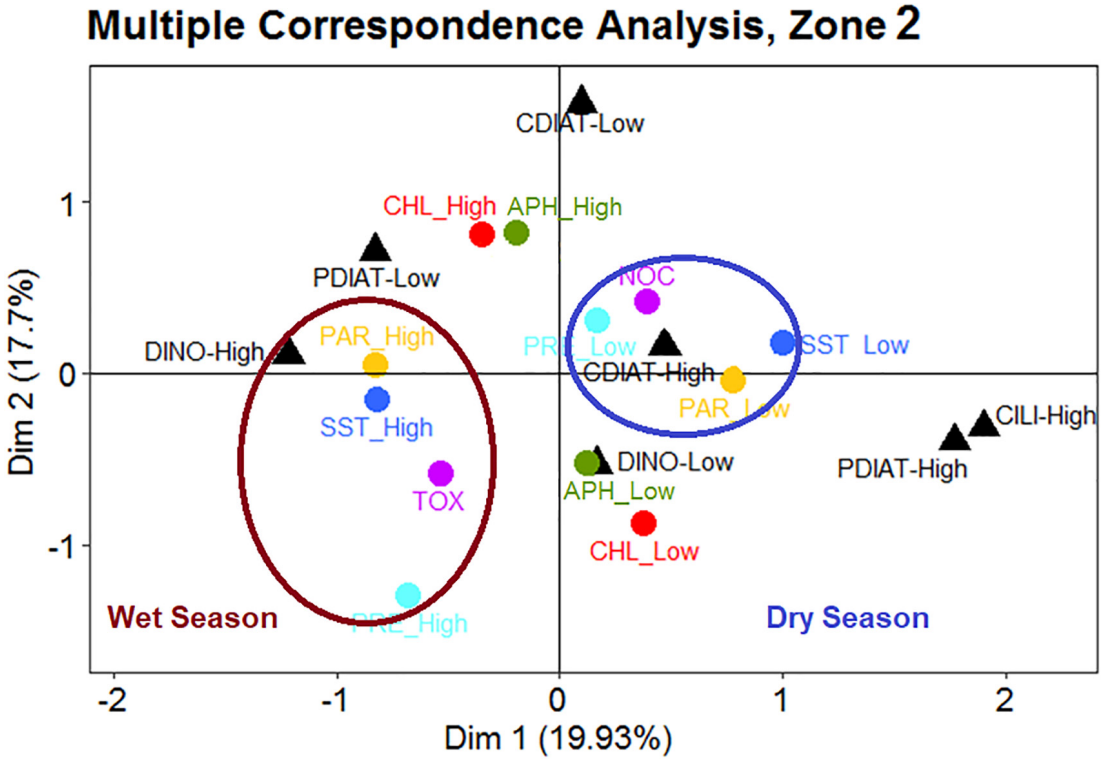


FIGURE 8 | In Zone 2, occurrences of toxic HABs (TOX) are associated with high levels of sea surface temperature (SST), photosynthetic active radiation (PAR), and precipitation (PRE), accompanied by high concentrations of dinoflagellates during the wet season. Events of non-toxic HABs (NOC) seem to be associated with low levels of SST, PRE and PAR and high levels of centric diatoms during the dry season.

for their seafood, a HABs monitoring program, by research and academic institutions, and a preventive policy to protect tourism and public health should be developed as a priority.

A recent study on the risk perceptions of coastal managers, public health officers, and coastal communities in Ecuador revealed that there is limited knowledge of the health impacts of "red tides" and HABs (Borbor-Cordova et al., 2018). While there have not been any reports of human beings affected by HAB toxins in Ecuador to date, this may be explained by the lack of awareness of the syndromes they cause (Borbor-Cordova et al., 2018). *Gymnodinium catenatum*, *Prorocentrum cordatum*, *Prorocentrum mexicanum*, *Prorocentrum micans*, *Karenia brevis*, *Alexandrium* spp., *Dinophysis caudata*, and *Pseudo-nitzschia* spp. are species found along the Ecuadorian coast that are associated with syndromes related to seafood toxicity. The toxins associated with algal blooms and affected seafood for the aforementioned species are described in **Supplementary Table S3**. The main vectors of algal toxins to humans are filter-feeding bivalve mollusks and finfish that ingest toxic algae. The main shellfish toxic syndromes include: paralytic shellfish poisoning, associated with *Gymnodinium catenatum*, diarrhetic shellfish poisoning, associated with *Dinophysis* spp. and *Prorocentrum* spp., neurotoxic shellfish poisoning, associated with *Karenia brevis*, and amnesic shellfish poisoning, linked to *Pseudo-nitzschia* (**Supplementary Table S3**). Symptoms depend upon the toxin and can be temporary, chronic, or even lethal (Backer et al., 2003, 2005). A main limitation of this study is that no analysis of toxins was done during the HABs events, thus an important further step would be to implement a toxins analysis in Ecuador.

Finally, HAB research has shown that exotic species of dinoflagellates can be introduced into the environment through ballast water from shipping (Barry et al., 2008). The intense harbor activities carried out within the Gulf of Guayaquil, with its high volume of international traffic, makes it a site of great risk for the introduction of exotic harmful microalgae, which is therefore another important reason to develop a HABs monitoring program in Ecuador.

Management Implications for Monitoring Ecosystem Health on the Ecuadorian Coast

The findings of this study raise many questions about high levels of productivity and HABs, upwelling conditions and their interaction with extreme events, such as El Niño or La Niña, and the associated impact on human health on the Ecuadorian coast. There are many knowledge gaps in terms of how climate ocean drivers, vertical patterns of mixing and stratification, and upwelling nutrients trigger high levels of productivity, as well as HABs. Thus, this study recommends the design of an integrated ecosystem health research program for the Gulf of Guayaquil

and the central Ecuadorian coast. This program would attempt to increase knowledge of the drivers of algal growth, analyze the effects of extreme climatic events and upwelling systems on phytoplankton communities, monitor eutrophication indicators, and identify toxins (Hallegraeff, 2010; Anderson et al., 2017). The program would also develop an early warning system for potentially toxic algal blooms and introduced HAB species. Such an ecosystem health research program would be based on a collaborative framework of stakeholders from the scientific community, public health institutions, and marine resources management organizations, such as scientific institutions, health promoters, residents, and the tourism industry—whoever is interested in participating in citizen science (Fleming et al., 2006; Anderson, 2014). Finally, considering the intrinsic relationship between ocean and human health in relation to HABs, it is important to develop policies related to seafood safety, sanitary controls of shellfish, and deploy a fully equipped monitoring system for a biotoxin analysis program in Ecuador.

FINAL CONSIDERATIONS

Using remote sensing information combined with *in situ* historical data might help increase understanding of the drivers of high biomass phytoplankton within Ecuadorian coastal sites and indicate when those high biomass events might become toxic, harmful algal blooms. *In situ* sampling is critical to determine the composition and distribution of phytoplankton taxonomic groups and biotoxins, thus an integrated ecosystem health research program to monitor HABs in the Gulf of Guayaquil needs be developed and implemented in the near future. Upwelling studies linked to the ecology of the HABs in the Gulf of Guayaquil and the Humboldt Current are critical to maintaining the productivity of the coastal zone and managing its estuarine resources.

AUTHOR CONTRIBUTIONS

MB-C was responsible for the study design, developing an integrated framework, analyzing the data, and drafting the manuscript. GM-S and AC-T pre-processed and analyzed the data. GT collected and analyzed phytoplankton samples and analyzed the data. WR collected samples and reviewed all sections. Together, MB-C, BB, RB, and AC-T drafted the article. All authors discussed the results and reviewed and approved the final manuscript for submission.

FUNDING

This work was funded by the Escuela Superior Politécnica del Litoral (ESPOL) to the Transdisciplinary Project "Climate Variability and recurrence of harmful algae blooms and their impact on human health along with an estuarine-coastal gradient" (T2-DI-2014) and by the Instituto Oceanografico de la Armada del Ecuador (INOCAR).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2019.00013/full#supplementary-material

REFERENCES

Alexander, J., Benford, D., Boobis, A., Ceccatelli, S., Cravedi, J., Di Domenico, A., et al. (2009). Marine biotoxins in shellfish – Domoic acid scientific opinion of the Panel on Contaminants in the Food Chain adopted on 2 July 2009. *EFSA J.* 1181, 1–61. doi: 10.2903/j.efsa.2008.723

Alonso-Rodríguez, R., and Páez-Osuna, F. (2003). Nutrients, phytoplankton and harmful algal blooms in shrimp ponds: a review with special reference to the situation in the Gulf of California. *Aquaculture* 219, 317–336. doi: 10.1016/S0044-8486(02)00509-4

Andersen, G. N. (2012). *Ichthyotoxic Algae and Their Effects on Fish*. Ph.D. thesis, University of Copenhagen, Copenhagen.

Anderson, D. (2014). "HABs in a changing world: a perspective on harmful algal blooms, their impacts, and research and management in a dynamic era of climactic and environmental change," in *Proceedings of the 15th International Conference on Harmful Algae: October 29–November 2, 2012*, eds H. G. Kim, B. Reguera, G. M. Hallegraeff, and C. K. Lee (Changwon: CECO), 3–17.

Anderson, D. M., Boerlage, S. F. E., and Dixon, M. B. (eds) (2017). *Harmful Algal Blooms (HABs) and Desalination: A Guide to Impacts, Monitoring and Management*. Paris: Intergovernmental Oceanographic Commission of UNESCO.

Anderson, D. M., Kulis, D. M., Sullivan, J. J., Hall, S., and Lee, C. (1990). Dynamics and physiology of saxitoxin production by the dinoflagellates *Alexandrium* spp. *Mar. Biol.* 104, 511–524. doi: 10.1007/BF01314358

Backer, L., Fleming, L. E., Rowan, A., and Baden, D. (2003). "Epidemiology and public health of human illnesses associated with harmful marine phytoplankton," in *UNESCO Manual on Harmful Marine Algae*, eds G. M. Hallegraeff, D. M. Anderson, and A. D. Cembella (Geneva: WHO), 725–750.

Backer, L. C., Schurz, R. H., Fleming, L. E., Kirkpatrick, B., and Benson, J. (2005). "Phycotoxins in marine seafood," in *Chemical and Functional Properties of Food Components: Toxins in Food*, ed. W. Dabrowski (Boca Raton, FL: CRC Press), 155–190.

Balech, E. (1974). El Género "*Protoperidinium*" Bergh. 1881 ("*Peridinium*" Ehrenberg, 1831, Partim). *Rev. Museo Argentino Cienc. Nat. Inst. Nac. Inv. Cienc. Nat.* 4, 1–79.

Balech, E. (1988). Los dinoflagelados del Atlántico Sudoccidental. *Inst. Esp. Oceanogr.* 1, 1–300.

Barry, S. C., Hayes, K. R., Hewitt, C. L., Behrens, H. L., Dragsund, E., and Bakke, S. M. (2008). Ballast water risk assessment: principles, processes, and methods. *ICES J. Mar. Sci.* 65, 121–131. doi: 10.1093/icesjms/fsn004

Begum, M., Sahu, B. K., Das, A. K., Vinithkumar, N. V., and Kirubagaran, R. (2015). Extensive *Chaetoceros curvisetus* bloom in relation to water quality in Port Blair Bay, Andaman Islands. *Environ. Monit. Assess.* 187, 1–14. doi: 10.1007/s10661-015-4461-2

Bendix, J., Trachte, K., Palacios, E., Rollenbeck, R., Göttlicher, D., Nauss, T., et al. (2011). El Niño meets La Nina—anomalous rainfall patterns in the "traditional" El Niño region of Southern Ecuador. *Erdkunde* 65, 151–167. doi: 10.3112/erdkunde.2011.02.04

Berdalet, E., Fleming, L. E., Gowen, R., Davidson, K., Hess, P., Backer, L. C., et al. (2016). Marine harmful algal blooms, human health and wellbeing: challenges and opportunities in the 21st century. *J. Mar. Biol. Assoc. U.K.* 1, 61–91. doi: 10.1017/S0025315415001733

Blainville, H. M. D. (1825). *Dictionnaire des Sciences Naturelles*. Strasbourg: F.G. Levrault.

Blondeau-Patissier, D., Gower, J., Dekker, A., Phinn, S., and Brando, V. (2014). A review of ocean color remote sensing methods and statistical techniques

for the detection, mapping and analysis of phytoplankton blooms in coastal and open oceans. *Prog. Oceanogr.* 123, 123–144. doi: 10.1016/j.pocean.2013.12.008

Borbor-Cordova, M., Boyer, E., Mcdowell, W., and Hall, C. (2006). "Nitrogen and phosphorus budgets for a tropical watershed impacted by agricultural land use: Guayas, Ecuador," in *Nitrogen Cycling in the Americas: Natural and Anthropogenic Influences and Controls SE – 7*, eds L. Martinelli and R. Howarth (Dordrecht: Springer), 135–161. doi: 10.1007/978-1-4020-5517-1_7

Borbor-Cordova, M. J., Pozo-Cajas, M., Cedeno-Montesdeoca, A., Mantilla Saltos, G., Kislik, C., Espinoza-Celi, M. E., et al. (2018). Risk perception of coastal communities and authorities on harmful algal blooms in Ecuador. *Front. Mar. Sci.* 5:365. doi: 10.3389/fmars.2018.00365

Casey, K. S., Brandon, T. B., Cornillon, P., and Evans, R. (2010). "The past, present, and future of the AVHRR pathfinder SST program," in *Oceanography from Space*, eds V. Barale, J. Gower, and L. Alberotanza (Dordrecht: Springer), 273–287. doi: 10.1007/978-90-481-8681-5_16

Chavez, F. P., Ryan, J., Lluch-Cota, S. E., and Miguel, N. (2003). From anchovies to sardines and back: multidecadal change in the Pacific Ocean. *Science* 299, 217–221. doi: 10.1126/science.1075880

Chen, G. F., Wang, G. C., Zhang, B. Y., and Fan, X. L. (2007). Morphological and phylogenetic analysis of *Skeletonema costatum*-like diatoms (Bacillariophyta) from the China Sea. *Eur. J. Phycol.* 42, 163–175. doi: 10.1080/09670260601149784

Cleve, P. T. (1873). Examintaion of diatoms found on the surface of the sea of Java. *Bihang Till K. Svenska Vet. Akad. Handlingar.* 11, 1–17.

Cleve, P. T. (1889). "Pelagisk Diatomeer från Kattegat," in *Det Videnskabelige Udbytte Af Kanonbaaden "Hauchs" Togter I de Danske Have Indefor Skagen, I. Aarene 1883-86*, ed. C. G. J. Petersen (Kjøbenhavn: Andr. Fred. Høst & Sons Forlag), 53–56.

Coello, D. (2003). "Mareas rojas en el Ecuador", in *Proceedings of the Informe del Instituto Nacional de Pesca en Sexto Taller Regional de Planificación Científica sobre Floraciones de Algas Nocivas en Sudamérica*, Guayaquil.

Coello, D. (2010). Marea Roja ocasionada por Scripsiella trochoidea en el Estuario del río Chone (mayo 2007). *Rev. Ciencias Del Mar Limnol.* 4, 93–10.

Coello, D., and Cajas, J. (2007). *Plancton del Crucero Demersal. T07/06/02, junio de 2007*. Reporte Interno. Instituto Nacional de Pesca, Guayaquil.

Coello, D., Cajas, J., Macías, P., and Lindao, J. (2010). Floración algal ocasionada por *Prorocentrum mexicanum* en el canal de Jambelí. *Rev. Ciencias Del Mar Limnol.* 4, 25–33.

Conde, A., Hurtado, M., and Prado, M. (2018). Phytoplankton response to a weak El Niño event. *Ecol. Indic.* 95, 394–404. doi: 10.1016/j.ecolind.2018.07.064

Conde, A., and Prado, M. (2018). Changes in phytoplankton vertical distribution during an El Niño event. *Ecol. Indic.* 90, 201–205. doi: 10.1016/j.ecolind.2018.03.015

Corcoran, A. A., Richardson, B., and Flewelling, L. J. (2014). Effects of nutrient-limiting supply ratios on toxin content of *Karenia brevis* grown in continuous culture. *Harmful Algae* 39, 334–341. doi: 10.1016/j.hal.2014.08.009

Cortés, C., Cortés, R., and Sierra, A. (2004). Presencia de *Cochlodinium catenatum* (Gymnodiniales: Gymnodiniaceae) en mareas rojas de Bahía de Banderas, Pacífico mexicano. *Biol. Trop.* 52, 35–49.

Cortés-Lara, M. C. (2002). Primer registro de marea roja del 2002. Bahía de Banderas Jalisco- Nayarit (Enero de 2002). *Revista Biomédica* 13, 229–230. doi: 10.32776/revbiomed.v13i3.321

Cucalon, E. (1989). "Oceanographic characteristics off the coast of Ecuador," in *A Sustainable Shrimp Mariculture Industry for Ecuador*, eds S. Olsen and L. Arriaga (Narragansett, RI: Coastal Resources Center), 183–196.

Daneri, G., Dellarossa, V., Quiñones, R., Jacob, B., Montero, P., and Ulloa, O. (2000). Primary production and community respiration in the Humboldt current system off Chile and associated oceanic areas. *Mar. Ecol. Prog. Ser.* 197, 41–49. doi: 10.3354/meps197041

Daugbjerg, N., Hansen, G., Larsen, J., and Moestrup, Ø., (2000). Phylogeny of some of the major genera of dinoflagellates based on ultrastructure and partial LSU rDNA sequence data, including the erection of three new genera of unarmoured dinoflagellates. *Phycologia* 39, 302–317. doi: 10.2216/i0031-8884-39-4-302.1

Davidson, K., Gowen, R. J., Harrison, P. J., Fleming, L. E., Hoagland, P., and Moschonas, G. (2014). Anthropogenic nutrients and harmful algae in coastal waters. *J. Environ. Manage.* 146, 206–216. doi: 10.1016/j.jenvman.2014.07.002

Díaz, P. A., Reguera, B., Ruiz-Villarreal, M., Pazos, Y., Velo-Suárez, L., Berger, H., et al. (2013). Climate variability and oceanographic settings associated with interannual variability in the initiation of *Dinophysis acuminata* blooms. *Mar. Drugs* 11, 2964–2981. doi: 10.3390/md11082964

Díaz, P. A., Ruiz-Villarreal, M., Pazos, Y., Moita, T., and Reguera, B. (2016). Climate variability and *Dinophysis acuta* blooms in an upwelling system. *Harmful Algae* 53, 145–159. doi: 10.1016/j.hal.2015.11.007

Diesing, K. M. (1866). Revision der Prothelminthen. Abtheilung: Mastigophoren. Sitzungsberichte Der Kaiserlichen Akademie Der Wissenschaften. Mathematisch-Naturwissenschaftliche Classe. *Abt. 1 Mineral. Botanik Zool. Anat. Geol. Paläontologie*, 52, 287–401.

Dodge, J. D. (1975). The Prorocentrales (Dinophyceae). II. Revision of the taxonomy within the genus *Prorocentrum*. *Bot. J. Linn. Soc.* 71, 103–125. doi: 10.1109/ICTD.2009.5426693

Edwards, M., Johns, D. G., Richardson, A. J., Leterme, S. C., Svendsen, E., and Richardson, A. J. (2006). Regional climate change and harmful algal blooms in the northeast Atlantic. *Limnol. Oceanogr.* 51, 820–829. doi: 10.4319/lo.2006.51.2.0820

Ehrenberg, C. G. (1834). Dritter Beitrag Zur Erkenntniss Grosser Organisation in Der Richtung Des Kleinsten Raumes. *Abh. Königl. Akad. Wiss. Berlin* 1833, 145–336.

Ehrenberg, C. G. (1839). Über die Bildung der Kreidefelsen und des Kreidemergels durch unsichtbare Organismen. *Abh. Königl. Akad. Wiss. Berlin* 1838, 59–147.

Fleming, L. E., Bean, J. A., Katz, D., and Hammond, R. (2001). "The epidemiology of seafood poisoning," in *Seafood and Environmental Toxins*, eds Y. H. Hui, D. D. Kits, and P. S. Stanfield (Amsterdam: Marcel Dekker), 287–310.

Fleming, L. E., Broad, K., Clement, A., Dewailly, E., Elmira, S., Knap, A., et al. (2006). Oceans and human health: emerging public health risks in the marine environment. *Mar. Pollut. Bull.* 53, 545–560. doi: 10.1016/j.marpolbul.2006.08.012

Food and Agriculture Organization [FAO] (2004). *Marine Biotoxins*. Rome: FAO.

Gárate-Lizárraga, I. (2014). Proliferation of Levanderina fissa and Polykrikos hartmannii (Dinophyceae: Gymnodiniales) in Bahia de la Paz, Gulf of California, Mexico. *CICIMAR Oceán.* 29, 25–35.

Gárate-Lizárraga, I., Band, C., Cervantes, R., and Escobedo, D. (2002). Mareas rojas de *Mesodinium rubrum* (Lohmann) Hamburger y Buddenbrock en el Golfo de California. *Hidrobiológica* 12, 15–20.

Gómez, F. (2013). Reinstatement of the dinoflagellate genus *Tripos* to replace *Neoceratium*, marine species of *Ceratium*(Dinophyceae, Alveolata). *CICIMAR Oceán.* 28, 1–22.

Gómez, F., Claustre, H., and Souissi, S. (2008). Rarely reported dinoflagellates of the genera Ceratium, Gloeodinium, Histioneis, Oxytoxum and Prorocentrum (Dinophyceae) from the open southeast Pacific Ocean. *Rev. Biol. Mar. Oceanogr.* 43, 25–40. doi: 10.4067/S0718-19572008000100004

Gómez, F., Richlen, M. L., and Anderson, D. M. (2017). Molecular characterization and morphology of *Cochlodinium strangulatum*, the type species of *Cochlodinium*, and *Margalefidinium* gen. nov. for *C. polykrikoides* and allied species (Gymnodiniales, Dinophyceae). *Harmful Algae* 63, 32–44. doi: 10.1016/j.hal.2017.01.008

Graham, H. W. (1943). *Gymnodinium catenatum*, a new dinoflagellate from the Gulf of California. *Trans. Am. Microsc. Soc.* 62, 259–261. doi: 10.2307/3223028

Grunow, A. (1863). Über einige neue und ungenugend bekkante Arten und Gattungen von Diatomaceen. *Verh. Kaiserlich Königlichen Zool. Bot. Ges. Wien* 13, 137–162.

Gul, S., and Saifullah, S. M. (2011). The dinoflagellate genus Prorocentrum (Prorocentrales, Prorocentraceae) from the North Arabian Sea. *Pak. J. Bot.* 43, 3061–3065.

Halim, Y. (1960). *Alexandrium minutum*, n. gen. n. sp. dinoflagellé provocant des "eaux rouges". *Vie Milieu* 11, 102–105.

Hallegraeff, G. M. (1993). A review of harmful algal blooms and their apparent global increase. *Phycologia* 32, 79–99. doi: 10.2216/i0031-8884-32-2-79.1

Hallegraeff, G. M. (2004). "Harmful algal blooms: a global overview," in *Manual on Harmful Marine Microalgae (UNESCO)*, eds G. M. Hallegraeff, D. Anderson, and A. Cembella (Paris: IOC-UNESCO), 1–45. doi: 10.1016/B978-0-12-391499-6.00008-6

Hallegraeff, G. M. (2010). Ocean climate change, phytoplankton community responses, and harmful algal blooms: a formidable predictive challenge. *J. Phycol.* 46, 220–235. doi: 10.1111/j.1529-8817.2010.00815.x

Hasle, G. R. (1973). Thalassiosiraceae, a new diatom family. *Nor. J. Bot.* 20, 67–69.

Heil, C. A., Glibert, P. M., and Fan, C. (2005). *Prorocentrum minimum* (Pavillard) Schiller: a review of a harmful algal bloom species of growing worldwide importance. *Harmful Algae* 4, 449–470. doi: 10.1016/j.hal.2004.08.003

Horner, R. A., Garrison, D. L., and Plumley, F. G. (1997). Harmful algal blooms and red tide problems on the U.S. west coast. *Limnol. Oceanogr.* 42, 1076–1088. doi: 10.4319/lo.1997.42.5_part_2.1076

Ibrahem, H., and Al-Shawi, I. (2015). Potential harmful algae of Iraqi coastal marine waters. *Int. J. Mar. Sci.* 5, 1–6. doi: 10.5376/ijms.2015.05.0060

Ignatiades, L., and Gotsis-Skretas, O. (2010). A review on toxic and harmful algae in Greek coastal waters (E. Mediterranean Sea). *Toxins* 2, 1019–1037. doi: 10.3390/toxins2051019

Jiménez, R. (1983). Diatomeas y dinoflagelados del fitoplancton del Golfo de Guayaquil. *Acta Oceanogr. Pac.* 2, 1993–1282.

Jiménez, R., and Gualancañay, E. (2006). Floraciones de *Mesodinium rubrum* en los procesos de surgencia en el Pacífico Ecuatorial. *Acta Oceanogr. Pac.* 13, 65–72.

Jiménez, R., and Intriago, P. (2001). Red tides in Ecuador during La Niña 2000. *Harmful Algae* 22:7.

Jugnu, R., and Kripa, V. (2008). Prevalence of non toxic and harmful algal blooms along Kerala coast. *Mar. Fish. Inform. Serv.* 195, 3–5.

Kempton, J. W., Wolny, J., Tengs, T., Rizzo, P., Morris, R., Tunnell, J., et al. (2002). *Kryptoperidinium foliaceum* blooms in South Carolina: a multi-analytical approach to identification. *Harmful Algae* 1, 383–392. doi: 10.1016/S1568-9883(02)00051-3

Kofoid, C. A., and Swezy, O. (1921). *The Free-Living Unarmored Dinoflagellata*. Berkeley, CA: University of California Press.

Konno, S., Inoue, N., Hernández-Becerril, D. U., and Jordan, R. W. (2010). *Chaetoceros affinis* blooms in palauan meromictic marine lakes. *Vie Milieu* 60, 257–264.

Kudela, R., Pitcher, G., Probyn, T., Figueiras, F., Moita, T., and Trainer, V. (2005). Harmful algal blooms in coastal upwelling systems. *Oceanography* 18, 184–197. doi: 10.5670/oceanog.2005.53

Kudela, R. M., and Gobler, C. J. (2012). Harmful dinoflagellate blooms caused by *Cochlodinium* sp.: Global expansion and ecological strategies facilitating bloom formation. *Harmful Algae* 14, 71–86. doi: 10.1016/j.hal.2011.10.015

Lauder, H. S. (1864). Remarks on the marine Diatomaceae found at Hong Kong, with descriptions of new species. *Trans. Microsc. Soc. Lond. New Ser.* 12, 75–79.

Lee, K.-W., Yeh, S.-W., Kug, J.-S., and Park, J.-Y. (2014). Ocean chlorophyll response to two types of El Niño events in an ocean-biogeochemical coupled model. *J. Geophys. Res.* 119, 933–952. doi: 10.1002/2013JC009050

Lee, T. C. H., Fong, F. L. Y., Ho, K. C., and Lee, F. W. F. (2016). The mechanism of diarrhetic shellfish poisoning toxin production in *Prorocentrum* spp.: physiological and molecular perspectives. *Toxins* 8, 2–23. doi: 10.3390/toxins8100272

Lindemann, E. (1924). Der Bau der Hülle bei Heterocapsa und *Kryptoperidinium foliaceum* (Stein) n. nom. (Zugleich eine vorläufige Mitteilund). *Bot. Arch.* 5, 114–120.

Lloyd, J. K., Duchin, J. S., Borchert, J., Quintana, H. F., and Robertson, A. (2013). Diarrhetic shellfish poisoning, Washington, USA, 2011. *Emerg. Infect. Dis.* 19, 1314–1316. doi: 10.3201/eid1908.121824

Lo, W.-T., Hsu, P.-K., Fang, T.-H., Hu, J.-H., and Hsieh, H.-Y. (2016). Phytoplankton communities impacted by thermal effluents off two coastal nuclear power plants in subtropical areas of northern taiwan. *Terr. Atmos. Ocean. Sci.* 27, 107–120. doi: 10.3319/TAO.2015.09.24.01

Lohmann, H. (1908). Untersuchungen zur Feststellung des vollständigen Gehaltes des Meeres an Plankton. *Wiss. Meeresunters. N. F. Abt. Kiel* 10, 129–370.

Longhurst, A. (2006). *Ecological Geography of the Sea*. Cambridge, MA: Academic Press.

Lundholm, N. (2011). "Bacillariophyta," in *Taxonomic Reference List of Harmful Micro Algae*, ed. N. Lundholm (Paris: IOC-UNESCO).

Masó, M., and Garcés, E. (2006). Harmful microalgae blooms (HAB): problematic and conditions that induce them. *Mar. Pollut. Bull.* 53, 620–630. doi: 10.1016/j.marpolbul.2006.08.006

Mathew, K. J., Thomas, P. A., George, R. M., Girijavallabhan, K. G., Siraimeetan, P., Naomi, T. S., et al. (1988). Plankton blooms along the Indian coasts, some highlights. *Mar. Fish. Inf. Serv. Tech. Ext. Ser.* 84, 11–13.

Matsuoka, K., Iwataki, M., and Kawami, H. (2008). Morphology and taxonomy of chain-forming species of the genus *Cochlodinium* (Dinophyceae). *Harmful Algae* 7, 261–270. doi: 10.1016/j.hal.2007.12.002

McClain, C. R., Feldman, G. C., and Hooker, S. (2004). An overview of the SeaWiFS project and strategies for producing a climate research quality global ocean bio-optical time series. *Deep Sea Res.Part II* 51, 5–42. doi: 10.1016/j.dsr2.2003.11.001

McKibben, S. M., Watkins-Brandt, K. S., Wood, A. M., Hunter, M., Forster, Z., Hopkins, A., et al. (2015). Monitoring Oregon coastal harmful algae: observations and implications of a harmful algal bloom-monitoring project. *Harmful Algae* 50, 32–44. doi: 10.1016/j.hal.2015.10.004

Mélin, F., and Hoepffner, N. (2011). "Case Study 6 - Monitoring phytoplankton productivity from satellite: an aid to marine resources management," in *Handbook of Satellite Remote Sensing Image Interpretation: Applications for Marine Living Resources Conservation and Management*, eds J. Morales, V. Stuart, T. Platt, and S. Sathyendranath (Dartmouth: EU PRESPO and IOCCG), 79–93.

Mereschkowsky, C. (1902). Liste des Diatomées de la Mer Noire. *Scr. Bot.* 19, 51–88.

Merino-Virgilio, F. C., Okolodkov, Y. B., Aguilar-Trujillo, A. C., Osorio-Moreno, I., and Herrera-Silveira, J. A. (2014). "Florecimientos algales nocivos en las aguas costeras del norte de Yucatán (2001-2013)," in *Golfo de México. Contaminación e Impacto Ambiental: Diagnóstico y Tendencias*, eds A. V. Botello, J. Rendón von Osten, J. A. Benítez, and G. Gold-Bouchot (Mérida: CINVESTAV- Unidad Mérida).

Miller, P., Shutler, J., Moore, G., and Groom, S. (2006). SeaWiFS discrimination of harmful algal bloom evolution. *Int. J. Remote Sens.* 27, 2287–2301. doi: 10.1080/01431160500396816

Moestrup, Ø., Akselmann, R., Fraga, S., Hoppenrath, M., Iwataki, M., Komárek, J., et al. (2009). *IOC-UNESCO Taxonomic Reference List of 427 Harmful Micro Algae*. Available at: http://www.marinespecies.org/hab/

Moestrup, Ø., Hakanen, P., Hansen, G., Daugbjerg, N., and Ellegaard, M. (2014). On Levanderina fissa gen. & comb. nov. (Dinophyceae) (syn. Gymnodinium fissum, Gyrodinium instriatum, Gyr. uncatenum), a dinoflagellate with a very unusual sulcus. *Phycologia* 53, 265–292. doi: 10.2216/13-254.1

Moisan, J. R., Moisan, T. A. H., and Linkswiler, M. A. (2011). An inverse modeling approach to estimating phytoplankton pigment concentrations from phytoplankton absorption spectra. *J. Geophys. Res.* 116, 0148–0227. doi: 10.1029/2010JC006786

Moore, S. K., Trainer, V. L., Mantua, N. J., Parker, M. S., Laws, E. A., and Backer, L. C. (2008). Impacts of climate variability and future climate change on harmful algal blooms and human health. *Environ. Health* 7(Suppl. 2):S4. doi: 10.1186/1476-069X-7-S2-S4

Muciño-Márquez, R. E., Gárate-Lizárraga, I., and López-Cortés, D. J. (2015). Variación estacional del género Prorocentrum (Dinophyceae) en dos granjas atuneras en la Bahía de La Paz México. *Acta Biol. Colomb.* 20, 195–206. doi: 10.15446/abc.v20n1.42442

Nagai, S., Suzuki, T., Nishikawa, T., and Kamiyama, T. (2011). Differences in the production and excretion kinetics of okadaic acid, dinophysistoxin-1, and pectenotoxin-2 between cultures of *Dinophysis acuminata* and *Dinophysis fortii* isolated from western Japan. *J. Phycol.* 2011, 1326–1337. doi: 10.1111/j.1529-8817.2011.01076.x

Naha, S., Rakshit, D., Kumar, S., and Kumar, R. (2014). Impact of multispecies diatom bloom on plankton community structure in Sundarban mangrove wetland, India. *Mar. Pollut. Bull.* 85, 306–311. doi: 10.1016/j.marpolbul.2014.04.015

Nair, A., Sathyendranath, S., Platt, T., Morales, J., Stuart, V., Forget, M., et al. (2008). Remote sensing of phytoplankton functional types. *Remote Sens. Environ.* 112, 3366–3375. doi: 10.1016/j.rse.2008.01.021

Nitzsch, C. L. (1817). Beitrag zur Infusorienkunde oder Naturbeschreibung der Zerkarien und Bazillarien. *Neue Schrift. Naturforsch. Ges. Halle* 3, 1–128.

NASA Ocean Biology Processing Group (2017). *MODIS-Aqua Level 3 Mapped Chlorophyll Data Version R2018.0 [Data Set]*. Greenbelt, MD: NASA Ocean Biology DAAC. doi: 10.5067/aqua/modis/l3m/chl/2018

NASA Ocean Biology Processing Group (2018a). *MODIS-Terra Level 3 Mapped Absorption Due to Phytoplankton at 443 nm Data Version R2018.0 [Data Set]*. Greenbelt, MD: NASA Ocean Biology DAAC.

NASA Ocean Biology Processing Group (2018b). *MODIS-Terra Level 3 Mapped Photosynthetically Available Radiation Data Version R2018.0 [Data Set]*. Greenbelt, MD: NASA Ocean Biology DAAC. doi: 10.5067/orbview-2/seawifs/l3m/par/2018

NASA Ocean Biology Processing Group (2018c). *SEAWIFS-ORBVIEW-2 Level 3 Mapped Absorption Due to Phytoplankton at 443 nm Data Version R2018.0 [Data Set]*. Greenbelt, MD: NASA Ocean Biology DAAC.

NASA Ocean Biology Processing Group (2018d). *SEAWIFS-ORBVIEW-2 Level 3 Mapped Chlorophyll Data Version R2018.0 [Data Set]*. Greenbelt, MD: NASA Ocean Biology DAAC. doi: 10.5067/orbview-2/seawifs/l3m/chl/2018

NASA Ocean Biology Processing Group (2018e). *SEAWIFS-ORBVIEW-2 Level 3 Mapped Photosynthetically Available Radiation Data Version R2018.0 [Data Set]*. Greenbelt, MD: NASA Ocean Biology DAAC. doi: 10.5067/orbview-2/seawifs/l3m/par/2018

NOAA NESDIS COASTWATCH and NOAA SWFSC ERD (2018). *Day and Night, Western Hemisphere Level 3 Sea Surface Temperature from the Geostationary Operational Environmental Satellites (GOES) Imager on the GOES-11 Satellite (GDS Version 1)*. Silver Spring, MD: National Oceanographic Data Center.

Okamura, K. (1916). Akashio ni Tsuite (On red-tides). *Suisan Koushu Sikenjo Kenkyu Hokoku* 12, 26–41.

Osorio-Tafall, B. F. (1942). Notas sobre algunos dinoflagelados planctónicos marinos de México con descripciön de nuevas especies. *An. Esc. Nal. Cienc. Biol.* 2, 435–447.

Oyarzún, D., and Brierley, C. M. (2018). The future of coastal upwelling in the Humboldt Current from model projections. *Clim. Dyn.* 51, 1–17. doi: 10.1007/s00382-018-4158-7

Padmakumar, K. B., Thomas, L. C., Salini, T. C., and Vijayan, A. (2018). Subsurface bloom of dinoflagellate *Gonyaulax polygramma* Stein in the shelf waters off Mangalore- South Eastern Arabian Sea. *Int. J. Geo Mar. Sci.* 47, 1658–1664.

Pennington, J. T., Mahoney, K. L., Kuwahara, V. S., Kolber, D. D., Calienes, R., and Chavez, F. P. (2006). Primary production in the eastern tropical Pacific: a review. *Prog. Oceanogr.* 69, 285–317. doi: 10.1016/j.pocean.2006.03.012

Peragallo, H., and Peragallo, M. (1900). *Diatomées Marines de France et des Districts Maritimes Voisins* (Grez-sur-Loing: M. J. Tempére), 73–80. doi: 10.5962/bhl.title.13501

Perumal, P., Sampathkumar, P., and Karuppasamy, P. K. (1999). Studies on the bloom-forming species of phytoplankton in the Vellar estuary, southeast coast of India. *Indian J. Mar. Sci.* 28, 400–403.

Pesantes, F. (1983). Dinoflagelados del fitoplancton del Golfo de Guayaquil. *Acta Oceanogr. Pac.* 2, 284–318.

Pierce, R. H., and Henry, M. S. (2008). Harmful algal toxins of the Florida red tide (*Karenia brevis*): Natural chemical stressors in South Florida coastal ecosystems. *Ecotoxicology* 17, 623–631. doi: 10.1007/s10646-008-0241-x

Pitcher, G. C., and Calder, D. (2000). Harmful algal blooms of the southern Benguela current: a review and appraisal of monitoring from 1989–1997. *S. Afr. J. Mar. Sci.* 22, 255–271. doi: 10.2989/025776100784125681

Pitcher, G. C., Jiménez, A. B., Kudela, R. M., and Reguera, B. (2017). Harmful algal blooms in eastern boundary upwelling systems: a GEOHAB Core Research Project. *Oceanography* 30, 22–35. doi: 10.5670/oceanog.2017.107

Prado, M., Trocholli, L., and Moncayo, E. (2015). Cambios estructurales del microfitoplancton en la zona 442 costera de la provincia El Oro-Ecuador en temporada seca. *Bol. Inst. Oceanogr. Venez.* 54, 139–152.

Pritchard, A. (1861). *A History of Infusoria, Including the Desmidiaceae and Diatomaceae, British and Foreign*, eds J. T. Arlidge, B. A. Lond, W. Archer, J. Ralfs, and W. C. Williamson (London: Whittaker & Co).

Razali, R. M., Leaw, C. P., Lim, H. C., Nyanti, L., Ishak, I., and Lim, P. T. (2015). Harmful microalgae assemblage in the aquaculture area of Aman Island, Northern Strait of Malacca. *Malays. J. Sci.* 34, 20–32. doi: 10.22452/mjs.vol34no1.3

Reguera, B., Riobó, P., Rodríguez, F., Díaz, P., Pizarro, G., Paz, B., et al. (2014). Dinophysis toxins: causative organisms, distribution and fate in shellfish. *Mar. Drugs* 12, 394–461. doi: 10.3390/md12010394

Reguera, B., Velo-Suárez, L., Raine, R., and Park, M. G. (2012). Harmful *Dinophysis* species: a review. *Harmful Algae* 14, 87–106. doi: 10.1016/j.hal.2011.10.016

Rhodes, L., Scholin, C., and Garthwaite, I. (1998). Pseudo-nitzschia in New Zealand and the role of DNA probes and immunoassays in refining marine biotoxin monitoring programmes. *Natural Toxins* 6, 105–111. doi: 10.1002/(SICI)1522-7189(199805/08)6:3/4<105::AID-NT13>3.0.CO;2-9

Saburova, M., Polikarpov, I., and Al-Yamani, F. (2012). First record of *Kryptoperidinium foliaceum* (Dinophyceae: Peridiniales) from a hypersaline environment in Kuwait, north-western Arabian Gulf. *Mar. Biodivers. Records* 5, 3–7. doi: 10.1017/S1755267212000838

Saville-Kent, W. (1881). *A Manual of the Infusoria, Including a Description of all Known Flagellate, Ciliate, and Tentaculiferous Protozoa, British and Foreign and an Account of the Organization and Affinities of the Sponges*. David Bogue: London, 289–720.

Schrank, F. P. (1793). Mikroskopische Wahrnehmungen. *Naturforscher* 27, 26–37.

Schütt, F. (1895). Die peridineen der plankton-expedition. *Ergeb. Plankton Exp. Humboldt Stift.* 4, 1–170.

Schütt, F. (1896). "Bacillariales (Diatomeae)," in *Die Natürlichen Pflanzenfamilien*, eds A. Engler and K. Prantl (Leipzig: Verlag von Wilhelm Engelmann), 31–150.

Sellner, K. G., Doucette, G. J., and Kirkpatrick, G. J. (2003). Harmful algal blooms: causes, impacts and detection. *J. Ind. Microbiol. Biotechnol.* 30, 383–406. doi: 10.1007/s10295-003-0074-9

Shen, L., Xu, H., and Guo, X. (2012). Satellite remote sensing of harmful algal blooms (HABs) and a potential synthesized framework. *Sensors* 12, 7778–7803. doi: 10.3390/s120607778

Shi, K., Zhang, Y., Liu, X., Wang, M., and Qin, B. (2014). Remote sensing of diffuse attenuation coefficient of photosynthetically active radiation in Lake Taihu using MERIS data. *Remote Sens. Environ.* 140, 365–377. doi: 10.1016/j.rse.2013.09.013

Shin, H. H., Li, Z., Kim, E. S., Youn, J. Y., Jeon, S. G., Oh, S. J., et al. (2016). Morphology and phylogeny of *Triadinium polyedricum* (Pouchet) Dodge (Dinophyceae) from Korean coastal waters. *Ocean Sci. J.* 51, 647–654. doi: 10.1007/s12601-016-0049-1

Smayda, T. J., and Reynolds, C. S. (2003). Strategies of marine dinoflagellate survival and some rules of assembly. *J. Sea Res.* 49, 95–106. doi: 10.1016/S1385-1101(02)00219-8

Smayda, T. J., and Trainer, V. L. (2010). Dinoflagellate blooms in upwelling systems: seeding, variability, and contrasts with diatom bloom behaviour. *Prog. Oceanogr.* 85, 92–107. doi: 10.1016/j.pocean.2010.02.006

Sorooshian, S., Hsu, K., Braithwaite, D., Ashouri, H., and NOAA CDR Program (2014). *NOAA Climate Data Record (CDR) of Precipitation Estimation from Remotely Sensed Information Using Artificial Neural Networks (PERSIANN-CDR), Version 1 Revision 1 [Data Set]*. Asheville, NC: NOAA National Climatic Data Center. doi: 10.7289/v51v5bwq

Stein, F. (1878). *Die Organismus der Flagellaten*. Leipzig: Verlag von Wilhelm Engelmann.

Suaréz, B., and Guzmán, L. (1994). *Floraciones de Algas Nocivas : Mareas Rojas y Toxinas Marinas*. Santiago: Editorial Universitaria, 40–46.

Suparna, M. (2005). "Harmful phytoplankton blooms indicate the existence of allelopathic interactions," in *Proceedings of the 4th World Congress on Allelopathy*, Wagga Wagga, 355–358.

Taylor, F. J. R., Fukuyo, Y., and Larsen, J. (1995). "Taxonomy of toxic dinoflagellates," in *Manual on Harmful Marine Microalgae*, eds G. M. Hallegraeff, D. M. Anderson, and A. D. Cembella (Paris: UNESCO), 283–317.

Taylor, M. H. (2008). *The Northern Humboldt Current Ecosystem and its Resource Dynamics: Insights from a Trophic Modeling and Time Series Analysis*. Ph.D. dissertation, Bremen University, Bremen.

Thangaraja, M., Al-Aisry, A., and Al-Kharusi, L. (2007). Harmful algal blooms and their impacts in the middle and outer ROPME sea area. *Int. J. Oceans Oceanogr.* 2, 85–98.

Torres, G. (2000). Mareas Rojas durante 1989-1999 en aguas ecuatorianas. *Acta Oceanogr. Pac.* 10, 127–136.

Torres, G. (2006). Eventos de Mareas Rojas en el Pacífico Ecuatorial (Ecuador). Reporte en el Curso COI-AECI-IEO de Taxonomía y Biotoxinas de Fitoplancton toxico, Vigo-España, febrero 2006.

Torres, G. (2011). *Eventos de Mareas Rojas: Estrategias de Manejo Preventivas en Ecuador*. Master's thesis, Universidad de Guayaquil, Guayaquil.

Torres, G. (2013). *Eventos de Mareas Rojas: Estrategias de Manejo Preventivas en Ecuador*. Master's thesis, Universidad de Guayaquil, Guayaquil.

Torres, G. (2015). Evaluación de Mareas Rojas durante 1968-2009 en Ecuador. *Acta Oceanogr. Pac.* 20, 89–98.

Torres, G. (2016). *Informe Sobre las Ocurrencias de Mareas Rojas en Ecuador*. Guayaquil: INOCAR.

Torres, G. (2017). *Evaluacion del Fitoplacnton Como un Mecanismo Preventivo a la Ocurrencia de Bloom Algal Frente a las Costas de Esmeraldas, Manta, La Libertad y Puerto Bolivar en Ecuador 2013-2015*. Ph.D. thesis, Universidad Nacional Mayor de San Marcos, Lima.

Torres, G., and Palacios, C. (2007a). Bloom de Noctiluca Scintillans y Ceratium dens en el Golfo de Guayaquil (2004). *Acta Oceanogr. Pac.* 14, 125–130.

Torres, G., and Palacios, C. (2007b). Calidad Ambiental del Blomm Algal en area urbana del Estero Salado (Ciudad de Guayaquil) en junio 2005. *Acta Oceanogr. Pac.* 14, 115–124.

Torres, G., Recalde, S., Narea, R., Renteria, W., Troccoli, L., and Tinoco, O. (2017). Variabilidad espacio temporal del fitoplancton y variables oceanográficas en El Golfo de Guayaquil durante 2013-15. *Rev. Inst. Invest.* 20, 70–79.

Torres, G., and Tapia, M. (2002). Distribución del fitoplancton en la región costera del mar ecuatoriano, durante diciembre 2000. *Acta Oceanogr. Pac.* 11, 63–72.

Torres, G., Tapia, M. E., Coello, D., and Jiménez, R. (2004). "Monitoreo de fitoplancton y eventos de mareas rojas en el Pacífico Ecuatorial (Ecuador)," in *Sexto Taller Regional de Planificación Científica sobre Floraciones de Algas Nocivas en Sudamérica* (Guayaquil: Comisión Oceanográfica Intergubernamental), 56–64.

Trainer, V. L., Bates, S. S., Lundholm, N., Thessen, A. E., Cochlan, W. P., Adams, N. G., et al. (2012). Pseudo-nitzschia physiological ecology, phylogeny, toxicity, monitoring and impacts on ecosystem health. *Harmful Algae* 14, 271–300. doi: 10.1016/j.hal.2011.10.025

Trainer, V. L., Pitcher, G. C., Reguera, B., and Smayda, T. J. (2010). The distribution and impacts of harmful algal bloom species in eastern boundary upwelling systems. *Prog. Oceanogr.* 85, 33–52. doi: 10.1016/j.pocean.2010.02.003

Vila, M., and Masó, M. (2005). Phytoplankton functional groups and harmful algal species in anthropogenically impacted waters of the NW Mediterranean Sea. *Sci. Mar.* 69, 31–45. doi: 10.3989/scimar.2005.69n131

Vyas, A. A., Chhaya, N. D., Buch, A. U., Bhaskaran, M., Patel, A. J., and Mohile, V. (1986). Mass culture of centric diatom *Chaetoceros affinis* (Lauder) - A live feed for prawn larvae. *Indian J. Mar.Sci.* 15:55.

Wall, D., and Dale, B. (1971). A reconsideration of living and fossil Pyrophacus Stein, 1883 (Dinophyceae). *J. Phycol.* 7, 221–235. doi: 10.1111/j.1529-8817.1971.tb01507.x

Wall, S., and Cicily, L. (2015). First report on a bloom of marine dinoflagellate Pyrophacus steinii (Schiller). *Harmful Algae News* 51, 8–9.

Wang, D. Z. (2008). Neurotoxins from marine dinoflagellates: a brief review. *Mar. Drugs* 6, 349–371. doi: 10.3390/md6020349

Wang, S. F., Tang, D. L., He, F. L., Fukuyo, Y., and Azanza, R. V. (2008). Occurrences of harmful algal blooms (HABs) associated with ocean environments in the South China Sea. *Hydrobiologia* 596, 79–93. doi: 10.1007/s10750-007-9059-4

Wei, G. F., Tang, D. L., and Wang, S. (2008). Distribution of chlorophyll and harmful algal blooms (HABs): a review on space based studies in the coastal environments of Chinese marginal seas. *Adv. Space Res.* 41, 12–19. doi: 10.1016/j.asr.2007.01.037

Wells, M. L., Trainer, V. L., Smayda, T. J., Karlson, B. S. O., Trick, C. G., Kudela, R. M., et al. (2015). Harmful algal blooms and climate change: learning from the past and present to forecast the future. *Harmful Algae* 49, 68–93. doi: 10.1016/j.hal.2015.07.009

White, A. E., Watkins-Brandt, K. S., McKibben, S. M., Wood, A. M., Hunter, M., Forster, Z., et al. (2014). Large-scale bloom of Akashiwo sanguinea in the Northern California current system in 2009. *Harmful Algae* 37, 38–46. doi: 10.1016/j.hal.2014.05.004

Yasumoto, T., Murata, M., Oshima, Y., Sano, M., Matsumoto, G. K., and Clardy, J. (1985). Diarrhetic shellfish toxins. *Tetrahedron* 41, 1019–1025. doi: 10.1016/S0040-4020(01)96469-5

Yurimoto, T., Aue-umneoy, D., Meeanan, C., and Tsutsui, I. (2015). Bloom of the two dinoflagellates *Ceratium furca* and *Diplopsalis lenticula* in a mangrove estuary of Thailand. *Int. Aquat. Res.* 7, 133–141. doi: 10.1007/s40071-015-0099-5

Zingone, A., and Enevoldsen, H. (2002). The diversity of harmful algal blooms: a challenge for science and management. *Ocean Coast. Manage.* 43, 725–748. doi: 10.1016/j.watres.2018.12.048

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Borbor-Cordova, Torres, Mantilla-Saltos, Casierra-Tomala, Bermúdez, Renteria and Bayot. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Marine Science

REVIEW
published: 21 February 2019
doi: 10.3389/fmars.2019.00042

Paralytic Toxin Producing Dinoflagellates in Latin America: Ecology and Physiology

Christine J. Band-Schmidt[1], Lorena M. Durán-Riveroll[2], José J. Bustillos-Guzmán[3], Ignacio Leyva-Valencia[4], David J. López-Cortés[3†], Erick J. Núñez-Vázquez[3], Francisco E. Hernández-Sandoval[3] and Dulce V. Ramírez-Rodríguez[1]*

[1] Instituto Politécnico Nacional-Centro Interdisciplinario de Ciencias Marinas, La Paz, Mexico, [2] CONACYT, Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Ciudad de México, Mexico, [3] Centro de Investigaciones Biológicas del Noroeste, La Paz, Mexico, [4] CONACYT-Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas, La Paz, Mexico

OPEN ACCESS

Edited by:
Jorge I. Mardones, Instituto de Fomento Pesquero (IFOP), Chile

Reviewed by:
Patricio A. Díaz, University of Los Lagos, Chile
Claudio Fuentes-Grünewald, Swansea University, United Kingdom

***Correspondence:**
Christine J. Band-Schmidt
cjband@yahoo.com;
cbands@ipn.mx

†Deceased

Specialty section:
This article was submitted to Marine Ecosystem Ecology, a section of the journal Frontiers in Marine Science

Received: *31 July 2018*
Accepted: *28 January 2019*
Published: *21 February 2019*

Citation:
Band-Schmidt CJ, Durán-Riveroll LM, Bustillos-Guzmán JJ, Leyva-Valencia I, López-Cortés DJ, Núñez-Vázquez EJ, Hernández-Sandoval FE and Ramírez-Rodríguez DV (2019) Paralytic Toxin Producing Dinoflagellates in Latin America: Ecology and Physiology. Front. Mar. Sci. 6:42. doi: 10.3389/fmars.2019.00042

In this review we summarize the current state of knowledge regarding taxonomy, bloom dynamics, toxicity, autoecology, and trophic interactions, of saxitoxin producing dinoflagellates in this region. The dinoflagellates *Gymnodinium catenatum*, *Pyrodinium bahamense* and several species of *Alexandrium* are saxitoxin producers, and have been responsible of paralytic shellfish poisoning in different regions of Latin America, causing intoxications and important fisheries losses. The species distribution differ; most harmful algal blooms of *G. catenatum* are from the northern region, however this species has also been reported in central and southern regions. Blooms of *P. bahamense* are mostly reported in North and Central America, while blooms of *Alexandrium* species are more common in South America, however this genus is widely spread in Latin America. Species and regional differences are contrasted, with the aim to contribute to future guidelines for an international scientific approach for research and monitoring activities that are needed to increase our understanding of paralytic toxin producing dinoflagellates in this region.

Keywords: *Alexandrium*, dinoflagellates, *Gymnodinium catenatum*, Latin America, paralytic toxins, *Pyrodinium bahamense*, review, strains

INTRODUCTION

Neurotoxic paralytic shellfish toxins (PSTs) are produced in the marine environment mainly by dinoflagellates of three genera associated with harmful algal blooms (HABs). These include about a dozen species of *Alexandrium*, a single species of *Gymnodinium* (*G. catenatum*) and a single species of *Pyrodinium* (*P. bahamense*).

PSTs molecules comprise saxitoxin (STX) and over 57 analogs have been described (Wiese et al., 2010) that vary in toxicity, being the carbamoyl [STX, neosaxitoxin (NEO), gonyautoxins (GTX)] the most potent, followed by decarbamoyl (dcSTX, dcNEO, dcGTX) and the deoxydecarbamoyl analogs (doSTX, doGTX2, doGTX3). The least toxic analogs are the *N*-sulfocarbamoyl (B and C toxins). Only *G. catenatum* produces benzoyl analogs (GC), and out of 18 theoretical toxins (Negri et al., 2003a, 2007; Vale, 2008, 2010), 15 benzoyl analogs have been confirmed (Durán-Riveroll et al., 2017) (**Figure 1**).

Consumption of PSTs-contaminated seafood results in a variety of gastro-intestinal and neurologic symptoms known as paralytic shellfish poisoning (PSP) that depend on the toxin concentration and can lead, in extreme cases, to human death. PSTs are usually transferred to humans by the consumption of mollusks such as clams, oysters, and mussels; other toxin vectors that have been reported are gastropods, crustaceans and fish (Llewellyn et al., 2006; Deeds et al., 2008; McLeod et al., 2017). These toxins act at the nervous system level by blocking the voltage-gated sodium channels (Na_V) in mammals (Llewellyn, 2006). They can also bind to voltage-gated calcium (Ca_V) (Su et al., 2004) and potassium (K_V) channels (Wang et al., 2003). In the case of GC analogs, their binding to Na_V channels has only been demonstrated by *in silico* analyses (Durán-Riveroll et al., 2016). PSTs have been related to the death and intoxication of diverse organisms such as shrimp, fish, sea birds, turtles, and whales; however, the knowledge of toxin action in marine organisms is still scarce (Pérez-Linares et al., 2008; Núñez-Vázquez et al., 2011; Costa et al., 2012).

In Latin America (LAm), ∼1,410 people have been intoxicated (94 fatalities) by PSP from 1970 to 2016 (**Table 1**). *Pyrodinium bahamense* has caused the highest number of intoxications (819 cases), followed by *Alexandrium* spp. (350 cases), and *G. catenatum* (241 cases). In the genus *Alexandrium* the highest mortalities have been caused by *A. catenella* (Lagos, 2003), causing 10 M USD losses in salmon industry (Mardones et al., 2015). In spite that intoxications and economic losses due to PSP are becoming an important public concern, few Latin American countries have established and/or maintained monitoring programs.

In this review, we summarize the current state of knowledge regarding PSTs producing dinoflagellates in LAm, contrasting the regional differences with the aim to contribute to future guidelines for an international scientific approach for research and monitoring activities that are needed to increase our understanding of PSP events in this region.

Gymnodinium catenatum

Gymnodinium catenatum is the only gymnodinioid known to produce PSTs. The first description of the species was in the northern region of the Gulf of California (GOLCA) in 1939 (Graham, 1943). Forty years later, in 1979, the first PSP related to this species in LAm was reported along the coasts of Sinaloa, Mexico, during an upwelling event, causing an extensive fish kill (∼200 km), 19 human intoxications and 3 fatalities (de la Garza-Aguilar, 1983; Mee et al., 1986).

Although it was rarely observed after being described, from the decade of 1970 on, blooms have been reported and associated with human poisonings and fatalities in several countries (de la Garza-Aguilar, 1983; Hallegraeff et al., 2012; Cembella and Band-Schmidt, 2018). An apparent increase in frequency, duration, and distribution of blooms are probably related to anthropogenic activities (Hallegraeff, 1993, 1995; Hallegraeff et al., 2012). To date, it has been reported in nine countries of LAm (**Figure 2**). In Mexican coasts there have been numerous reports since 1990 (Band-Schmidt et al., 2010; Gárate-Lizárraga et al., 2016; Medina-Elizalde et al., 2018), associated with human intoxications and death of marine organisms (de la Garza-Aguilar, 1983; Mee et al., 1986; Núñez-Vázquez et al., 2011, 2016). HABs of this species have resulted in 241 cases of PSP (14 fatalities) in Mexico and Venezuela (**Table 1**), and epizootic diseases with mass mortalities of fish, seabirds, and marine mammals. Losses in shrimp cultures have also been reported. Also, prolonged sanitary closures by contamination with PSTs in shellfish, have affected commercialization, causing un-estimated but significant economic losses due to the closure of important shellfish fisheries in the upper GOLCA (García-Mendoza et al., 2016).

Gymnodinium catenatum Bloom Dynamics

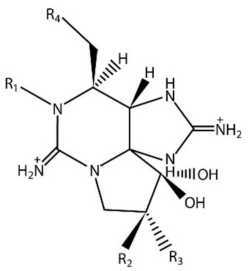
Mexico

One of the most recent and lengthy HABs of this species occurred in the northern part of the GOLCA with maximum abundances of 152×10^3 cell L^{-1} (Medina-Elizalde et al., 2018). This bloom lasted from January to March in 2015, affecting a large number of seabirds, mammals and people (García-Mendoza et al., 2016). In Bahía Concepción (BACO), *G. catenatum* is often present without forming blooms and is more abundant during hydrographic transitional periods during spring and early fall (Morquecho and Lechuga-Devéze, 2004). BACO is one of the few bays where studies on cyst dynamics have been realized, cyst yields are low, but seem to be a constant inoculum that sustains the population for long periods (Morquecho and Lechuga-Devéze, 2004).

In Bahía de La Paz (BAPAZ), in the south of the California Peninsula, SW GOLCA, there is only one report of a HAB in February/March 2007, with abundances from 6×10^5 to 2.4×10^6 cell L^{-1}, an average sea surface temperature (SST) of $20.9 \pm 0.7°C$ and low nitrate ($1.0 \pm 0.3\,\mu M$) and phosphate ($0.8 \pm 0.8\,\mu M$) concentrations (Hernández-Sandoval et al., 2009). In the same bay, in 2010, this dinoflagellate was reported in low abundances (21×10^3 cell L^{-1}) (Muciño-Márquez, 2010).

On the NE GOLCA, in Bahía Bacochibampo, Sonora, a 24-year study was performed on HABs (1970–1994), recording 43 events, where one of the main species was *G. catenatum* (Manrique and Molina, 1997); this led to conclude that NW winds during colder months promoted blooms. They also agreed with Cortés-Altamirano (1987) that HABs had an inverse relationship with El Niño. Unfortunately, this is the only study for this region.

In April 1979 a HAB was reported in Bahía de Mazatlán (BAMAZ), Mexico, (SE GOLCA) with high abundances (6.6×10^6 cell L^{-1}) (Mee et al., 1986). A thermal gradient up to 5°C was registered between 0 and 10 m, associating the bloom with an upwelling event. From 1979 to 1985, several HABs occurred in this bay, being this species one of the most frequently reported (Morey-Gaines, 1982; Cortés-Altamirano, 1987). In 1997, it was concluded that blooms in this region were inhibited due to the influence of El Niño, that attenuated the upwelling of deep nutrient rich waters (Cortés-Altamirano, 2002).



Analog	R_1	R_2	R_3	R_4
STX	H	H	H	carbamoyl
NEO	OH	H	H	
GTX1	OH	H	OSO_3^-	
GTX2	H	H	OSO_3^-	
GTX3	H	OSO_3^-	H	
GTX4	OH	OSO_3^-	H	
B1	H	H	H	N-sulfocarbamoyl
B2	OH	H	H	
C1	H	H	OSO_3^-	
C2	H	OSO_3^-	H	
C3	OH	H	OSO_3^-	
C4	OH	OSO_3^-	H	
dcSTX	H	H	H	decarbamoyl
dcNEO	OH	H	H	
dcGTX1	OH	H	OSO_3^-	
dcGTX2	H	H	OSO_3^-	
dcGTX3	H	OSO_3^-	H	
dcGTX4	OH	OSO_3^-	H	
GC1	H	H	OSO_3^-	*p*-hydroxibenzoyl
GC2	H	OSO_3^-	H	
GC3	H	H	H	
GC4*	OH	H	OSO_3^-	
GC5*	OH	OSO_3^-	H	
GC6*	OH	H	H	
GC1a*	H	H	OSO_3^-	di-hydroxibenzoyl
GC2a*	H	OSO_3^-	H	
GC3a*	H	H	H	
GC4a*	OH	H	OSO_3^-	
GC5a*	OH	OSO_3^-	H	
GC6a*	OH	H	H	
GC1b*	H	H	OSO_3^-	sulfo-benzoyl
GC2b*	H	OSO_3^-	H	
GC3b*	H	H	H	
GC4b*	OH	H	OSO_3^-	
GC5b*	OH	OSO_3^-	H	
GC6b*	OH	H	H	

FIGURE 1 | Paralytic shellfish toxins produced by dinoflagellates. Benzoyl toxins are only produced by *Gymnodinium catenatum.* (Modified from Durán-Riveroll and Cembella, 2017).

TABLE 1 | Paralytic shellfish poisoning impacts in Latin America.

Dinoflagellate	Locality/date	Impacts	References
Alexandrium spp.	Argentina/1980, 1993	*Public health*: 2 intoxicated (2 deaths)	Vinuesa, 1993; Vinuesa and Labal, 1996; Montoya and Carreto, 2007; Carreto et al., 1998b; Montoya et al., 1998; Ferrari, 2001; Sunesen et al., 2014.
		Epizootic: fish and bird mass mortality	
	Chile/1972–1998, 2002, 2005, 2006, 2009, 2016	*Public health*: 348 intoxicated (27 deaths)	Fuentealba et al., 1981; Montebruno, 1993; Lagos, 1998; Suárez-Isla and Guzmán-Méndez, 1998; Carvajal, 2002 in FAO (2004); Guzmán et al., 2002; García et al., 2004; Hernández et al., 2016.
		Epizootic: fish cultured mass mortality. Probably related to marine mammals mass mortality	Fuentes et al., 2008; Mardones et al., 2015; Häussermann et al., 2017; Montes et al., 2018.
Gymnodinium catenatum	Mexico/1979, 1988, 2015	*Public health*: 37 intoxicated (3 deaths)	de la Garza-Aguilar, 1983; Mee et al., 1986; Cortés-Altamirano and Núñez-Pasten, 1992; Band-Schmidt et al., 2010, 2011; Núñez-Vázquez et al., 2011, 2016; Bustillos-Guzmán et al., 2016; García-Mendoza et al., 2016.
		Epizootic: wild fish, shrimp (adult and larvae cultured), bird and marine mammals mass mortality	Mee et al., 1986; Cortés-Altamirano and Núñez-Pasten, 1992; Alonso-Rodríguez and Páez-Osuna, 2003; Alonso-Rodríguez et al., 2004a,b; Band-Schmidt et al., 2010, 2011; Núñez-Vázquez et al., 2011, 2016; García-Mendoza et al., 2016.
	Venezuela/1979, 1981, 1991	*Public Health*: 204 intoxicated (11 deaths)	IPCS, 1984; La Barbera-Sánchez et al., 2004
	Ecuador/1998 (*Gymnodinium* sp.)	*Epizootic*: sea bird mortality	Torres, 2000.
Pyrodinium bahamense	Mexico/1976, 1985, 1989, 1991, 1992, 1995, 2001, 2010	*Public Health*: 476 intoxicated (18 deaths)	Saldate-Castañeda et al., 1991; Cortés-Altamirano et al., 1993; Parrilla-Cerrillo et al., 1993; Sotomayor-Navarro and Domínguez-Cuellar, 1993; Ramírez-Camarena et al., 1996, 2002; Ochoa et al., 1998; Orellana-Cepeda et al., 1998; Sierra-Beltrán et al., 1998; Meave del Castillo et al., 2006; Núñez-Vázquez et al., 2007b, 2016; Licea et al., 2008; Band-Schmidt et al., 2011; Gárate-Lizárraga et al., 2011; Sánchez-Flores, 2011; Bustillos-Guzmán et al., 2016.
	Costa Rica/1989–1990	*Epizootic*: wild fish and sea turtle mass mortality	Mata et al., 1990; Vargas-Montero and Freer, 2004.
	Guatemala/1987	Public Health: 70 Intoxicated (6 deaths)	Rosales-Loessener, 1989; Rosales-Loessener et al., 1989; Rodríguez et al., 1990.
	El Salvador/ 2001–2001, 2009, 2013, 2017	*Public Health*: 187 Intoxicated (26 deaths)	Enrique Barraza et al., 2004; Barraza, 2009
		Public Health: 41 Intoxicated	
		Epizootic: sea turtle mass mortality	Amaya et al., 2014, 2017, 2018
	Nicaragua/2005	*Public Health*: 45 Intoxicated (1 death)	Callejas et al., 2015

In the Mexican Pacific coast, in Nayarit, two HABs were reported in December 2005. The average abundances were 1010 $\times$ 10^3 cell L^{-1}, at a SST of 25°C and high nutrient concentrations (Castillo-Barrera and García-Murillo, 2007). Further south, in Bahía Manzanillo (BMANZ), Colima, HABs have increased in frequency, duration, and coverage. During winter and spring of 1999, HABs were reported with abundances of 35 $\times$ 10^2 cell L^{-1} (Blanco-Blanco et al., 1999; Morales-Blake et al., 2000). The temperature during the initiation of the bloom was 23°C, and at the highest abundance it reached 25°C. In the same bay, in spring of 2007, blooms were reported, with abundances of 3532 $\times$ 10^6 cell L^{-1}, SST ranged between 24.8 and 26.3°C, salinity from 31.8 to 32.6, and dissolved O_2 from 5.1 to 6.1 mg L^{-1} (González-Chan et al., 2007). The authors suggested that HABs were caused by upwelling events documented by satellite images.

In May 2010, the species proliferated again in BMANZ, and Bahía de Santiago (Quijano-Scheggia et al., 2012). Water temperature was 21°C at the beginning of May, with salinities of 32.5–34.6. Again, during the HAB, temperature raised from 25.5 to 27.5°C, and when temperature decreased to 22.5°C, the highest abundances (3.7 $\times$ 10^6 cell L^{-1}) were found. Also, high mean values of dissolved inorganic nitrogen (6.16–6.45 μM), orthophosphates (0.27–0.51 μM), and silicates (7.49–21.07 μM) were reported, coinciding with an upwelling event in the Central Mexican Pacific that lasted 2 weeks and was more intense than previous events. Morning winds

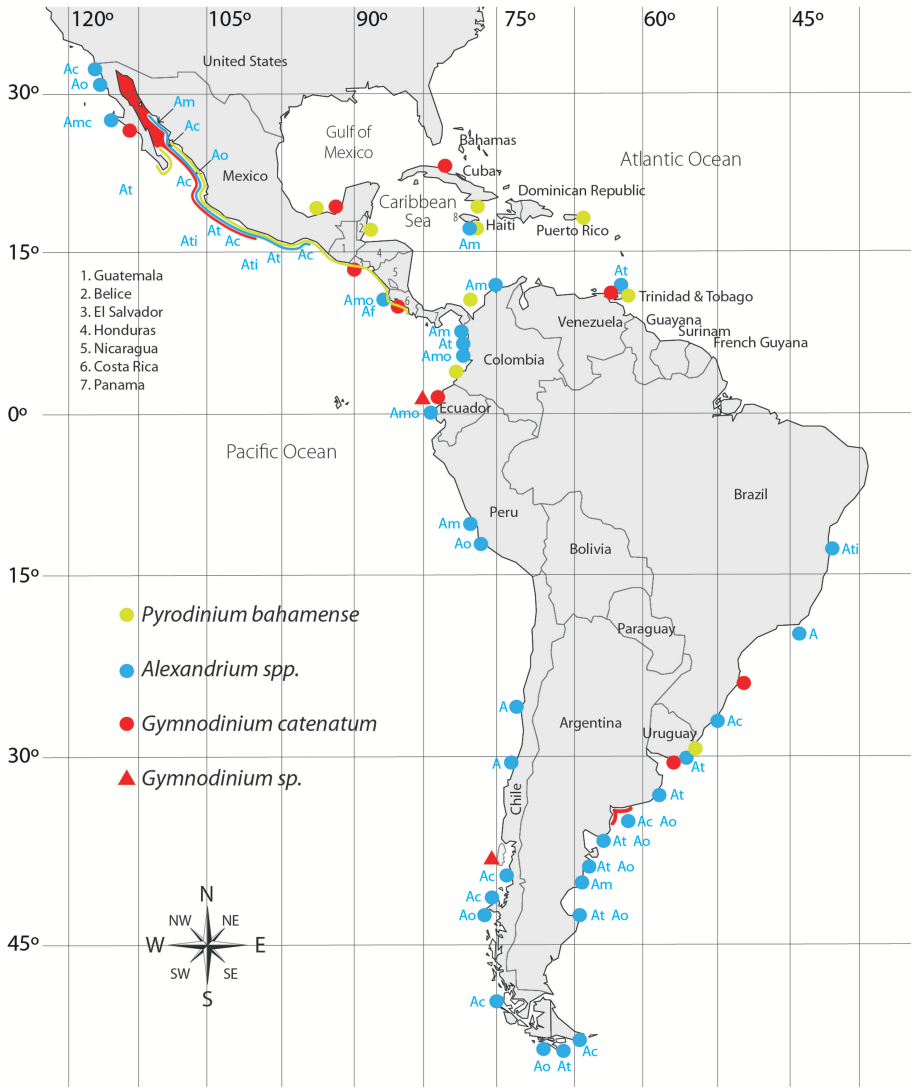


FIGURE 2 | Distribution of PST producing dinoflagellates in Latin America. Ac, *A. catenella;* Am, *A. minutum;* Amo, *A. monilatum;* Ao, *A. ostenfeldii;* At, *A. tamarense;* Ati, *A. tamiyavanichi;* A, *Alexandrium* sp.

flowed from the coast to the ocean, carrying the HAB off the coast; and in the early afternoon, the flow was in the opposite direction, causing cells to accumulated near the coast, suggesting that this dinoflagellate is present in the oceanic zone in low abundances, and that when the abundance increases, cells are transported to coastal areas during more favorable conditions, such as relaxation periods during upwellings (Quijano-Scheggia et al., 2012).

In the southern Mexican Pacific, in Bahía de Acapulco (BACA), the first record occurred in March 1999 with low abundances from 7 to 78 $\times$ 10^3 cell L^{-1} (Gárate-Lizárraga et al., 2009). Co-occurrence with *M. polykrikoides* was also reported during a HAB from December 2005 to February 2006, with an abundance of *G. catenatum* from 141 $\times$ 10^3 cell L^{-1} to 604 $\times$ 10^3 cell L^{-1}. In December 2007 once again there was a HAB of both species at a SST of 26–27°C.

In the last decade, the species has been reported in the Gulf of Mexico, with the highest densities in October 2008, between 183 and 1797 cell L^{-1} (Zamudio et al., 2013). *Gymnodinium* cf. *catenatum* was reported in oyster beds in Campeche (Poot-Delgado et al., 2015), but no HABs have occurred.

Band-Schmidt et al. (2010), reviewed the reports in the Mexican Pacific, including the GOLCA. They concluded that this dinoflagellate is more abundant during March/April, associated with a SST from 18 to 25°C, and an increase of nutrients from coastal upwelling and river runoffs. Also, that blooms are inhibited by El Niño. Later studies have confirmed these assertions.

Other Latin American Countries

In the coasts of Ecuador several HAB events occurred in the 1990s (Torres, 2000). Within the list of species, *Gymnodinium* sp. proliferated in February 1993 in the Península of Santa Elena, extending for several kilometers, with a SST of 25°C and a wind speed of 8 kn. HABs of *Gymnodinium* sp. were reported in March 1993, in Estero Salado (Golfo de Guayaquil, GGUAY) and Apri 1997 in Isla del Muerto (Santa Clara). In August of the same year, in island Santa Clara, this species proliferated ($<5 \times 10^4$ cell L^{-1}) and remained during 5 months. In February/March 1999, abundances of 3.5×10^5 cell L^{-1} were reported in GGUAY, with phosphates concentrations of 10.1 μg-at L^{-1} and silicates of 170.69 μg-at L^{-1}. Nutrient values were high in comparison with previous data recorded in the same area. In January 1998 a bloom of *Gymnodinium* sp. occurred at a SST of 25–29.7°C, and was related with ENSO and associated with a bird mortality. In 1999 (April/May) blooms of *Gymnodinium* sp. were reported near shrimp farms and associated to turtle mortality (Torres, 2000). Torres-Chuquimarca (2011) reviewed HABs events from 1968 to 2009, and concluded that from March to May, HABs are recurrent in the coasts of Ecuador and again, the most frequent genus was *Gymnodinium* sp. Areas with the highest incidence of blooms and mortality of organisms were in the GGUAY (80%), particularly Estero Salado, and Río Guayas, Puna island and the coastline of the province El Oro, a region where shrimp farming is an important activity. The mortality of organisms associated to these blooms suggests that the responsible species could be *G. catenatum*.

In Brazil, this species was recorded in 1997 (Proença et al., 2001) in the estuarine complex of Paranaguá (Southern Brazil), where fisheries and aquaculture are important economic activities. From 1913 to 2000 several HAB species that impacted wildlife off the coast of Brazil were reported, including *Gymnodiniun* sp., although *G. catenatum* was also reported (Odebrecht et al., 2002). From August 2002 to October 2003, it was reported in Paranaguá, among various toxic species, with a maximum abundance of 6.4×10^3 cell L^{-1} in February (spring-fall) at 29°C (Mafra-Junior et al., 2006).

In Chile, during March-April 1999, in the archipelago of Chiloé and inland waters, a massive fish mortality that extended >1,330 km took place, from the latitude 42° to 54° S, affecting salmon farming (a loss of ∼1,500 t), eels, sea stars, sea urchins, mussels, octopuses and snails (Clément et al., 2001). Mortalities were associated with *Gymnodinium* sp. ($4–8 \times 10^6$ cell L^{-1}). Also, densities of 3 to 43×10^4 cell L^{-1} were recorded in the Magellan region (MAGR). The bloom was more intense during neap tides and strong solar radiation. A higher abundance was found in surface waters during the day, and at night at 10 m depth. The HAB originated outside the fjords and channels in the hydrographic front between the open waters (>30 salinity) and the inland waters (salinity15–25) (Clément et al., 2001). In the archipelago of Chiloé, temperature was 1.5°C higher (15°C) than the expected, suggesting that the growth of dinoflagellates could be stimulated with high irradiance, additionally to prolonged minimal amounts of discharged fresh water in the channels and fjords, providing suitable conditions for oceanic species. HABs were associated with the front structure of the body of water masses.

In Argentina, this species was recorded for the first time in 1964 (Balech, 1964). Until 1995, during March-April, it was observed in low densities (80 cell L^{-1}) in Mar del Plata (MPlat), but in April 1997, an abundance of 1.9×10^3 cell L^{-1} was reported (Akselman et al., 1998). This species was also recorded on the coast of Uruguay in 1991 at the end of summer, and in autumn 1993 it was reported in both, Uruguay and MPlat. From 1991 to 2004 blooms occurred in Uruguay at a SST between 21.8 and 24.0°C and salinities between 18.4 and 32 (Méndez, 2006). In March 2003, in coastal waters of MPlat, the species was found in high abundances (8.9×10^4 cell L^{-1}) (Montoya et al., 2006). As toxin profiles differed within both locations, authors suggested that the MPlat HAB was a local event and was not caused by the transportation of the population from the estuarine region.

In Cuba, *G. catenatum* was found in the NE region coinciding with higher nutrient inputs from rivers and anthropogenic discharges. Nevertheless, during dry season, at a SST between 26.8 and 27.3°C, salinity 36.3, abundances were reported between 400 and 800 cell L^{-1} (Leal et al., 2001). In the southern region, in Bahía de Cienfuegos, it was also reported (2.3×10^3 cell L^{-1}) at a wider SST range (between 23.6 and 27.0°C) (Moreira-González et al., 2013).

Toxin Profiles in Environmental Samples

In Mexico, two review papers have been published regarding the toxicity and toxin profile of phytoplankton and shellfish samples during HABs of *G. catenatum*. The first report included information from the Mexican Pacific coast (Band-Schmidt et al., 2010), and the second only considered data from the GOLCA (Bustillos-Guzmán et al., 2016). As in many other geographic zones, natural phytoplankton PSTs analysis linked to this species are scarce in LAm, and reports have only been done in Uruguay, Argentina and Mexico (Méndez et al., 2001; Gárate-Lizárraga et al., 2006; Montoya et al., 2006; Quijano-Scheggia et al., 2012).

Toxicity of this species is relatively low in natural phytoplankton samples and, according to reported analysis, it oscillates between 1 and 3.7 pg STXeq $cell^{-1}$ in BAPAZ (Gárate-Lizárraga et al., 2006), and in BMANZ from 1.4 to 10.9 pg STXeq $cell^{-1}$, with an average of 4.2 pg STXeq $cell^{-1}$ (Quijano-Scheggia et al., 2012). The PSTs analogs are predominantly sulfocarbamoyl C1/2 (73.0% in molar basis), followed by carbamoyl, STX (16.2%) and NEO (3.3%). Decarbamoyl analogs (dcGTX2/3, dcSTX) are less represented (<5%). In coastal waters of MPlat, toxin content was 122 fmol $cell^{-1}$ (Montoya et al., 2006), and presented a profile dominated by C1/2 (82 mol%), followed by GTX2/3 (9%), GTX4 (6%), and dcGTX2/3 (4%). The calculated toxin cell content was similar to that reported previously for Uruguayan isolates (Méndez et al., 2001). These values also agreed with the calculated cell toxin concentration related to the first lethal poisoning in Mexico, of 10 pg $cell^{-1}$ (Mee et al., 1986). Both reviews emphasized the low quantities of PSTs in natural phytoplankton samples of the dinoflagellate. This remark, as shown in this work, could also be extended to other zones in LAm.

Concerning the toxin profile of phytoplankton samples, two patterns can be clearly distinguished: One where sulfocarbamoyl analogs (B, C) are dominant (>50% in molar basis), and a second one, where decarbamoyl analogs (dcGTX2/3, dcSTX) dominate, representing >70% (Gárate-Lizárraga et al., 2004, 2006). The first pattern has been reported in bloom samples of Argentina (Montoya et al., 2006) and Mexico (Quijano-Scheggia et al., 2012). The second was found in samples from Mexico: Bahía Concepción (BACO) and BAPAZ (Gárate-Lizárraga et al., 2004, 2006) in the GOLCA. However, the liquid chromatography with fluorescence detection (LC-FLD) method commonly used for these analyses has some withdrawals, and miss-identification may occur depending on the extraction methods, if two or more toxins have the same retention time or if phantom peaks are present they can compromise the identification of analogs (Bustillos-Guzmán et al., 2015). These patterns have to be further confirmed and analyzed by confirmatory methods such as LC coupled to mass spectrometry detectors alone or in tandem (LC-MS or LC-MS/MS). Benzoyl analogs should also be analyzed in natural phytoplankton samples of *G. catenatum*, since it has been reported that at least 15 of them have been identified in cultured strains (Durán-Riveroll et al., 2017).

During HABs of this species in Mexico, PSTs content of several species of mollusks have been reported, including oysters, clams, and scallops (Bustillos-Guzmán et al., 2016; Quijano-Scheggia et al., 2016). Dominance of B and C, or dc analogs (dcGTX2/3, dcSTX) are usually found in mollusks (Gárate-Lizárraga et al., 2004; Hernández-Sandoval et al., 2009). In *Panopea globosa* analogs could be linked to the bloom phase, with a higher content of C toxins during the HAB occurrence, and a higher content of GTX5, dcGTX2, and dcSTX several weeks afterwards (Medina-Elizalde et al., 2018).

In Venezuela, from August to December 1991, in mussels (*Perna perna)* from Isla Margarita, the most abundant analog was dcSTX; NEO and GTX1-4 were also registered (La Barbera-Sánchez et al., 2004). The presence of dcGTX2/3 was attributed to *G. catenatum,* however, during this bloom cells of *A. tamarense* were also documented. In Uruguay, during a HAB, the toxin profile in *Mytilus edulis* and the clam *Donax hanleyanus* presented N-sulfocarbamoyl analogs (C1/2, 53% in molar basis) and significant amounts of GTX2/3 and STX for the former bivalve and dc toxins, mainly dcGTX2 (∼63%), for the later species (Méndez et al., 2001). Analog variation was also linked to the bloom phase, as well as the cell abundance, ingested cells or specific biotransformations of each bivalve.

Autoecology Studies

Several autoecology studies have been performed with strains of *G. catenatum* from the GOLCA and the Pacific coast of Mexico (reviewed by Band-Schmidt et al., 2010, 2016). Under laboratory conditions, this species tolerates a wide salinity, temperature, and N:P ratio. Strains are able to grow at salinities from 15 to 40; the optimal range changes with seawater source. In general, highest growth rates (from 0.28 to 0.31 div day^{-1}) occur between 28 and 38 (Band-Schmidt et al., 2004). Strains can grow at temperatures between 11.5 and 33°C, with higher growth rates (from 0.32 to 0.39 div day^{-1}) between 21 and 24°C, and maximum cell concentration between 4,700 and 5,500 cell mL^{-1} (Band-Schmidt et al., 2004, 2014). This species also tolerates a high range of N:P ratio (from 5.4 to 74.3 μM) with growth rates between 0.20 and 0.24 div day^{-1}, and a maximum cell concentration (5,852 cell mL^{-1}) at a N:P ratio of 23.5 (Bustillos-Guzmán et al., 2011). This wide tolerance to environmental factors probably explains partially the wide distribution of this species along the Mexican Pacific coast.

There are only two studies in LAm where photosynthetic pigments are reported. The first one determined the effect of two light intensities (120 and 350 μmol quanta $m^{-2}s^{-1}$) on a strain from BACO [Murillo-Martínez, 2015, in (Paredes-Banda et al., 2016)], finding that, at the highest light intensity (HL) growth rates were of 0.28 ± 0.06 div day^{-1}, with maximum cell concentration of 5902 cell mL^{-1}; at a lower light intensity (LL) growth decreased in 50% (0.14 div day^{-1}) and the maximum abundance decreased by 45%. At LL the concentration of chl *a* and peridinine was higher than at HL. Also, at HL diadinoxanthine and diatoxanthine increased (18%) indicating the photoprotection in cells is higher compared with LL conditions. No differences where observed in the concentration and toxin profile under both light conditions indicating that toxin synthesis is not related with light intensity. The second study, in an isolate from Santa Catarina, Brazil, determined the pigment profile, detecting chl *a*, chl *c2*, peridinine, diadinoxanthine, and minor carotenoids (Proença et al., 2001).

Few studies have been performed in LAm to understand the effect of planktonic species on its growth and survival. In the GOLCA, grazing studies have been performed with the copepod *Acartia clausi* and the dinoflagellate *Noctiluca scintillans*, demonstrating that both are important predators of the dinoflagellate, suggesting they have an important ecological role in the regulation of its blooms (Palomares-García et al., 2006; Bustillos-Guzmán et al., 2013). It was also demonstrated the allelopathic effect of the raphidophyte *Chattonella marina* var. *marina* and the dinoflagellate *Margalefidinium polykrikoides* toward *G. catenatum.* Both species inhibited the growth of *G. catenatum* and caused changes in its morphology, and chain formation. Inhibition was stronger and occurred in a shorter time with *Chattonella*; mortality occurred with and without direct cell contact, indicating that toxic metabolites are released to the culture medium (Fernández-Herrera et al., 2016; Band-Schmidt et al., 2017). These results suggested that biotic factors affect its growth providing new insights on the interactions between this dinoflagellate and co-occurring planktonic species.

Gymnodinium catenatum Toxin Profiles Under Laboratory Conditions

In LAm, several strains of *G. catenatum* have been isolated and maintained in culture for laboratory studies (**Figure 3**). Toxin profiles in laboratory conditions, however, have been reported only from Brazil and Mexico. In Brazil, one isolate has been studied, isolated from Armaçao do Itapocoroy (AdI). In Mexico, the isolates used for autoecological studies come from Baja California Sur (BAPAZ, BACO); Sinaloa (BAMAZ); Colima (BMANZ); Bahía de Topolobampo, Sinaloa (TOPO) and Lázaro

FIGURE 3 | Origin of isolates from where toxin production in laboratory conditions have been analyzed.

Cárdenas, Michoacán (CAR). In these studies, toxin analyses have been performed mostly by HPLC-FLD. This technique, though reliable for most of the common STX analogs, has some drawbacks that have been mostly reduced by several improvements of the methods. Nevertheless, it is important to keep in mind that some of these drawbacks are persistent and have led to important confusions in toxin analysis.

In 2001, laboratory studies started in Brazil. The only available standards for the analysis were GTX1-4, NEO, and STX, but none of these analogs were found in the culture samples, and peaks eluted in retention times previously reported for N-sulfocarbamoyl toxins. The production of these analogs was later proven by acidic hydrolysis of the extract and further analysis, showing a peak for GTX2/3, equivalent to the C1/2 toxins, characteristic of this species (Proença et al., 2001).

Since 2005, toxin profiles from Mexican isolates started to be studied. These isolates have been mainly from the GOLCA region, and the Mexican Pacific. Their toxin profile included NEO*[1], dcSTX, dcGTX2/3, B1, and C1/2 (**Table 2**). The low molar percentages of B toxins was considered a distinctive characteristic of Mexican strains, compared to reports from Portugal and Spain (Negri et al., 2001; Ordás et al., 2004; Gárate-Lizárraga et al., 2005), but a huge variation in profiles and toxin content among strains was found, and it was also proven that they can vary with culture age and culture conditions (Gárate-Lizárraga et al., 2005).

It has been reported that the toxin content was slightly variable with culture age, but differences were not always significant (Band-Schmidt et al., 2005). As cultures aged, a decrease in the number of isolates producing NEO*, GTX2/3, C1/2, and B2 was registered, and a there was significant increase of decarbamoyl

[1]In 2015, Bustillos-Guzmán and collaborators, using LC-MS/MS found that the analog reported as NEO in previous analysis could have corresponded to dcNEO.

analogs (dcSTX, dcGTX2/3) (**Table 2**). Nevertheless, in another study, no significant differences in toxin content or toxicity related to culture age were found, neither differences between strains isolated from vegetative cells and cysts. They also reported STX during certain days of the culture, a toxin that is almost never reported in Mexican isolates, which could indicate that the rest of the time it is produced in non-detectable concentrations (Band-Schmidt et al., 2006).

Later, the toxin profile of one isolate from BACO was analyzed, though the main goal was to determine its toxic capacity when fed to the copepod *A. clausi*. Toxin profile of the isolate showed only the commonly reported analogs C1/2, dcSTX, and dcGTX2/3, and no B1, B2, dcNEO or any carbamoyl toxins that were previously reported (Palomares-García et al., 2006) (**Table 2**). Also, the effects of this dinoflagellate were demonstrated on the clam *Nodipecten subnodosus*. In this case, toxins were extracted with a different acetic acid concentration (0.1 N vs. 0.03 N in former experiments) (Estrada et al., 2007). The authors found a vast amount of GTX toxins, but did not specify the analogs, and no N-sulfocarbamoyl toxins, such as B1, B2, and C1-4 were reported (**Table 2**), though these analogs have been often described as the main toxin component for the species. A year later, the effect of the PSTs were studied in the white leg shrimp, *Litopenaeus vannamei*, using an isolate from BACO (Pérez-Linares et al., 2008). Cultures were grown in f/2 medium and the toxin profile was analyzed prior to injecting the shrimp with the toxic extracts. The profile was very different from previous reports from the same isolate, probably related to differences in culture media (f/2 vs. GSe), temperature (21°C vs. 26 ± 1°C), and toxin extraction, that was performed with hydrochloric acid instead of acetic acid. The described profile for this isolate was composed by STX, NEO, GTX1/4, GTX2/3, and dcSTX (Pérez-Linares et al., 2008), and no N-sulfocarbamoyl toxins or other decarbamoyl toxins (dcNEO, dcGTX1-4) (**Table 2**), were reported. Probably, the acidic conditions of the extraction could modify the toxin profile, or the lack of N-sulfocarbamoyl standards prevented the detection of these toxins. As reported in many other countries, the presence of dcSTX, dcGTX2/3, and C1/2 are considered usual components of *G. catenatum* strains from the Pacific Mexican coasts, also, being the decarbamoilated analogs the most important in terms of toxin potency while the latest are usually the most important in terms of molar contribution (Band-Schmidt et al., 2010).

In the search for a better understanding of how the environmental factors affect the toxin profile and toxicity, *in vitro* experiments have been performed using different nutrient concentrations and temperature regimes. In 2012, the analysis of an isolate from BACO in the GOLCA, with different N:P ratios was reported (Bustillos-Guzmán et al., 2012). Toxins were extracted with 0.03 N acetic acid and a subsample was hydrolized to detect N-sulfocarbamoyl toxins. No significant differences in cell toxicity or toxin profile were recorded, and by the end of the experiment, an increase in total toxicity was evident in all treatments, as reported previously (see Band-Schmidt et al., 2005, 2006; Gárate-Lizárraga et al., 2005). The toxins found in these experiments were STX, NEO*, dcSTX, dcGTX2/3, B1, B2, and C1-3 (**Table 2**). This toxin profile varied slightly with previous reports of isolates from the same geographical area, where STX and/or GTX1/4 and no N-sulfocarbamoyl toxins were described (Estrada et al., 2007; Pérez-Linares et al., 2008).

The effect of temperature in toxin profile, toxicity and growth was studied in 2014 in eight Mexican isolates from four locations in the Pacific coast: BAPAZ, BAMAZ, TOPO, and CAR (Band-Schmidt et al., 2014). All isolates produced the same ten analogs (**Table 2**), though significant differences were found among them in terms of GTX2/3 and B toxins, all of them produced higher proportions of B and C toxins. At lower temperatures (16–19°C) the production of C toxins was greater and at higher temperatures (30–33°C) B toxins represented up to 63.4% of the toxin profile, whereas at lower temperatures the maximum was 12.7% in molar basis. Decarbamoyl toxins (dcSTX, dcGTX2) were more abundant at 21°C, but since the main changes in toxin profile were within the low-potency toxins, no changes in cell toxicity related to temperature were found. An interesting relationship between dcSTX and dcGTX2 was found since both increased or decreased simultaneously (Band-Schmidt et al., 2014). This toxin profile change related to temperature, from mostly C1/2 toxins in lower temperatures to mostly B1/2 at high temperatures could be explained by enzyme activity, but more studies are necessary.

In 2001, new analogs reported for *G. catenatum* that brought attention to this species (Negri et al., 2001). Chromatographic peaks eluted late in the C toxin chromatograms. These peaks were later confirmed in Spanish isolates (Ordás et al., 2004), and reported as C5 and C6 compounds or possible artifacts, but it was later confirmed to be new toxin analogs containing a benzoyl ring in the lateral chain. These toxins were named GC1-3 toxins or "hydrophobic toxins" (Negri et al., 2003b). These peaks were previously noted in isolates from different countries, and in 2007 Negri and collaborators reported the widespread presence of these analogs in isolates from Australia, China, Japan, Portugal, Uruguay, and Spain, comprising between 10 and 63 mol% of the total toxin in cultured vegetative cells or cysts (Negri et al., 2007). One year later, a Portuguese strain was analyzed and several unknown oxidation products were found, in addition to the three GC toxins previously reported (Vale, 2008). Further mass spectrometry analysis confirmed the existence of a wide range of GC analogs (**Figure 1**) (Vale, 2008). In 2011, these analogs were searched for and found in Mexican isolates (Bustillos-Guzmán et al., 2011). The isolate used for these analysis was from BACO, and the toxin detection was done by HPLC-FLD, using the methodology previously described by Vale (2008) and comparing the retention times of the later peaks. The GC toxins found in this isolate were abundant and mostly composed by the sulfobenzoylated, the C-11 sulfated and the non-sulfated analogs (corresponding to the GCb series, GC1/2 and GC3 analogs, respectively). Apart from GC toxins, this analysis found the commonly reported B and C analogs but no STX or NEO, bringing out the question about previous analysis and the possibility of having misidentified dcSTX and dcNEO for STX and NEO (Bustillos-Guzmán et al., 2011). A second report of GC toxins in Mexico was from an isolate from the Pacific coast (MANZ), using nuclear magnetic resonance (NMR) in semi-purified

TABLE 2 | *Gymnodinium catenatum* toxin analogs reported under laboratory conditions.

N	Origin	Culture media	STX	NEO	GTX1/4	GTX2/3	B1	B2	C1/2	C3/4	dcSTX	dcNEO	dcGTX1/4	dcGTX2/3	GC	Reference
1	Armaçao do Itapocoroy, Bra	K + SE	–	–	–	–	?	?	1/1	?	?	?	?	?	?	Proença et al., 2001
3	BAPAZ, B.C.S. Mx	GSe	–	3/3	–	–	3/3	3/3	3/3	–	3/3	–	–	3/3	?	Gárate-Lizárraga et al., 2005
5	BACO, B.C.S. Mx	GSe	–	5/5	–	–	5/5	3/5	5/5	–	5/5	–	–	5/5	?	
2	BAMAZ, Sin, Mx	GSe	1/2	2/2	–	–	1/2	1/2	2/2	–	2/2	–	–	2/2	?	
16	BACO, B.C.S. Mx	f/2 + H_2SeO_3	–	?/16	–	t	–	?/16	16/16	–	16/16	–	–	16/16	?	Band-Schmidt et al., 2005
3	BACO, B.C.S. Mx	GSe	?	3/3	–	–	3/3	3/3	3/3	?	–	–	–	3/3	?	Band-Schmidt et al., 2006
2	BAPAZ, B.C.S. Mx	GSe	?	2/2	–	–	2/2	2/2	2/2	?	–	–	–	2/2	?	
2	BAMAZ, Sin, Mx	GSe	?	2/2	–	–	2/2	2/2	2/2	?	–	–	–	2/2	?	
1	BACO, B.C.S. Mx	f/2 + H_2SeO_3	–	–	–	–	–	–	1/1	–	1/1	–	–	1/1	?	Palomares-García et al., 2006
1	BACO, B.C.S., Mx	GSe	–	–	1/1	1/1	–	–	–	–	–	–	–	–	?	Estrada et al., 2007
1	BACO, B.C.S., Mx	f/2	1/1	1/1	1/1	1/1	–	–	–	–	1/1	–	–	–	?	Pérez-Linares et al., 2008
1	BACO, B.C.S., Mx	f/2 + H_2SeO_3	1/1	1/1	–	–	–	–	1/1	1/1	1/1	–	–	1/1	?	Bustillos-Guzmán et al., 2012
1	BACO, B.C.S., Mx	f/2 + H_2SeO_3	–	–	–	–	1/1	1/1	1/1	1/1	1/1	1/1	–	1/1	1/1	Bustillos-Guzmán et al., 2011
1	MANZ, Col, Mx	GSe	?	?	?	?	?	?	?	?	?	?	?	?	1/1	Durán-Riveroll et al., 2013
2	BAPAZ, B.C.S., Mx	f/2 + H_2SeO_3	–	–	–	2/2	2/2	2/2	2/2	–	2/2	2/2	–	2/2	?	Band-Schmidt et al., 2014
3	TOPO, Sin, Mx	f/2 + H_2SeO_3	–	–	–	2/2	2/2	2/2	2/2	–	2/2	2/2	–	2/2	?	
1	BAMAZ, Sin, Mx	f/2 + H_2SeO_3	–	–	–	2/2	2/2	2/2	2/2	–	2/2	2/2	–	2/2	?	
2	CAR, Mich, Mx	f/2 + H_2SeO_3	–	–	–	2/2	2/2	2/2	2/2	–	2/2	2/2	–	2/2	?	
2	BACO, B.C.S., Mx	GSe	–	–	–	–	2/2	2/2	2/2	2/2	–	2/2	2/2	–	2/2	Bustillos-Guzmán et al., 2012
2	BACO, B.C.S., Mx	f/2 + H_2SeO_3	–	–	–	–	2/2	2/2	2/2	2/2	–	2/2	2/2	–	2/2	
2	BAPAZ, B.C.S., Mx	GSe	–	–	–	–	2/2	2/2	2/2	2/2	–	2/2	2/2	–	2/2	
2	BAPAZ, B.C.S., Mx	f/2 + H_2SeO_3	–	–	–	–	2/2	2/2	2/2	2/2	–	2/2	2/2	–	2/2	
2	BAMAZ, Sin, Mx	GSe	–	–	–	–	2/2	2/2	2/2	2/2	–	2/2	2/2	–	2/2	
2	BAMAZ, Sin, Mx	f/2 + H_2SeO_3	–	–	–	–	2/2	2/2	2/2	2/2	–	2/2	2/2	–	2/2	
1	CAR, Mich, Mx	GSe	–	–	–	–	1/1	1/1	1/1	1/1	–	1/1	1/1	–	1/1	
1	CAR, Mich, Mx	f/2 + H_2SeO_3	–	–	–	–	1/1	1/1	1/1	1/1	–	1/1	1/1	–	1/1	
1	MANZ, Colima, Mx	GSe	–	–	–	–	1/1	1/1	1/1	1/1	–	1/1	1/1	–	1/1	
1	MANZ, Colima, Mx	f/2 + H_2SeO_3	–	–	–	–	1/1	1/1	1/1	1/1	–	1/1	1/1	–	1/1	
3	BACO, BAPAZ, B.C.S. and CAR, Mich, Mx (mix)	GSe	?	?	?	?	?	?	?	?	?	?	?	?	3/3	Durán-Riveroll et al., 2017

N, number of isolates analyzed; t, trace amounts; ?/No, positive but data not shown in original publication; ?, these analogs were not searched for. Epimeric pairs have been considered together because they easily epimerise under extraction and analytical conditions, so their measurements do not necessarily reflect their original composition in plankton cells (Krock, B., personal communication).

BAPAZ, Bahía de La Paz, Baja California Sur; BACO, Bahía Concepción, Baja California Sur; BAMAZ, Bahía de Mazatlán, Sinaloa; MAN: Manzanillo, Colima; TOPO, Bahía de Topolobampo, Sinaloa; CAR: Port of Lázaro Cárdenas, Michoacán.

Mx, Mexico. Bra, Brazil.

water-methanol fractions, where the *para*-hydroxylated benzoyl ring was visible (Durán-Riveroll et al., 2013).

In 2015, Bustillos-Guzmán and collaborators analyzed the toxin profile of *G. catenatum* from five locations along the Mexican Pacific coast that were cultivated in two different media (GSe, f/2 + H_2SeO_3). Analyses were performed by hydrophilic interaction liquid ion chromatography (HILIC) coupled to tandem mass spectrometry (MS/MS). In these analysis, C1/2 analogs were the most abundant, with >85% on molar basis. Decarbamoyl toxins, such as dcSTX and dcNEO represented a mean average content <5% and no differences were found between the two culture media (Bustillos-Guzmán et al., 2015). The richness of GC analogs in Mexican isolates was confirmed but due to the lack of standards, they could not be quantified, but in terms of relative abundance (peak area cell^{-1}) an interesting geographical increasing trend from strains from northern to southern regions was found (Bustillos-Guzmán et al., 2015). Also, the study was interesting since it was not possible to confirm the presence of GTX2/3 that had been reported in previous analysis done by HPLC-FLD. The same occurred for STX, and NEO, which led to the question on the existence of phantom peaks, particularly in dcNEO, which probably had been misreported as NEO. These findings raised doubts on the reliability of the most used technique in Mexico and LAm in general, especially for analogs for which reference standards are unavailable, such as GC toxins (Bustillos-Guzmán et al., 2015). For these reasons, it has been proposed that a second detection method, such as MS, should be performed in order to overcome the normal drawbacks of the identification methods.

The most recent research on GC analogs has been performed by Durán-Riveroll et al. (2017). They confirmed the production of fifteen GC analogs in the mixture of three Mexican isolates from the GOLCA, and the coasts of Michoacán, in the Pacific coast. The microalgal extract was fractionated by reverse-phase column chromatography and a MS/MS-guided fractionation was performed. Confirmation was done using MS on the search of the precursor ions and their fragments, using HILIC-MS/MS. For the analogs with same mass and mass fragments, ^{1}H-NMR spectroscopy was used to discriminate between them (GC4/5 and GC1a/2a). Fifteen GC analogs were identified: GC1-6, GC1a-5a, GC1b/2b, and GC4b/5b. During this study, the chromatographic behavior of the previously named "hydrophobic analogs" was analyzed, and it was revealed that GC analogs are as hydrophilic as many of the "common hydrophilic" analogs. In light of these results, authors proposed a better designation as "benzoyl analogs" and subdivide them into *para*-hydroxybenzoyl, di-hydroxybenzoyl, and sulfo-benzoyl analogs (Durán-Riveroll et al., 2017). The combination of high resolution techniques, such as HILIC-MS/MS and ^{1}H-NMR represented an important progress on the GC analogs identification and proved the need of the use of confirmatory techniques, mostly when relatively unknown analogs are investigated.

Cell Toxin Content

Reported toxicities from different studies are shown in **Table 3**. Differences in toxicity have been found among isolates (Band-Schmidt et al., 2006), related mainly to the toxin profile: isolates from BAMAZ have been found to be the most toxic with a higher amount of the more potent carbamoyl toxins; isolates from BAPAZ were in second place and the ones from BACO, where the least toxic. A wide variation in toxicity among isolates from the same area have also been found, which has been reported previously in other geographical areas (Bolch et al., 2001; Seok Jin et al., 2010). Also, toxicity in all isolates reached their highest point during the exponential growth phase (Band-Schmidt et al., 2006), as described for other isolates. The only other report of the toxin content in cultured cells is from a Brazilian isolate were the toxicity was estimated by mouse bioassay to be 29 pg STXeq cell^{-1} (Proença et al., 2001).

According to previous studies, cultured dinoflagellates tend to produce less toxin per cell (cell toxin quota) due to the "forced growth" by high nutrient conditions, compared to natural populations (Cembella, 1998). In nature, cell division could take longer and toxins tend to concentrate inside the cell. Nevertheless, *in vitro* observations from *G. catenatum* cultures in Mexico have shown higher cell toxin quotas than natural populations, probably related to differences in nutrients between natural and culture conditions (Gárate-Lizárraga et al., 2005; Band-Schmidt et al., 2006). However, the experiments with different N:P ratios did not shown effects in toxicity and/or toxin profile (Bustillos-Guzmán et al., 2012).

Docking Studies

Since the properties of GC analogs, such as mammalian toxicity, receptor-binding affinities and biological action mechanisms are mostly unknown due to the lack of analytical standards and the difficulties in producing and purifying these toxins, computational tools were used as an alternative strategy for their exploration. Only one docking study on *G. catenatum* toxins has been done in LAm, by Durán-Riveroll et al. (2016). Docking modeling is a useful *in silico* tool that can reveal molecular interaction mechanisms in great detail, generating information that cannot be deduced from electrophysiological approaches (Gordon et al., 2013). Molecular docking approaches have been key in the description of the interactions of several toxins with Na_V channels, like with μ-conotoxins (Li et al., 2001). This approximation, even though it allows screening of many ligands for a given protein (Halperin et al., 2002; Brooijmans and Kuntz, 2003), also has certain disadvantages, related to its accuracy (Warren et al., 2006). In any case, it is possible to obtain important clarifying information about the mode of action and affinity of toxins in a protein target.

Members of the Na_V channels family are the membrane-protein targets for STX and its analogs. These interactions are mediated by the guanidinium groups in the toxin molecules, which are formed by a central carbon and three nitrogen atoms, and have a positive charge at physiological pH, directly implicated in their binding capacity to the Na_V, and allows the toxins to block the Na^+ influx to the cell (Durán-Riveroll and Cembella, 2017). Docking studies have been useful to gain a better understanding of the blocking capacity, and due to the absence of information on GC analogs, theoretical studies are considered essential to increase the knowledge about their properties. In this study, authors found that all eighteen GC

TABLE 3 | Toxin content of cultured *G. catenatum* under laboratory conditions.

No. of isolates	Origin	Culture media	Toxicity (pg STXeq cell^{-1})	References
1	Armaçao do Itapocoroy, Bra	K + SE	28.0	Proença et al., 2001
3	BAPAZ, B.C.S. Mx	GSe	15.1	Gárate-Lizárraga et al., 2005
5	BACO, B.C.S. Mx	GSe	13.3	
2	BAMAZ, Sin, Mx	GSe	20.3	
16	BACO, B.C.S. Mx	f/2 + H_2SeO_3 17 days	25.7 ± 6.2*	Band-Schmidt et al., 2005
16	BACO, B.C.S. Mx	f/2 + H_2SeO_3 22 days	27.9 ± 17.7*	
3	BACO, B.C.S. Mx	GSe	13–47	Band-Schmidt et al., 2006
2	BAPAZ, B.C.S. Mx	GSe	36–73	
2	BAMAZ, Sin, Mx	GSe	37–101	
1	BACO, B.C.S. Mx	f/2 + H_2SeO_3	60.3	Palomares-García et al., 2006
1	BACO, B.C.S. Mx	f/2 + H_2SeO_3	21.8 ± 2.9	Bustillos-Guzmán et al., 2012

BAPAZ, Bahía de La Paz, Baja California Sur; BACO, Bahía Concepción, Baja California Sur; BAMAZ, Bahía de Mazatlán, Sinaloa. Mx, Mexico. Bra, Brazil. SE, Soil Extract.
**Average toxicity was not significantly different.*

analogs theoretically interacted with the Na_V 1.4 (muscular Na_V channel) residues in the two protein models used for the experiment. They also reported high affinity values (as low binding energies ΔG), for some of the GC toxins, raising the hypothesis that at least some of them could be toxic to mammals because they are able to reach key protein residues by electrostatic interactions (Durán-Riveroll et al., 2016). As the model of ion channel of Na_V eukariotic structure continues improving (Shen et al., 2017) instead of using homologation models of the bacterial Na_V, as in this analysis, docking studies will generate more precise information. The greatest obstacle, however, is that docking programs, in order to reduce computational costs, consider the protein as a rigid body, which is far from real. The omission of conformational protein changes due to ligand binding can yield unrealistic results, and for that reason, the use of molecular dynamics (MD) is highly recommended to be used in combination with docking approach (Deeb et al., 2010), though the later greatly increases computational costs and it is more time consuming than molecular docking simulations.

Molecular Studies

The first study of genetic variation among strains of *G. catenatum* from Asia, Europe, and Australia was published by Bolch and collaborators in 1999. Populations from Japan, Spain, and Portugal showed higher genetic similarities than with those from Australia, where a recent dispersal could have happened (Bolch et al., 1999). In LAm, molecular analyses of the species are scarce. In 2008 the LSUrDNA sequences and morphology of strains from the GOLCA were reported, where a single nucleotide polymorphism was identified in sequences from strains from BACO, suggesting a mutation or genetic isolation (Band-Schmidt et al., 2008). Authors proposed that the Western Pacific population could be an ancestral population of this species. In 2013, the gene expression and histological injuries in the Japanese oyster *Crassostrea gigas* when exposed to *G. catenatum* toxins were analyzed, determining changes in the transcription level cell-cycle regulation genes and epithelial damages associated to inflammatory responses, concluding that the toxins induced DNA damage in this mollusk (García-Lagunas et al., 2013).

The origin of STX genes and the relationship between copy number and genome size in *G. catenatum* has not been well established, recently Mendoza-Flores et al. (2018) carried out a study to determine the origin of *sxt*A (domains *sxt*A1 and *sxt*A4) and their gene copy number. The phylogenetic tree with partial sequences of *sxt*A1 of *G. catenatum* showed a separated subclade of toxic and non-toxic *Alexandrium* species, while sequences of *sxt*A4 showed two well-supported clades within the dinoflagellates group, separating *G. catenatum* from *Alexandrium* species. Authors concluded that *G. catenatum* did not acquire *sxt*A gene by horizontal gene transference from *Alexandrium*. Moreover, differences in copies number of *sxt*A1 and *sxt*A4 between *G. catenatum* and *Alexandrium* species were observed.

Pyrodinium bahamense

Pyrodinium bahamense is an important member of PST-producing marine dinoflagellates, especially in tropical waters, and has caused more human illnesses and fatalities than any other PST producing dinoflagellate (Usup et al., 2012). This species was originally described from New Providence Island, Bahamas (Plate, 1906). For many years, two varieties were assigned to the genus: the Indo-Pacific variety designated "*compressum*," and the Atlantic-Caribbean variety "*bahamense*." However it was demonstrated that the range of distribution of both varieties extended beyond these original locations (Martínez-López et al., 2007; Morquecho, 2008), and that both varieties co-occur in several areas (Glibert et al., 2002; Gárate-Lizárraga and González-Armas, 2011). In addition to several morphological attributes, one of the primary differences between both varieties was the

absence of toxin production in var. *bahamense* (Steidinger et al., 1980). However, a recent re-evaluation found no consistent morphological or molecular traits that could be used to separate the varieties, since toxin production was also demonstrated in var. *bahamense* (Landsberg et al., 2006; Mertens et al., 2015).

Its distribution in the Pacific coast extends from southern GOLCA to Colombia (Martínez-López et al., 2007; Rodríguez-Salvador and Meave del Castillo, 2007; Morquecho, 2008; Gárate-Lizárraga and González-Armas, 2011; Usup et al., 2012). In the Atlantic coast it has been reported from the Gulf of Mexico to Uruguay (Licea et al., 2013; Limoges et al., 2015; Mertens et al., 2015; Poot-Delgado et al., 2015; Cusick et al., 2016). HABs have been linked in several occasions to human poisoning (Rosales-Loessener, 1989; Rodríguez et al., 1990; Saldate-Castañeda et al., 1991; Núñez-Vázquez et al., 2011; Gárate-Lizárraga et al., 2012; Callejas et al., 2015) as well as to epizootic events (fish and sea turtles) (Núñez-Vázquez et al., 2011; Amaya et al., 2018) (**Table 1**). The first HAB of this dinoflagellate in LAm was reported in the coast of Guatemala in 1987, causing 175 intoxications and 26 fatalities (Rosales-Loessener, 1989). PSP cases produced by this species have affected several Latin American countries, notably in the southern Mexican Pacific, and Central America (**Figure 2**).

Bloom Dynamics

Mexico

Vegetative cells of *P. bahamense* were reported for first time in 1942 in Mexico (Osorio-Tafall, 1943). In several occasions, this species has been registered from the coasts of Michoacán to Chiapas (**Figure 2**) (Cortés-Altamirano et al., 1993; Orellana-Cepeda et al., 1998). In winter 1995, a HAB was recorded on the SW coast, affecting invertebrates, fish, and causing the death of 145 turtles (Orellana-Cepeda et al., 1998).

In the GOLCA, it was recorded for the first time in May 2005, in the lagoon system of Topolobampo-Santa María-Ohuira, with an abundance of 100 cell L^{-1}. Cysts were also recorded (Martínez-López et al., 2007). Shortly afterwards, vegetative cells in the island San José in BAPAZ were reported (Morquecho, 2008). The author mentioned that the relatively low frequency of cysts of *P. bahamense* in this zone may indicate the influence of warm water from the tropical Pacific, and therefore it is likely that El Niño event contributed to the transport of vegetative cells or cysts from Central America. High temperatures prevailed in summer (July-October) when outbreaks occurred close to San José Island. From July to November 2010, in the southern peninsula of Baja California, it was found with abundances between 800 and 1110 cell L^{-1} at a SST of 28–28.5°C and salinity of 35.2 (Gárate-Lizárraga and González-Armas, 2011). These contributions widen the northern distribution of this species in the Pacific coastline of LAm. The presence of this dinoflagellate from July to November was considered unusual, and again authors attributed this to El Niño event. It was suggested that this is an invasive species and that it was probably transported by surface tropical waters moving within the GOLCA (Cortés-Altamirano et al., 2006; Gárate-Lizárraga and González-Armas, 2011). Also, the species was recorded in coastal lagoons of Sinaloa (Alonso-Rodríguez et al., 2015). To date, no HABs of *P. bahamense* have been reported in this region.

During December 1989 and February 2002, in the southern Pacific coast of Mexico, HABs of this species were registered (Licea et al., 2008). Environmental conditions based on satellite images reported a positive thermal anomaly of 1.5°C between southern Mexico and Costa Rica. The dinoflagellate was recorded on the coast of Oaxaca from September 2009 to June 2010, with abundances from April to June 2010 of 4×10^3 cell L^{-1} and 3.3×10^4 cell L^{-1}, respectively. The SST ranged from 27.4 to 31.3°C and from 28.4 to 30°C, respectively (Alonso-Rodríguez et al., 2015).

In 2001, in BACA, cysts were reported (Meave del Castillo et al., 2006). Ten years later, in July 2010 vegetative cells were recorded at low abundances, from 1×10^3 to 1462×10^6 cell L^{-1} (Gárate-Lizárraga et al., 2012).

Based on a cyst study in the Gulf of Tehuantepec, it was suggested that the species has been present in this region since 1860, and that cells have been transported recently from the Indo-Pacific population (Sánchez-Cabeza et al., 2012). They mentioned that, since 1950, its influx increased in this region, due to La Niña events, however historical data of strong El Niño anomalies, recorded from 1892 to 1983, indicated there was a minimum cyst flow. Emphasizing that in 81% of cases these fluxes coincided with heavy rain (>400 mm) and high nutrients, and that high cyst flow was correlated with lower temperatures (<24.5°C). The authors proposed that low SST (conditions during La Niña) could favor the dinoflagellate growth. When a rapid transition to high SST conditions occur, rainfall increased with an input of large amounts of nutrients, making it possible to maintain the bloom. At the termination of the bloom, cysts could be incorporated into the sediments. They suggested that this situation may occur during strong upwelling conditions, hypothesis that needs to be confirmed. It was also suggested that the exceptional records in the coastline and offshore waters of the Mexican central Pacific during the 1999–2000 La Niña could suggest a link with the recurrence of HABs (Hernández-Becerril et al., 2007).

In the southern Gulf of Mexico, according to data recorded from 1966 to 1996, in coastal lagoons this species is widely distributed throughout the year (Gómez-Aguirre, 1998). In Laguna de Términos, the highest reported abundance has been 3×10^3 cell mL^{-1}, and blooms are more frequent in autumn-winter. HAB formation could be associated with seawater fluxes and northern winds that remove the water column. Mangrove forests could also provide suitable conditions for cell accumulation (Gómez-Aguirre, 1998). In the Gulf of Mexico, this dinoflagellate is limited to the SE region (Aké-Castillo and Poot-Delgado, 2016).

Other Latin American Countries

In November 2005 and March 2006, in the coast of El Salvador, satellite images showed a positive thermal anomaly of 1.5°C between southern Mexico and Costa Rica during a HAB of *P. bahamense* (Licea et al., 2008). In December 2005, its abundance reached 489×10^5 cell L^{-1} and in March 2006 it decreased to 15 cell mL^{-1}. The authors mentioned that blooms could be influenced by SST, strong winds and heavy precipitation.

From November to June 2010 in the same area, a bloom of ∼13 km was recorded with abundances of 15.3×10^6 cell L^{-1}. Offshore concentrations were of 22×10^3 cell L^{-1} (Licea et al., 2012). They stated that HABs of this dinoflagellate in Central America are influenced by smaller scale gyres, upwelling and local hydrographic conditions. Cysts were detected in March and August 2012 in the Gulf of Fonseca (Alvarado et al., 2014).

In November 2005, a HAB was detected on the Pacific coast of Nicaragua, with a maximum abundance of 17.3×10^6 cell L^{-1}, being likely that this HAB was a reflection of a major climate event scale that covered a wide area of the Central Pacific (Chow et al., 2010).

Toxin Profiles in Environmental Samples

Data regarding toxicity and toxin content for *P. bahamense* is scarce. Only two reports on toxin profiles in natural samples exist. The first one is from El Salvador, during a bloom in 2005–2006 that caused human intoxications and mortality of sea turtles (Licea et al., 2008). The second report is from phytoplankton samples of a HAB in the coast of Guerrero, Mexico in 2010. Sulfocarbamoyl analogs dominated (>80 mol%), followed by STX and GTX3, with a contribution of 12.0 and 6.8 mol%, respectively (Gárate-Lizárraga et al., 2012).

In 1987, during a HAB in Guatemala, the clam *Amphichaena kindermanni* presented mainly B1 toxin, accompanied by small amounts of STX and NEO (Rodríguez et al., 1990). The net toxicity calculated from the concentrations of these three toxins corresponded to 7,500 mg STX dihydrochloride 100 g^{-1}; 93.7 times above the maximum level for most regulations in Latin American countries that limits PSTs content at 80 μg STXeq 100 g^{-1} (400 MU 100 g^{-1}) for human consumption. During this event, 175 people were intoxicated, and 26 people died (Rosales-Loessener, 1989).

In 1991 and 1993, in Uruguay, toxin profile of *M. edulis* was linked to HABs of this species. The mollusk presented C1/2 (up to 53 mol%), with significant amounts of GTX2/3, and STX, while *D. hanleyanus* presented decarbamoyl analogs, mainly dcGTX2 (∼63 mol%) (Méndez et al., 2001).

In Mexico, most of the human intoxications related with this species have been due to the consumption of diverse bivalve species (Saldate-Castañeda et al., 1991; Cortés-Altamirano et al., 1993; Gárate-Lizárraga et al., 2012). In 1995, in BACA, STX, GTX2, dcGTX2/3, dcSTX as well as B1 were reported in the oyster *C. iridescens* (Núñez-Vázquez et al., 2007a). In 2010, a dominance of sulfocarbamoyl analogs (59 mol%), mainly B1/2 and C1, followed by STX (12%) and GTX3 (6.8%) were recorded in *C. mexicana*, in the same bay (Gárate-Lizárraga et al., 2012), with toxin concentrations ranging from 579.0 to 894.7 μgSTX 100 g^{-1}. In 2001, in the coasts of Chiapas, STX, NEO, GTX2/3, B1 were detected in *D. gracilis*, and STX, GTX2/3 and B1 in *M. capax* (Núñez-Vázquez et al., 2007a). No data on toxin concentrations were given.

During a bloom in Nicaragua, 45 people developed symptoms of PSP with one fatal case in 2005. The toxin profile of *A. tuberculosa* presented STX as the dominant toxin with small quantities of NEO and B1 (Callejas et al., 2015).

Molecular Studies

Limited genetic information exists regarding *P. bahamense*. Morphological differences, as well as the LSUrDNA sequences were analyzed in vegetative cells and cysts of both varieties: *compressum* and *bahamense*, from distinct geographic regions such as Jamaica, Puerto Rico, Guatemala and Colombia (Mertens et al., 2015). No morphological differences between vegetative cells of the isolates were found. Cyst morphology, however, showed differences between specimens from Indo-Pacific and Atlantic-Caribbean regions. Results agreed with ribosomal sequences, where a distinct ribotype was identified for each geographical region. Based on these results, the authors suggested that it is a species complex.

As many other dinoflagellates, this species has bioluminescence capacity. Molecular diversity based on 18S rRNA and on the luciferase gen (*lcf*) was examined, using single cell isolates from Florida, USA and Puerto Rico. Phylogenetic analysis revealed that all sequences clustered together and were closely related to *Alexandrium* spp. With *lcf* sequences from Florida and Puerto Rico, two distinct clusters formed, defined by a set of core amino acid substitutions, and the inclusion of *lcf* sequences from the Indo-Pacific strain resulted in a third cluster. *Lcf* sequences of *P. bahamense* were more closely related to *Pyrocystis* spp. than *Alexandrium* spp. suggesting a much greater variation than that seen in bioluminescent species with known gene variants (Cusick et al., 2016).

Alexandrium Species

One of the most studied harmful algal blooming genera worldwide is the genus *Alexandrium*. More than 30 species have been defined and at least half of them are known to be toxic or have harmful effects (Anderson et al., 2012). Three different families of known toxins are produced by species of this genus: STX, spirolides, and goniodomins. In LAm this genus is widely distributed (**Figure 2**), and several toxic species have been reported: *A. catenella*, *A. minutum*, *A. monilatum*, *A. ostenfeldii*, *A. tamarense*, and *A. tamiyavanichi*. There are also reports of *A. peruvianum,* however Kremp et al. (2014) proposed that *A. peruvianum* should be considered as a synonym of *A. ostenfeldii* due to inconsistent morphological and gradual genetic divergence of groups, together with no evidence of compensatory base changes indicating reproductive isolation (Kremp et al., 2014). For the purpose of this review *A. peruvianum* is considered as a synonym of *A. ostenfeldii*.

HABs of *Alexandrium* are mostly reported in South America and the main responsible species are *A. catenella* (Chile) and *A. tamarense* (Uruguay, Argentina). The intensity and frequency of these HABs have increased since 1990, causing severe economic losses in the fishing and aquaculture sector (Álvarez et al., 2009; Menezes et al., 2010; Aguilera-Belmonte et al., 2011, 2013; Mardones et al., 2015).

A total of 350 cases have corresponded to the consumption of seafood associated with HABs caused by *Alexandrium* species in South America (**Table 1**). Chile is the most affected country; HABs in this area have also caused massive mortalities of

invertebrates, fish, both wild and cultivated, as well as seabirds and whales.

Bloom Dynamics

Chile

In Chile, the main *Alexandrium* species reported is *A. catenella*. From 1972 to 1994, outbreaks in the MAGR and Aysén regions (AYSR) were attributed to this species. HABs have increased in their frequency, extension, duration and intensity (Guzmán et al., 2002, 2010). Until this date the distribution limits of this species were: Cailin in the southern sector of Los Lagos region, and in the north Seno Ponsonby, in the MAGR. In October 1972, after a period of high irradiance and thermohaline stratification, a HAB lasted for 5 weeks, with densities from 2.4 to 6×10^5 cell L^{-1} at the surface, and 157×10^2 and 3201×10^2 cell L^{-1} in the sub-surface layer (5–10 m), respectively. The same hydrographic conditions were recorded in Bahía Bell in 1973, when this species bloomed. In subsequent years (1972–1994) HABs continued emerging (Guzmán et al., 2002). The authors suggested that blooms occurred in response to global changes related to El Niño, which causes a decrease in salinity and stronger stratification, proposing that blooms are related to the terminal period of La Niña and the initiation of intense El Niño episodes. The authors concluded that this dinoflagellate is able to bloom under calm conditions, high insolation and a stable water column.

There is a list of data of HABs of this species from 1994 in the MAGR, with maximum abundance values of 30,902 cell L^{-1}, and in the AYSR of 5,808 cell L^{-1}. Cells are found from spring to autumn, when high temperatures trigger the blooms (Guzmán et al., 2002). From 1993 to 1998, also in AYSR, several dinoflagellates were identified, including *A. catenella*. Temperature ranged from 5.2 to 16.2°C; the lowest temperatures were recorded in 1996, and the highest in 1994 and 1998. HABs coincided with a temperature from 11 to 14°C, mainly between December and April, being more frequent in February (Cassis et al., 2002). Data suggested that 1998 was an anomalous year that presented an extensive HAB that covered the entire south of the country in March, overlapping with positive anomalous values. This event caused 20 human poisonings, two fatalities and losses of over USD 10 M to the salmon industry (Mardones et al., 2015).

From 1995 to 2002, several outbreaks with marked seasonality were reported in internal waters of the NW Patagonia (Molinet et al., 2003). Blooms occurred mainly from January to March, with a tendency to expand its distribution northwards. These events were attributed to the presence of cysts banks, and associated to thermal oscillations in adjacent ocean waters that affected the circulation in inland waters. Cyst dispersion was associated to the drift of surface waters, caused mainly by winds and circulation of inland waters (Molinet et al., 2003). Also, the structure of the water column suggested that these HABs originated from the mixture of Antarctic and sub-Antarctic surface waters, derived by wind action. Cyst germination is considered relevant in the recurrent HABs in inland seas, as well as in the fjords and MAGR channels (Uribe et al., 2010). In 2005 and 2006, another bloom in the X region occurred at 12°C (Fuentes et al., 2008). Data suggested that stratification conditions favored dinoflagellates growth and coincided with the presence of PSTs (Díaz et al., 2014).

In March 2009, a HAB covered a wide geographical area from 46° to 43° 45′ S. During late February in Easter Island, this dinoflagellate reached a maximum of 2230 cell mL^{-1}; in March, in the same geographic region it reached 12×10^2 cell mL^{-1}, and in Cuptana 6×10^3 cell mL^{-1}. These data demonstrated an expansion of the northern distribution of this species with respect to their historical distribution range, being the first record of *A. catenella* in oceanic waters of the Pacific coast SE outside Chiloé Island. The new distribution suggested a cell transport toward inland waters through Chacao channel. Cells probably also reached Chiloé water fjord and channels, by oceanic water masses (Mardones et al., 2010). Northern distribution of this species continues expanding, during the last intense bloom of *A. catenella*, in the late summer of 2016, PSP-affected areas reached for the first time as far north as Los Ríos (39°S) (Hernández et al., 2016).

In the Southern Austral Ecosystem, this species was associated to temperatures between 9 and 13°C and salinities of 28 and 33 (Cruzat et al., 2018), with higher abundances at 10°C. The high genetic diversity in terms of population differentiation suggested a possible recent dispersion. Many factors could be influencing this dispersion, as the frequent use of transportation related with aquaculture operations, ocean currents, and natural dispersion. In the AYSR there has been a tendency of intense HABs in summer. Cysts play a key factor for repopulation during spring-summer and they can be re-suspended by the advection of the water column (Pizarro et al., 2018). In southern Chile fjords, resting cyst of *A. catenella* are sparce and there have been doubts on their importance in the recurrence of massive toxic dinoflagellate blooms in the region. Mardones et al. (2016) under laboratory conditions demonstrated with strains isolated from this region that moderate vegetative cell abundances (>400 cell mL^{-1}) can produce high amounts of cysts which have a short dormancy period (minimum 69 days). These results are in agreement with the finding by Díaz et al. (2014) of empty cysts found a few months (∼3 months) after a bloom event, representing less than 10%. Mardones et al. (2016) suggest that the short cyst dormancy for Chilean strains explains the rapid cyst depletion from the sediments of the inner fjords. However, dense cyst aggregation cysts can be accumulated and preserved in selected areas of the fjords. Authors point out the need to investigate the oceanographic conditions that lead to massive outbreaks.

Argentina

In the Argentine sea, *A. tamarense* is the most frequently reported harmful dinoflagellate species. During 1980–1984, several HABs of this species were reported. In 1980 a high abundance (1.8×10^6 cell L^{-1}) restricted to a hydrographic frontal system was found outside PVAL, characterized by high nutrients and turbulence (Carreto et al., 1985). The initial outbreaks expanded widely, and covered most of the Argentine coasts, in the frontal area, where vegetative cells, cysts, and high levels of toxicity are found. Also, a decoupling zooplankton-phytoplankton arrangement was found, being the main predator the dinoflagellate *Polykrikos schwartzii*

(Carreto et al., 1985). In 1981, a HAB lasted 4 months (September to December), and was also restricted to a frontal system. In areas with stratified waters, cysts were found in sediments and were re-suspended during the mixing of the water column. Also seedbeds of hypnozygotes were observed which are known to serve as dispersion centers and increase the extension of toxic areas (Carreto et al., 1985). In 1995, during an annual cycle, cysts were found most of the year in coastal waters in MPlat (151–2758 cysts cm^{-3}) (Carreto et al., 1998a). *In situ* germination was the main source of vegetative cells that were initially found in August at 30 m (80 cells L^{-1}) depth and in September at 0 m (15×10^3 cells L^{-1}). A broad temperature range was recorded, from 9°C to 20°C in summer, during a well-developed thermocline. It has been suggested that the presence of vegetative cells in winter could be attributed to the biological mechanism that controls cyst germination before temperature rises (Carreto et al., 1998a). A retrospective analysis of HAB and toxicity concluded that most records of this species occur in a wide coastal strip from 34° to 47° S at a depth between 50 and 100 m. Maximum abundances are reported in the frontal system of the Patagonian tidal region. Advection transport from external waters toward the coast explained the accumulation of cells in the intertidal zone. The Patagonian current contributed to the northward spread of cysts, the thermocline, hydrographic fronts and ocean currents were also key factors for their growth and distribution (Carreto et al., 1998a).

In Golfo Nuevo (GNVO) this species was recorded in August 1995 (Gayoso, 2001), at a lower temperature (9°C), with a peak in September of the same year of 6.1×10^3 cell L^{-1}. Cysts were recorded in surface sediments (300 cysts cm^{-3}). Heavy precipitation in winter and high solar radiation during spring could have influenced the formation of HABs. However, during blooms in July and December 2000, with a maximum abundance of 21.8×10^3 cell L^{-1}, and temperature from 9 to 12.5°C, a multiple linear regression of the cell abundance with environmental variables (solar radiation, temperature, wind speed, rainfall, and nutrients), did not show a high correlation (Gayoso and Fulco, 2006), remaining to determine the factors that influence its blooms in this region.

Waters in the north coast of the Patagonian region, are ecosystems poor in nitrates (Montoya et al., 2010). On the other hand, phosphate concentrations are relatively high throughout the year, reason why the N:P ratio is heavily imbalanced with respect to the Redfield ratio. On the contrary, nitrate concentration is high in the sub-Antarctic waters and the Patagonian current mainly in southern latitudes, where N limitation was not found during summer. Therefore, it is assumed that the nutrient ratio and cell toxin content from natural populations of *A. tamarense* are correlated with the availability of nutrients in the southern region of South America, and the toxin content is inversely correlated with water temperature.

Other *Alexandrium* species, such as *A. catenella, A. excavatum* and *A. ostenfeldii* have been reported in Argentina. During spring/summer of 1980 PSTs were detected for the first time in GNVO (Esteves et al., 1992). The responsible species was *A. excavatum*, with abundances from 7.5×10^5 to 3.1×10^3 cell L^{-1} during an upwelling event. The SST was 15°C and prevailing winds were from the SW (>20 km h^{-1}). In late December northern winds dominated, the average temperature was 18.3 ± 0.9°C and salinity was 33.9. Nitrate concentration remained constant, 0.3 ± 0.1 μg-at L^{-1}. However, in February nitrates, phosphates and ammonium raised to 1.9, 7.1, and 9.3 μg-at L^{-1}, respectively.

In the Beagle Channel, during 2006 and 2007 *A. catenella, A. tamarense* and *A. ostenfeldii* were observed (Almandoz et al., 2011). Temperature ranged from 4.9 to 10.1°C, and salinity from 30.7 to 31.2. In 2006, nitrate and phosphate were higher in winter (15.2 and 1.4 μM, respectively). The highest abundance of *A. tamarense* was detected in spring 2006 with 11.7×10^5 cell L^{-1} (Almandoz et al., 2011). Outbreaks in the Beagle Channel of this dinoflagellate, have been proposed to have a relationship with the decrease in the ozone layer (Benavides et al., 1995). In the same region, in July 2005 and December 2006, *A. ostenfeldii* had a low abundance (5860 and 3850 cell L^{-1}, respectively). Temperature and salinity varied between 7.5 and 10°C and 30–30.5. Considering the apparent absence of records of this species in the Antarctic waters, the Beagle channel is considered as the most southerly distribution of *A. ostenfeldii* (Almandoz et al., 2014).

Other Latin American Countries

In El Salvador, in September 2014 an intense HAB of *M.* cf. *polykrikoides* co-occurred with *Alexandrium* cf. *globosum*. Fish deaths were reported and attributed to *M. polykrikoides* (Alvarado et al., 2015).

In the Pacific coast of Colombia, in Bahía Tumaco and in the island Gorgona, in April/May 2001, an *A. tamarense* bloom with maximum abundances of 7.5×10^6 cell L^{-1} occurred (García-Hansen et al., 2004). A year afterwards, in March 2002, another event was recorded from northern Cabo Corrientes to Bahía Solano, with a lower abundance (1.6×10^6 cell L^{-1}). In both occasions, temperature was between 24 and 24.6°C, and salinities between 33 and 34. These parameters were abnormal, since temperature was three degrees lower and salinity was 2.5 higher than the average for this region. The authors assumed that these conditions reflected upwelling events, and suggested that this could be a recently introduced species.

In Venezuela, *A. tamarense* was recorded forming HABs since 1972, during March and June with abundances of 1×10^6 cell L^{-1}. Cells were also detected in mussel's viscera, in raft-cultures in the Gulf of Cariaco. In 1975, in April-March, this species proliferated with other dinoflagellates. This HAB was associated with the predominance of trade winds that influenced the water circulation, causing coastal upwellings. Another bloom occurred in the NW coast of island Margarita in May 1977, intoxicating 171 people.

In February 2010 and May 2014, in Peruvian shellfish extraction areas, HABs of *Alexandrium* were reported. In May 2012 *A. ostenfeldii* bloomed at a temperature between 18 to 23°C, phosphate concentration ranged from 0.7 to 11.6 μM and nitrate concentrations from 0.8 to 9.8 μM. In 2015, *A. minutum* was reported for the first time off the coast of Peru, with a maximum abundance of 16×10^6 L^{-1}, suggesting that this species was

transported by ballast water or that its presence had not been detected previously (Baylón et al., 2015).

In Brazil, the distribution of *A. tamarense* apparently expanded into the southern hemisphere during the last two decades. The first HAB was documented in 1996 (Persich et al., 2006). Through satellite images, a cold water front that moved from northern Uruguay to Brazil was detected, which may have been the transportation vector. The high similarity among toxic profiles of *Alexandrium* strains from Brazil and Uruguay, suggested a Uruguayan origin of the Brazilian strain. Also, the low number of cysts in Brazilian sediments supported the hypothesis of a recent dispersal from Uruguay. The authors concluded that the southern Brazilian coastline may have experienced additional outbreaks of PSP originated from local cyst germination or by recent populations that were transported by the Uruguayan coastal current (Persich et al., 2006). In 2007, *A. minutum* was registered on the coast of Río de Janeiro, with abundances from 4.3×10^5 to 1×10^2 cell L^{-1}; cysts were also registered (Menezes et al., 2007).

In Uruguay, frequent HABs of *A. tamarense* were registered during the 90s. From 1991 to 1993, recurrent blooms were reported in early spring, near La Paloma, with abundances of 80×10^3 cell L^{-1}. In the coastal area, in a transition zone between the stream and the Malvinas current, abundance was of 355×10^2 cell L^{-1} with a SST from 11 to 15°C (Méndez et al., 2001). A wide and strong thermal-saline front during spring-winter coincided with the HABs; blooms initiated when the discharge of the Río de la Plata decreased, as well as the front of the Falklands current (Méndez et al., 1996). From 1991 to 2004, HABs of *A. tamarense* and *G. catenatum* have been reported, blooms of *Alexandrium* occurred at abundances of 10×10^3 cells L^{-1}, temperatures between 11 and 14°C, and salinities of 32.2.

Toxin Profiles in Environmental Samples

Alexandrium catenella

Paralytic toxins have affected ∼35% of the Southern Pacific coast of Chile (Oyaneder-Terrazas et al., 2017). In 1996, samples of the mussel *M. chilensis* from the regions XI and XII, were analyzed, and in general they showed a molar dominance of GTX analogs, with a dominance of GTX2/3 in samples collected in the XI region, whereas GTX1/4 were the major analogs in the XII region (Lagos et al., 1996). The more potent analogs STX and NEO represented, together, 12 and 16 mol%, for the XI and XII region, respectively. A clear contrasting pattern was observed for the XII region when comparing *M. chilensis* profiles between years with a dominance of STX and NEO (45–46 mol%) in 1992 (Lagos et al., 1996). In several bivalves collected in 2012 in the AYSR (Region XI) a clear dominance of GTXs analogs was evident in most bivalves (García et al., 2015). Record toxicity values have been found in the AYSR with maximal values oscillating between 22,000 and 28,340 μg STX eq 100 g^{-1} (Guzmán et al., 2002; Molinet et al., 2003).

Compartmentalization of PSTs showed that GTX analogs are mainly found in the digestive tract. The case of *M. chilensis* is relevant since in the adductor muscle 99% (in molar basis) NEO was found, whereas in the mantle and digestive glands, GTX1/4 were detected. In a recent study of the PSTs content in regulated and non-regulated aquatic organisms from regions with a variable presence of *A. catenella* (Oyaneder-Terrazas et al., 2017), the analogs GTX1/4, GTX2/3, NEO, dcSTX, STX were detected. Dominant analogs in rocky strata-dwelling species were 58.8 mol% STX, followed by 15.4 mol% GTX2/3, 4.8 mol% NEO, and 3.3 mol% dcSTX; while in sandy bottom-dwelling species, 77.7 mol% of GTX2/3 were detected, 19.3 mol% STX, 2.1 mol% NEO and 0.9 mol% dcSTX. Data clearly showed the variability of toxin profiles in this zone and that this variation is not only associated to the sampling season, but also to the distinct basal analogs produced by this dinoflagellate as reported previously (Varela et al., 2012) and the bivalve species.

In phytoplankton samples, sulfocarbamoyl toxins C1/2 and B1 represented 70% in molar basis of the total analogs, followed by carbamoyl analogs GTX1/4 (24 mol%), contributing in smaller proportions GTX2/3 (≈3 mol%). NEO, STX, dcGTX2/3 and dcSTX were found in trace levels. Cell toxicity was estimated in ≈15 pg STXeq $cell^{-1}$ (Oyaneder-Terrazas et al., 2017). The authors pointed out that this was a characteristic toxin profile for species blooming during January and March in the austral fjords of Chile. In other seasons the toxin profile differed, for instance, toxin profiles in spring predominantly had, in molar basis, β-epimers (C2, GTX4, GTX3), and in autumn α-epimers (C1, GTX1, GTX2) (Oyaneder-Terrazas et al., 2017), which is a bit surprising since it has been proven that these enantiomers do not reflect the original composition in plankton due to their easy epimerization under extraction and analytical conditions (Bustillos-Guzmán et al., 2015). Size fractionated plankton samples obtained from the XI region of Chile only presented sulfocarbamoyl analogs C1/2 in concentrations <0.7 ng μL^{-1} (Pizarro et al., 2018). The authors pointed out the low abundance of *A. catenella* during the cruise. In autumn, during an exceptional toxic event in the Beagle Channel in 1991/1992, the cell toxicity was calculated, also via mouse bioassay, to be 325 pg STXeq $cell^{-1}$ (Benavides et al., 1995).

In El Rincón, Argentina in 2015 during a bloom of *Pseudonitzschia*, cells of *A. catenella* and *A. ostenfeldii* were found in low numbers (up to 1×10^3 cell L^{-1}). Phytoplankton net tows (NT) samples presented PST in low concentrations ranging between 114.4 ng NT^{-1} and 2593.8 ng NT^{-1}. Toxin profiles were dominated by GTX1/4; C1/2 and GTX2/3 were also detected (Guinder et al., 2018).

Alexandrium tamarense

In Argentinian coasts, based on historical data and on shellfish toxicity, it was suggested that *A. tamarense* could grow offshore and that cells were transported inshore toward shellfish populations. This phenomena also occurred in the Uruguayan coast, where shellfish reached toxicity values between 80 and 10,000 μg STXeq 100 g^{-1}, whereas in the Argentinian coast the toxicity values were higher, with exceptional values up to 50,000 μg STXeq 100 g^{-1} (Carreto et al., 1998b). Toxin profile in phytoplankton samples during a bloom from MPlat to Bahía Grande, were dominated by GTX1/4 analogs (from 69.1 to 93.6 mol%), with an exception of a sample from MPlat, where GTX2/3 represented 88.5 mol% (Montoya et al., 2010). Sulfocarbamoyl toxins (C1/2) were scarce, and their contribution

was <8.5 mol%. Cell toxicity varied between 9.8 and 93.0 pg STXeq cell^{-1}. Toxin profiles contrasted with those obtained from culture strains, where sulfocarbamoyl analogs represented >60 mol%. Therefore, cell toxicity from field samples, in general, was higher when compared with cultured strains (1.8–10.3 pg STXeq cell^{-1}) grown in nutrient replete conditions. These results were confirmed with NT samples from the Argentinian coast containing *A. tamarense* cells and minor quantities of *A.* aff. *minutum*, which again showed a higher concentration of GTXs (Fabro et al., 2017). Gonyautoxin concentrations (GTX1/4) ranged between 0.3 and 1104 ng NT^{-1} and GTX2/3 ranged between 0.1 and 31.7 ng NT^{-1}. Cell quotas of total GTX estimated for *A. tamarense* species complex plus *A. minutum* were <0.8 pg cell^{-1}. N-sulfocarbamoyl toxins (C1/2) were also detected at low concentrations. The high potency carbamoyl analogs STX and NEO were the least frequent analogs (Fabro et al., 2017).

Mussels (*M. edulis*) were sampled during two blooms, in spring and autumn. In both occasions, the toxin profile in mussels was dominated by GTX1/4 and to a lesser extent by C1/2 that constituted >80 mol% (Carreto et al., 2004). However, toxin profile changed according to the mussel toxin content. Thus whereas GTX1/4 represented more than 60 mol% during the maximum toxicity and detoxification rate period, an important increase of STX and GTX2/3 was observed at a low toxin content. Toxin profile changes reflected the compositional shifts resulting from differential toxin retention and toxin bioconversions within the mussel (Oshima, 1995). Maximum values of toxicity in the mussel were between 450 and 500 μg STXeq 100 g^{-1}.

Other *Alexandrium* Species

In Trinidad and Tobago, PSTs were first reported in 1999 in the mussel *Perna viridis* from Chaguaramas (Yen et al., 2004). STX, dcSTX, GTX1/4, GTX2/3 and dcGTX2/3 were detected, with the last three toxins being the most abundant. During 2000, STX, dcSTX, NEO, GTX2/3, and dcGTX2/3 were detected, with the last three analogs in the highest concentrations. No causative organism was identified. Known PSTs-producing dinoflagellates in this region are *A. catenella*, *A. tamarense*, *G. catenatum*, and *P. bahamense* (Heileman and Siung-Chang, 1990; Sánchez-Suárez and Troncone-Osorio, 1994; La Barbera-Sánchez et al., 2004).

Although *A. minutum* has been reported in Peru and Argentina, to our knowledge, no further toxin research on this species has been performed. In Brazil only one bloom in the coast of Río de Janeiro in 2007 was analyzed, being NEO (73.8 mol%), GTX2/4 (22.6 mol%), and STX (3.6 mol%) the reported toxins (Menezes et al., 2007).

In the northern coast of Chile, phytoplankton toxin profiles related to *Alexandrium* blooms did not present PSTs (Álvarez et al., 2009). However, the mollusks *Semimytilus algosus* and *Argopecten purpuratus* presented low quantities of PSTs, between 27.5 and 34.4 μg STXeq 100 g^{-1} (determined by mouse bioassay). The toxin profile was characterized by the presence of C2 and GTX2/3 in both bivalves, concluding that *A. catenella* was unlikely to be the responsible species for these PSP events.

Autoecological Studies

Few strains of the genus *Alexandrium* have been isolated and autoecological studies of the group are scarce in LAm (**Figure 3**). Since Chile has been historically adversely affected by their blooming off its coasts, it is in this country where most of studies have been performed, followed by Argentina and Brazil.

Alexandrium catenella

The effect of temperature, salinity, L:D cycle, cyst or vegetative strains, and nutrients on the growth of *A. catenella* from diverse regions of Chile have been determined (**Table 4**) (Navarro et al., 2006; Aguilera-Belmonte et al., 2011, 2013; Ávila et al., 2015). In general, this species tolerates temperatures between 10 and 16°C, producing cell concentrations above 50,000 cell mL^{-1} at 15°C with growth rates from 0.54 to 0.73 div day^{-1} (Navarro et al., 2006; Aguilera-Belmonte et al., 2011, 2013); indicating that growth is temperature-dependent. Studies for the same species from other geographical sites have reported a higher optimal temperature for growth 20–25°C (Siu et al., 1997), the optimal temperature in this study being 14°C demonstrated its great capacity of this species to adapt to a wide range of temperatures. Maximum biomasses are reached between 15 and 65 days; this wide time difference could be related to the L:D cycle used in the different culture conditions or could be inherent to strain differences (**Table 4**) (Navarro et al., 2006; Aguilera-Belmonte et al., 2011, 2013). Their salinity tolerance is also wide; growing under laboratory conditions at salinities from 15 to 35. Chain formation was observed mainly during the first week of culture, reducing their presence (>7%) when cultures reached ∼5,000 cell mL^{-1} (Navarro et al., 2006). With these studies, a wide range of tolerance to salinity and temperature was proved, which helps explain, at least partially, its ecological success.

The effect of temperature on the cell dry weight was also determined. Maximum biomass was inversely proportional to temperature, with values of 117.3 ± 0.9, 120.3 ± 3.2, 90.9 ± 0.6 and 23.3 ± 1.4 μg mL^{-1} at 10, 12, 14, and 16°C, respectively, with a direct relationship between cell dry weight and cell abundance. Cell dry weight was greater in the initial phase, decreased during the exponential phase, and increased again toward the terminal phase. The highest values for individual cell dry weights were obtained at 14°C (9.6 ± 1.1 ng cell^{-1}, day 3) and at 10°C (9.5 ± 0.9 ng cell^{-1} day 3). The lowest were obtained at 12°C (5.9 ± 0.3 ng cell^{-1}, day 3) and at 16°C (5.8 ± 1.7 ng cell^{-1}, day 6). The authors suggested that the increase of cell weight during the growth phase could be explained by the presence of thecae of dead cells that added to the total weight of the culture and also to the increase in cell size (Navarro et al., 2006).

These results suggested this species has also a wide photo-acclimation and that it presents a lower range of thermal tolerance in relation to strains from other geographic regions. The optimal temperature range correlated well with ambient water temperatures in southern Chile, in which important blooms have been reported. Noteworthy differences have been detected in growth among populations from different geographical origins obtained from the same toxic event, concluding that abiotic factors can differentially affect the

TABLE 4 | Ecophysiology studies of *Alexandrium* species in Latin America.

Species	Strain code	Culture conditions	Photoperiod L:D h	Temp (°C)	Salinity	Abundance (cel mL^{-1})	Growth rate (div day^{-1})	Geographic area/date of isolation	References
Alexandrium catenella	ACC02	L1 59.53 ± 0.25 μmol/m^2s	14:10	10	30	29,460 ± 460	0.18 ± 0.00	Aysén Region, Chile 1994	Navarro et al., 2006
				12		34,850 ± 1,641	0.11 ± 0.00		
				14		24,000 ± 448	0.30 ± 0.01		
				16		6,389 ± 300	0.10 ± 0.03		
	PFB36	L1 50 μmol photons/m^2s	16:08	15 ± 1	nd	52,320 ± 6,122	0.54 ± 0.04	San Pedro Island, Los Lagos Region, Chile March 2009	Aguilera-Belmonte et al., 2011
	PFB37				nd	24,608 ± 884	0.52 ± 0.02	Quemada Island, Aysén Region, Chile March 2009	
	PFB38				nd	48,581 ± 6,963	0.73 ± 0.02	Cailin Island, Los Lagos Region, Chile March 2009	
	PFB39				nd	43,520 ± 1,227	0.58 ± 0.01	Asasao Bay Los Lagos Region, Chile March 2009	
	PFB41				nd	54,288 ± 3,982	0.55 ± 0.02	Luz Island, Aysén Region, Chile March 2009	
	PFB42				nd	45,632 ± 2,563	0.42 ± 0.02	Jorge Island, Aysén Region, Chile March, 2009	
	PFB45				nd	49,104 ± 2,400	0.56 ± 0.03	Aysén Bay, Aysén Region, Chile March, 2009	
	AY2	L1 with silicates 24 μE/m^2s N:P (24:1)	14:10	15	30	5,093	0.49*	Aysén Region	Garrido et al., 2012
		N:P (12:1)				5,266	0.41*		
		N:P (3:1)				4,072	0.39*		
	PFB37	L1 medium 65 μmol photons/m^2s	16:8	10 ± 1	15	22,205 ± 4190	0.35 ± 0.04	Quemada Island, Aysén Region, Chile March 2009	Aguilera-Belmonte et al., 2013
					35	22,080 ± 2,437	0.47 ± 0.05		
				15 ± 1	15	14,750 ± 452	0.61 ± 0.04		
					35	24,608 ± 884	0.52 ± 0.03		
	PFB38			10 ± 1	15	17,954 ± 5,164	0.34 ± 0.02	Cailin Island, Los Lagos Region, Chile March 2009	
					35	44,800 ± 7,250	0.39 ± 0.05		
				15 ± 1	15	40,760 ± 3,123	0.48 ± 0.03		
					35	52,671 ± 4,650	0.73 ± 0.03		
	PFB41			10 ± 1	15	14,869 ± 1,099	0.25 ± 0.02	Luz Island, Aysén Region, Chile March 2009	

(Continued)

TABLE 4 | Continued

Species	Strain code	Culture conditions	Photoperiod L:D h	Temp (°C)	Salinity	Abundance (cel mL^{-1})	Growth rate (div day^{-1})	Geographic area/date of isolation	References
					35	15,287 ± 1,391	0.35 ± 0.04		
				15 ± 1	15	11,006 ± 2,337	0.28 ± 0.02		
					35	52,038 ± 1,554	0.56 ± 0.03		
	PFB42			10 ± 1	15	24,211 ± 2,260	0.34 ± 0.03	Jorge Island, Aysén Region, Chile March 2009	
					35	38,005 ± 3,880	0.42 ± 0.04		
				15 ± 1	15	36,713 ± 1,713	0.29 ± 0.03		
					35	45,632 ± 2,563	0.42 ± 0.02		
	AC090610-QU-Q	L1 medium 24 μmol photons/m^{2}s	16:8	10	31	>3,000	0.08	sediment cysts Punta Yenecura, zona Sur de Quellón (43°08′44,2″S; 73°39′30,6″W) Región de Los Lagos, Chile Summer 2010	Ávila et al., 2015
				15		>3,000	0.12		
				20		<1,500	0.10		
				10	15	2,324	0.12		
					20	2,018	0.12		
				10	25	??	??		
					30	3,788	0.18		
		L1 medium 24 μmol photons/m^{2}s	12:12		31	2,000	0.05		
		L1 medium 24 μmol photons/m^{2}s	16:08			2,000	0.07		
		L1 medium 24 μmol photons/m^{2}s	08:16			2,000	0.07		
		L1	??			> 2,800	0.08		
		L1/2	??			> 2,800	0.07		
		L1/10	??			< 1,000	0.06		
		L1/15	??			< 1,000	0.06		
		L1/-N-P	??			< 1,000	0.06		
	Strain cross: Q09 x CERES8	L1 100 μphotons PAR/m^{2}s	12:12	17	30	?	0.36	Los Lagos, Quellón x North Aysén, Ceres Island	Mardones et al., 2016
	Strain cross: Q09 x K7	L1/-N 100 μphotons PAR/m^{2}s	12:12	17	30	?	0.52	Los Lagos, Quellón x South Magallanes	Mardones et al., 2016
A. tamarense	MDQ1096	45 μmol photons/m^{-2}s	12:12	18	??	1,924	0.28	Mar del Plata, Argentina	Frangópulos et al., 2004

*, *maximum values.*

population dynamics of toxic genotypes, making it extremely difficult to predict the ecological behavior of this species in the field in terms of the intensity of a potential outbreak (Aguilera-Belmonte et al., 2011, 2013).

In 2015, Ávila and collaborators evidenced the effects of salinity, temperature, photoperiod and nutrients in cultures established from cysts. Cysts were isolated from Punta Yenecura, Los Lagos region, in Chile. Different experiments were designed, and only one parameter was tested at the time (salinity, temperature, photoperiod, nutrients) (details in **Table 4**). Maximum biomasses were never above 4,000 cell mL^{-1} and was reached between 28 and 51 days of culture; maximum abundances were found at 10 and 15°C and salinities of 30-31 with growth rates from 0.12 to 0.18 div day^{-1}. No clear effect of the L:D cycle was observed on cell abundance, whereas growth rate was higher at 16:08 and 08:16 L:D. Higher abundances where obtained with L1 and L1/2 media, but where much lower than biomasses obtained with strains obtained from vegetative cells in other studies. This difference could also be explained by the lower light intensity used (Ávila et al., 2015) (**Table 4**). With strain crosses obtained from cysts collected in Los Lagos and AYSR during the massive HAB event, Mardones et al. (2016) using the same medium with and without N, and higher light intensities also obtained high growth rates (0.36 and 0.52 div day^{-1}) (**Table 4**).

There is only one report of allelopathy regarding *Alexandrium* species from LAm. This study was performed with a strain of *A. catenella* from southern Chile (Arzul et al., 1999). The allelopathic and hemolytic effect of this, and other toxic *Alexandrium* species from Europe and Asia were determined on *Gymnodinium mikimotoi*, *Scrippsiella trochoidea*, and *Chaetoceros gracile*. Filtrates of *A. catenella* reduced the growth of *G. mikimotoi* and *C. gracile*, particularly in the exponential phase of culture, which was related with toxicity and haemolytic activity. No effect on *S. trochoidea* was observed. This information supported the environmental observations, where blooms of the later usually follow a PSP outbreak caused by *A. catenella* (Arzul et al., 1999).

Alexandrium tamarense

The growth rate under different N and P regimes in *A. tamarense* from MPlat was determined. The strain was compared with strains of *A. tamarense*, *A. minutum*, and *A. anderssoni* from Spain. The Argentinian isolate presented the lowest affinity for P. The half saturation coefficient (K_s) and the P concentration to which the specific culture cell growth rate was zero (K_{min}) under P limitation were 1.68 and 0.48, respectively, which were higher than the K_s and K_{min} of the other strains. The lower affinity for P indicated that the Argentinian isolate has a lower competitive ability under P limitation. The average abundance for this strain under non-nutrient limitation was 1,924 cell mL^{-1}, with growth rates from 0.22 to 0.28 div day^{-1} (**Table 4**). These values were also lower than those reported previously for *A. catenella*, with the exception of a strain obtained from cyst germination (Frangópulos et al., 2004).

Other *Alexandrium* Species

Another study reported a negative experimental effect on copepod nauplii larvae, and larvae of gastropods and tintinnids. Exudates of *A. tamiyavanichii* decreased the survival of copepod larvae, suggesting that the dinoflagellate has an impact on the trophic chain (Silva et al., 2013).

Toxin Profiles in *Alexandrium* Species Under Laboratory Conditions

Several studies on toxin profiles have been performed with *Alexandrium* species in LAm, mostly in countries where the blooms of species of this genus are common and are an important threat for the economic activities.

Alexandrium catenella

This species is particularly important along the Chilean coasts, and many research groups have performed toxin analysis in cultures for more than one decade. Data from toxin profiles, toxicity and toxin content are available in **Tables 5**, **6**, and the origins of the isolates are indicated in **Figure 3**.

One of the first autoecological studies performed in LAm was done to determine the role of temperature in growth rate, cell density and toxicity (Navarro et al., 2006). Cells of *A. catenella* isolated from the AYSR were cultured in L1 medium at four temperatures: 10, 12, 14, and 16°C. Toxins were extracted with 0.1 M HCl and later HCl 1 M was added. Toxicity was measured using patch clamp electrophysiology with human embryonic kidney cells expressing STX-sensitive rat skeletal muscle Na_V channels. Toxin content was variable during the experiment, but an inverse relationship was noted between temperature and toxin content, with the highest toxin content found at 10°C, and the lowest at 16°C. According to Cembella (1998), low temperatures contribute to higher PSTs levels, making *Alexandrium* strains from higher latitudes more toxic, which is probably related to effects on metabolic processes (Navarro et al., 2006).

Among the toxinological studies performed on *Alexandrium* species in LAm, Brazilian isolates have been analyzed. Persich et al. (2006) worked on *Alexandrium* isolates and reported the species as *A. tamarense*, but later molecular studies confirmed them as *A. catenella* (Menezes et al., 2018). Five isolates were cultured from cysts and one from vegetative cells, in f/2 medium at 20°C. Toxins were extracted with 0.05 M acetic acid and analyzed by LC-FLD with post-column derivatization. Toxin content ranged from 42 to 199 fmol $cell^{-1}$ and toxicity varied between 7.1 to 65.9 pg STXeq $cell^{-1}$, except for the culture originated from vegetative cells, which showed higher toxicity, mainly due to the higher GTX4 content (Persich et al., 2006).

Krock et al. (2007) analyzed the toxin composition of an isolate from the coastal Channel, XI Region of Chile. Toxins were extracted in 0.03 M acetic acid and analyzed by LC-FLD and LC-MS/MS. For the first time both techniques were used for comparison and precision. Detected toxins were C1/2, GTX1/7, B1, GTX2/3, NEO, and B2, in descending order according to their concentration. STX, dcSTX, dcGTX2/3, and C3/4 were present only in trace amounts (<1 mol%). *Alexandrium catenella* is considered the predominant, or even exclusive, source of PSTs in central Chile, and its toxin profile agreed with other profiles from

TABLE 5 | *Alexandrium* toxin analogs reported under laboratory conditions.

Isolate(s)	Origin	Culture media	STX	NEO	GTX1/4	GTX2/3	B1	B2	C1/2	C3/4	dcSTX	dcNEO	dcGTX1/4	dcGTX2/3	References
A. catenella															
6	Patos Lagoon, Bra	f/2	1/1	1/1	1/1	1/1	–	–	1/1	–	–	–	–	1/1	Persich et al., 2006
1	Coastal Channel, XI Region, Ch	L1	t	1/1	1/1	1/1	1/1	1/1	1/1	Tr	Tr	–	–	Tr	Krock et al., 2007
7	South Chiloé to south Aysén, Ch	L1	2/7	2/7	7/7	7/7	3/7	3/7	3/7	1/7	-	–	–	–	Aguilera-Belmonte et al., 2011
7	Los Lagos, Ch (cysts)	L1	T6/7	7/7	7/7	7/7	6/7	t1/7	7/7	T5/7	t7/7	–	–	–	Varela et al., 2012
2	Aysén, Ch (cysts)		t1/2	2/2	2/2	2/2	0/2	0/2	*t2/2	0/2	t1/2	–	–	–	
3	Aysén, Ch (veg. cells)		t3/3	3/3	3/3	3/3	3/3	*t3/3	3/3	3/3	t1/3	–	–	–	
2	Magellan, Ch (veg. cells)		0/2	2/2	2/2	2/2	0/2	0/2	2/2	0/2	0/2	–	–	–	
1	Adjacent shelf front, Bahía Blanca, Arg	½ K	1/1	1/1	1/1	1/1	–	–	1/1	–	–	–	–	–	Guinder et al., 2018
A. tamarense															
1	Mar del Plata, Arg	f/2	1/1	1/1	1/1	1/1	–	–	1/1	–	–	–	–	1/1	Carreto et al., 1996
6	Patos Lagoon, Bra	f/2	1/1	1/1	1/1	1/1	–	–	1/1	–	–	–	–	1/1	Persich et al., 2006
6	Golfo Nuevo, Arg	f/2	t1/6 1/6	t3/61/6	6/6	6/6	–	–	6/6	–	–	–	–	–	
3	Golfo San José, Arg		t1/3 1/3	3/3	3/3	3/3	–	–	3/3	–	–	–	–	–	
1	Península Valdés, Arg		1/1	1/1	1/1	1/1	–	–	1/1	–	–	–	–	–	
3	Mar del Plata, Arg (Nat)	Natural samples	1/3	1/3	3/3	3/3	–	–	3/3	–	–	–	–	–	
1	Península Valdés, Arg (Nat)		–	–	1/1	1/1	–	–	–	–	–	–	–	–	
1	Camarones, Chubut, Arg (Nat)		–	–	1/1	1/1	–	–	–	–	–	–	–	–	
1	Bahía grande Santa Cruz, Arg (Nat)		–	–	1/1	1/1	–	–	–	–	–	–	–	–	
2	Golfo de San Jorge, Arg	½ K	2/2	2/2	2/2	2/2	2/2	–	2/2	–	–	–	–	–	Krock et al., 2015
A. ostenfeldii															
1	Aysén, Ch	L1	1/1	–	–	1/1	–	–	–	–	*t1/1	–	–	–	Salgado et al., 2015
3	Adjacent shelf front, Bahía Blanca, Arg	½ K	3/3	–	–	3/3	–	–	3/3	–	–	–	–	–	Guinder et al., 2018
A. tamiyavanichi															
1	Bahía, Bra	f/2	1/1	1/1	1/1	1/1	–	?	?	?	?	?	?	1/1	Menezes et al., 2010

*T, trace amounts; *t, trace amounts in one of the isolates.*

Ch, Chile; Arg, Argentina; Bra, Brazil.

Nat, Natural samples (for comparison).

Enantiomeric pairs are expressed together because they easily epimerize under extraction and analytical conditions, so their measurements do not necessarily reflect their original composition in plankton cells (Krock B, pers. comm.).

TABLE 6 | Toxin content of cultured *Alexandrium* under laboratory conditions.

N	Origin	Culture media	Toxicity (pg STX eq cell^{-1})	Toxin content (fmol STX cell^{-1})	References
A. catenella					
1	Morlaix river, Fr	f/2	0.5		Arzul et al., 1999
1	Canal Costa, Ch		1.39		
1	Onagawa Bay, Jap		7.2		
1	Aysén region, Ch	L1		27.8 (10°C)	Navarro et al., 2006
1	Aysén region, Ch			13.3 (12°C)	
1	Aysén region, Ch			7.82 (14°C)	
1	Aysén region, Ch			3.5 (16°C)	
1	Aysén, Ch	f/2		5–8	Uribe et al., 2008
7	South Chiloé to south Aysén, Ch	L1		8.5–96.9	Aguilera-Belmonte et al., 2011
7	Los Lagos, Ch (cysts)		Max: 63.65; min: 3.51	42.6–295.5	Varela et al., 2012
2	Aysén, Ch (cysts)			41.6–235.9	
3	Aysén, Ch (veg. cells)			41.1–79.0	
2	Magellan, Ch (veg. cells)			64.5–67.2	
3	Aysén, Ch	L1		Min nd (10°C, 15 and 35 sal) Max 239 (10°C and 35 sal)	Aguilera-Belmonte et al., 2013
1	Los Lagos, Ch			Min 8.54 (15°C and 35 sal) Max 95.8 (10°C and 35 sal)	
A. tamarense					
1	Mar del Plata, Arg				Carreto et al., 1996
6	Patos Lagoon, Bra	f/2	7.1–65.9	42–199	Persich et al., 2006
6	Golfo Nuevo, Arg	f/2	1.8–6.5	13.3–35.4	Montoya et al., 2010
3	Golfo San José, Arg		1.1–5.8	17.2–26.9	
1	Península Valdés, Arg		10.3	51.3	
3	Mar del Plata, Arg (Nat)	Natural samples	9.8–62.8	45.5–164.0	
1	Península Valdés, Arg (Nat)		80.2	225.52	
1	Camarones, Chubut, Arg (Nat)		86.8	228.7	
1	Bahía grande Santa Cruz, Arg (Nat)		93.0	261.4	
2	Golfo de San Jorge, Arg	½ K	74–60		Krock et al., 2015
A. ostenfeldii					
1	Aysén, Ch	L1		105.8 ± 72.2	Salgado et al., 2015
A. tamiyavanichi					
1	Bahia, Bra	f/2		16.9	Menezes et al., 2010

Fr, France (for comparison); Ch, Chile; Arg, Argentina; Bra, Brazil.
Nat, Natural samples (for comparison).

populations from the Pacific Ocean in the northern hemisphere (Krock et al., 2007).

Aguilera-Belmonte et al. (2011) compared the phenotypic and genotypic characteristics, including toxin profiles of seven isolates from the same HAB event, but from different geographical zones. Isolates were cultured in L1 medium at 15 ± 1°C. Toxin analysis was performed by LC-FLD on 0.05 M acetic acid extracts. *N*-sulfocarbamoyl analogs were detected after hydrolysis. All isolates had different toxin profiles, but most of them showed high proportions of GTX1/4 followed by GTX2/3. In three out of seven isolates they reported B1, B2, and C toxins (43 mol%). In two, NEO and STX (29 mol%) (Aguilera-Belmonte et al., 2011), showing large intraspecific variability, as reported within a variety of dinoflagellates, even among clones from the same geographic area (Tillmann et al., 2009; Seok Jin et al., 2010). In addition, they found a cosmopolitan distribution of the morphospecies within the North American Clade, though interesting differences were found in their toxin profiles (Aguilera-Belmonte et al., 2011).

Research on these isolates continued, but the aim of this study was to analyze four combined conditions: two temperatures (10 and 15°C) and two salinities (15 and 35). Samples were extracted with 0.05 M acetic acid. Toxins were analyzed by UPLC-FLD, and samples from the four replicates were pooled to cover intrinsic variability among them (Aguilera-Belmonte et al., 2013). Toxin content varied widely among isolates, treatments, salinities and culture age. During stationary phase, most isolates accumulated toxins (from 1.4 to 4.5 times) compared to the exponential phase, with three exceptions of the sixteen analyses (19%). Concerning the toxin profile, NEO, GTX2/3 and GTX1/4

TABLE 7 | Abiotic factors during HABs of *G. catenatum*, *P. bahamense*, and *A. catenella* in different geographic regions of Latin America.

Species	Country/ocean	Location/date	Abundance (cells L^{-1})	Temp (°C)	Salinity	Hydrographic conditions	References
Gymnodnium catenatum	Mexico/North East Pacific (Gulf of California)	N GOLCA Mar 1939	1×10^6	14-17	35.07–35.5		Graham, 1943
		BACO Spring, early fall 1990, 1999, 2000, 2002	1.8×10^2-5.7×10^5	18–26	34.2–36	Hydrographic transitional periods	Morquecho and Lechuga-Devéze, 2004; Gárate-Lizárraga et al., 2006
		BAPAZ Feb-Mar 2007	6×10^5-2.4×10^6	20.9 ± 0.7		NO_2 0.22 ± 0.05 μM NO_3 1.04 ± 0.33 μM PO_4 0.81 ± 0.76 μM $Si0_3$ 8.85 ± 1.60 μM N:P 1.6 - 3.2 DO 6.9 ± 0.3 mg L^{-1}	Hernández-Sandoval et al., 2009
		Bahía Bacochibampo, Son 1970–1994 43 events				NW winds during colder months promoted HABs	Manrique and Molina, 1997
		Puerto Libertad, Son 1981	Maximum 9.6×10^4	16.5–17.2		Early Spring	Cortés-Altamirano et al., 1999a,b
		BAMAZ Apr 1979, apr 1980, may 1981, 1984, 1987, Apr-may 1988, Apr-may 1997, Sept-Oct 1997	18×10^3-5.15×10^6	20–21 Maximum abundances		Upwelling conditions ENSO influence 1997 year with higher frequencies of HABs attributed to nutrients from water plant treatment	Morey-Gaines, 1982; Mee et al., 1986; Cortés-Altamirano et al., 1995, 1999a,b; Cortés-Altamirano and Alonso-Rodríguez, 1997
	Mexico/ N East Pacific	Nayarit Dec 2005	1.01×10^6	20			Castillo-Barrera and García-Murillo, 2007
		Bahía Banderas, Nay-Jal 2000-2001	450-2134 $\times 10^3$	23			Gómez-Villarreal et al., 2008
		BMANZ winter and spring 1999 Mar-May 2007 May 2010	35×10^2-1.02×10^7	20.5–27.5	31.8–34.4	N-NO^3 1 μg at L^{-1} DIN 6.16–6.45 μM PO_4 0.37–0.78 μM $Si0_3$ 7.49–21.07 μM DO 5.09–6.08 pH 8.59–8.78 upwelling events	Blanco-Blanco et al., 1999; Morales-Blake et al., 2000; González-Chan et al., 2007; Quijano-Scheggia et al., 2012
		BACA Mar 1999 Dec 2005–Feb 2006 Dec 2007	78 x 10^3-10×10^6	26–27			Licea-Durán et al., 1999; Gárate-Lizárraga et al., 2009
		Costa Grande, Guerrero Dec 2010	Maximum 129 $\times 10^3$			Multispecies bloom	Gárate-Lizárraga et al., 2011
		Oaxaca coast (Santiago Astata and Puerto Escondido) Apr 2010	Maximum 4 $\times 10^3$	28–29	32–33		Alonso-Rodríguez et al., 2015

(Continued)

TABLE 7 | Continued

Species	Country/ocean	Location/date	Abundance (cells L^{-1})	Temp (°C)	Salinity	Hydrographic conditions	References
	Ecuador/ S East Pacific	Península of Santa Elena* Feb 1993		25	Wind speed 8 kn		Torres, 2000
		Isla del Muerto (Santa Clara)* Jun, Aug 1997 Jan 1998*	$<5 \times 10^4$	25–29.7		ENSO	
		GGUAY* Feb-Mar 1999	3.5×10^5			PO_4 10.1 μg-at L^{-1} $Si0_3$ 170.69 μg-at L^{-1}	
	Chile/ S East Pacific	MAGR* Mar-Apr 1999	$4–8 \times 10^6$	15 (1.5 higher than normal conditions)		Unusual climatic conditions, high irradiance, reduced rainfall. *A. catenella* also present	Clément et al., 2001
	Mexico/ Gulf of Mexico	S Gulf of Mexico 2006–2010	$16–2.0 \times 10^3$				Zamudio et al., 2013
		S Gulf of Mexico Campeche Laguna de Términos** Jun-sept 2013 (rainy season)	Maximum 9.5×10^4	29.7–30.6	11.6–14.3	NO_2 0.3–3.4 μmol L^{-1} NO_3 1.31–3.42 μmol L^{-1} PO_4 0.14–0.60 μmol L^{-1} $Si0_3$ 29.5–51.52 μmol L^{-1} OD 88.4–96.3 pH 7.4–7.8	Poot-Delgado et al., 2015
	Cuba/Caribbean Sea	Sabana-Camagüey May 1999		26.4–28.2	33.3–36.5	pH 7.95–8.08	Leal et al., 2001
		Bahía de Cienfuegos Jun 1999-rainy season	7.2×10^3-3.3×10^4	30.6	31.4	OD 7.06 mgL^{-1} chl *a* 3.12 ugL^{-1}	Moreira-González, 2009
		Laguna Guanaroca Nov 2004	2.3×10^3	23.6	24.9	NO_2 0.007 mgL^{-1} SO_4 1870 mgL^{-1} OD 6.4 mgL^{-1} pH 7.94	Moreira-González et al., 2013
	Uruguay/S West Atlantic	1991 to 2004		21.8–24.0	18.4–32		Méndez, 2006
	Brasil/ S West Atlantic	Paranaguá Feb-Mar 2003	6.4×10^3	29		High temperatures and high nutrient concentration Rainy season	Mafra-Junior et al., 2006
	Argentina/S West Atlantic	MPlat Mar-Apr 1997 Mar 2003	0.8×10^3-8.9×10^4			Chl *a* 171 μg L^{-1} SW winds High rainfall	Akselman et al., 1998; Montoya et al., 2006
	Mexico/N East Pacific (Gulf of California)	S peninsula of Baja California Jul-Nov 2010	800–1110	28–28.5	35.2	ENSO event	Gárate-Lizárraga and González-Armas, 2011
		Costa Chica, Guerrero Jul-dec 2010	$<1–194 \times 10^3$	28.5–30.5			Gárate-Lizárraga et al., 2013
		Oaxaca Dec 1989 Ago 2001–feb 2002 Ene-mar 2006 Apr-Jun 2010	3.25×10^4-3.0×10^6	27.4–31.3			Cortés-Altamirano et al., 1993; Ramírez-Camarena et al., 1999; Rojas-Crisóstomo et al., 2007; Gárate-Lizárraga et al., 2008; Alonso-Rodríguez et al., 2015

(Continued)

TABLE 7 | Continued

Species	Country/ ocean	Location/date	Abundance (cells L^{-1})	Temp (°C)	Salinity	Hydrographic conditions	References
	Mexico/ Gulf of Mexico	Several areas Gulf of Mexico 1966 to 1989	$<0.1–3 \times 10^6$		3–38	Northern winds Summer	Reviewed by Gómez-Aguirre, 1998; Licea et al., 2004
		Campeche Laguna de Términos** Jun–sept 2013 (rainy season)	Maximum 6×10^4	29.7–30.6	11.6–14.3	pH 7.4–7.8 OD 88.4–96.3 nitrites 0.3–3.4 μmol L^{-1} nitrates 1.31–3.42 μmol L^{-1} phosphates 0.14–0.60 μmol L^{-1} silicates 29.5–51.52 μmol L^{-1}	Poot-Delgado et al., 2015
	Mexico/Caribbean Sea	Sian Kaan Q. Roo 1996	1.5×10^6		7–30	Associated with seawater fluxes and northern winds	Gómez-Aguirre, 1998
	El Salvador/ S East Pacific	Nov 2005–Mar 2006 Nov 2009 Apr–Jun 2010 Aug 2011 Sept 2012	15×10^3-48.9×10^6			1.5 C positive thermal anomaly Chl *a* 0.5–1 mg m^{-3}	Licea et al., 2008, 2012; Chow et al., 2010; Alvarado et al., 2014
Alexandrium catenella	Chile/South West Atlantic	Coast of Valdivia (región de Los Lagos) Late Summer 2016	2.5×10^5			Extreme hydroclimatic conditions, ENSO, decrease wind force, higher SST, negative rainfall anomalies	Hernández et al., 2016
		Coast of Chile Diverse events 1972–1993	$100\text{-}6 \times 10^5$	4.88–10.3	8.14–31.51	Thermohaline stratification, precipitation, low salinity, upwelling, high nutrients, ENSO	Guzmán et al., 2002
		Patagonia X-XI region (Los Lagos-AYSR) 1994–2002 2005, 2006 Feb-mar 2009	$1.1–6 \times 10^6$	11–14		Jan-Mar Germination of cyst banks, biannual variation, northern expansion, water circulation patterns stratification	Cassis et al., 2002; Molinet et al., 2003; Fuentes et al., 2008; Mardones et al., 2010; Díaz et al., 2014
		S Austral Ecosystem		9–13	28–33	Ocean currents, and natural dispersion	Cruzat et al., 2018

**Gymnodinium sp., **Gymnodinium cf. catenatum.*

represented more than 85 mol% of the toxin content in all strains and culture conditions. B1 was detected only in one isolate from the AYSR, and this analog was detected only when cultured at 10°C. Interestingly, the highest toxin contents were reached at this temperature (in agreement with Navarro et al., 2006) and a salinity of 35. This isolate also presented the lowest (non-detectable) and the highest toxin content (239 fmol cell^{-1}) at those culture parameters, being the culture age the only difference (exponential and stationary, respectively) (Aguilera-Belmonte et al., 2013). The scale of the combined effects of temperature and salinity was not clear, and differences were found among strains during all the experimental process, even though, two of the isolates were apparently genetically identical at least in their nuclear ribosomal ITS sequences (Aguilera-Belmonte et al., 2011). Results showed differential effects of temperature and salinity on growth parameters and toxin content, being the first more affected by salinity and the second by temperature.

Toxin profiles research continued, and toxin profiles from cultures originated from cysts and vegetative cells from different Chilean zones (Los Lagos, AYSR, and MAGR) and different years of isolation were compared. Analyses were carried out in 0.05 M acetic acid extracts and separated by LC, but the detector is not reported. Identified toxins were STX, dcSTX, NEO, GTX1/4, GTX2/3, B1, B2, C1-3, though STX and dcSTX were reported only in trace amounts and not in all isolates (Varela et al., 2012). The authors recognized two toxin production patterns in the strains, according to their toxin profile: one constituted by a group where analogs C1/2 accounted for most of the toxin content (47–74%), and a second one, where GTX1/4 accounted for the main analogs (54–87%). This second group was also conspicuous because of the absolute absence of B1, previously reported as distinctive for Chilean isolates (Montoya et al., 2010). They also reported morphological, toxinological and genetic differences among strains of this species, in accordance to other studies that have also characterized the diversity of the species in other regions (MacKenzie et al., 2004; Orlova et al., 2007).

A recent work from an oceanographic expedition in 2015, reports the isolation of four *Alexandrium* strains from offshore the Argentine coasts, near Bahía Blanca Estuary, south Mar del Plata. The cells were isolated in 1/10 K medium, later transferred to 1/2 K medium flasks, and incubated at 15°C and 50 μmol photons $m^{-2}s^{-1}$ on a 16:8 LD photocycle. One of the four isolates was identified as *A. catenella/tamarense* morphotype, but further morphological and phylogenetic analysis conformed to the description of *A. catenella*. Toxin profile from this one culture was dominated by C1/2 (72%), GTX1/4 (15%), NEO (9%), GTX2/3 (2%), and STX (2%) (Guinder et al., 2018).

These studies confirm that the only way to differentiate species from the *Alexandrium tamarense/fundyense/catenella* complex is by the use of molecular tools, and also, that more studies on toxin production are needed in the area. The presence of toxigenic planktonic species has been reported, and this indicates a hazard for fisheries, shellfish production and harvest and for human and animal health.

Alexandrium tamarense

Carreto et al. (1996) isolated vegetative cells from MPlat and compared their toxin profile with the toxin profile of mussels (*M. edulis*) and marine snails [*Zidona angulata* and *Adelomedon (Pachycymbiola) brasiliana*]. Toxin profiles were analyzed in 0.1 M acetic acid extracts from dinoflagellate cells by LC-FLD with post column derivatization. They reported C1/2 toxins as the most abundant, followed by GTX1/4, NEO, GTX2/3, and dcGTX2/3; STX was also reported, but as a small fraction. Toxin profiles in gastropods were highly dissimilar to those from the dinoflagellate, confirming metabolic changes upon the ingested toxin cell content, though all presented high toxin levels, but mainly from STX with the exception of *M. edulis* that presented mostly GTX analogs (Carreto et al., 1996). This study evidenced the importance of toxin analysis in gastropods.

In Brazil, toxin content of isolates were determined. Five strains were cultured from cysts and one from vegetative cells, in f/2 medium at 20°C. Toxins were extracted with 0.05 M acetic acid and analyzed by LC-FLD with post-column derivatization. Toxin content ranged from 42 to 199 fmol cell^{-1} and toxicity varied between 7.1 to 65.9 pg STXeq cell^{-1}, except for the culture originated from vegetative cells, which showed higher toxicity, mainly due to the higher GTX4 content (Persich et al., 2006).

In 2010, a comparative toxin profile study from cultured strains and natural populations was performed. Cultures were established from vegetative cells isolated from MPlat, GNVO, Golfo de San José and off Península Valdés (PVAL), and maintained in f/2 medium at 15°C and 12:12 L:D. At the same time, they sampled in MPlat, close to PVAL, Bahía Camarones and Bahía Grande. They found toxins in all isolates and in natural samples, though those from natural samples were significantly more toxic, and the highest toxicity was found in natural samples from open coastal waters in comparison with semi-enclosed water bodies such as bays or gulfs (Montoya et al., 2010). In general, natural samples produced more carbamoyl analogs whereas cultures produced more of the less potent *N*-sulfocarbamoyl toxins C1/2. A clear correlation ($r^2 = 0.993$) between nitrate concentration and GTX1/4 in natural samples was found, while GTX2/3 decreased significantly ($r^2 = 0.987$). Nevertheless, in culture, where nitrate concentration is higher than in natural conditions, the production of GTXs was considerably lower. When temperature and toxin content was compared, an inverse linear correlation ($r^2 = 0.989$) was found, in agreement with previous studies on the genus.

The fact that natural populations were notably more toxic than those in culture raises the question about the natural factors involved, and one could be associated to bacteria (Uribe and Espejo, 2003), and that natural conditions are far from being imitated in culture. Also, the fact that populations from open waters (e.g., out from PVAL) were more toxic than those from enclosed water habitats remains enigmatic, and is probably related to specific responses to environmental conditions (Montoya et al., 2010). In many cases, cultured *Alexandrium* cells are less toxic than those from natural environments, and they can lose toxin production during routine culture maintenance, which has been stated previously, and this has led to the hypothesis that some aspects on toxin production might not be genetically determined (Ogata et al., 1987). Nevertheless, field samples displayed important variability just as in other *Alexandrium* species and other toxin-producing dinoflagellates, like *G. catenatum*. Authors stated a clear need of further research

on toxin-producing species to reach a better understanding on toxin metabolism, changes within culture conditions, and significance of environmental conditions and associated bacteria (Uribe and Espejo, 2003).

In 2012, during a research cruise, Krock et al. (2007) sampled 48 stations along the Argentinian coast. From one station, outside Golfo de San Jorge, they isolated *A. tamarense* and established two clonal cultures using half strength K medium, 15°C and 16:08 L:D. PSTs were analyzed from 0.03 M acetic acid extraction by LC-FLD, according to previous methodology. Toxin profiles of the cultures were almost identical, and consisted of GTX1/4, C1/2 and minor proportions of NEO, GTX2/3, and STX (Krock et al., 2015). This profile has been previously reported for Argentinean strains in culture and natural populations (Carreto et al., 1996; Montoya et al., 2010). In comparison, toxin profiles in field samples consisted only of GTX2/3, while this toxin accounted for minor proportion in cultures. One possible explanation for these results could be that a chemical conversion due to storage conditions, since field samples were analyzed 3 months after the sampling and were transported from Argentina to Germany, where the analysis was performed. Nevertheless, it is interesting that not only the lateral chain was lost in toxins C1/2 to be converted into GTX2/3, but a dehydroxilation implying the loss of the N-1 hydroxyl group on GTX1/4 and NEO happened, and were converted into GTX2/3 and STX, respectively. Undeniably, further studies are needed under controlled conditions to clarify these processes (Krock et al., 2015).

Alexandrium ostenfeldii

During the expedition carried out in 2015 by Guinder et al. (2018), three of the isolates were identified as *A. ostenfeldii.* Cells from this species were isolated as mentioned (in 1/10 K medium and transferred to 1/2 K medium flasks and incubated at 15°C and 50 μmol photons $m^{-2}s^{-1}$ on a 16:8 LD photocycle). These cultures were specifically analyzed for spiroimines and also for PSTs (Guinder et al., 2018).

Toxin analysis from these cultured strains showed GTX2/3, C1/2 and STX as the most abundant toxins (no percentages reported). The three isolates produced six spirolides, being SPX-1 the most abundant (83–93% of the total). The authors reported a novel analog, and named it spirolide M. The other four are still uncharacterized (named "compounds 1–4") (Guinder et al., 2018). The novel spirolides produced by the *A. ostenfeldii* isolates highlight the richness and structure variability of these toxins, and there are probably more analogs than previously thought, as often seen in toxin-producing plankton. This is the first toxinological research on *A. ostenfeldii* at higher latitudes of the Patagonian shelf, and the other isolated strain from a southern geographical area (Beagle Channel) did not produce PSTs, but only spirolides (Almandoz et al., 2014).

Nevertheless, during this expedition the PST profiles from field plankton samples and those from the isolates did not match. The dominant toxins in field samples were GTX1/4, while the dominant toxins in cultures, including *A. catenella* and *A. ostenfeldii*, were C1/2 and GTX2/3, respectively. According to the authors, this discrepancy could be the result of a lack of detection of GTX1/4 due to the high limit of detection (LOD) for this epimeric pair. Other possibilities are that the profile changed due to the growth conditions in culture or due to other PSTs producing organisms that have not been reported in the SW Atlantic (Guinder et al., 2018), which highlights the importance of enhanced research in the area.

Salgado et al. (2015) studied a Chilean strain of *A. ostenfeldii*. The authors analyzed and compared morphology and toxin production of three strains from the Baltic Sea (Finland), the Mediterranean Sea (Spain) and the Chilean coast. Strains were cultured L1 medium with a photoperiod of 12:12 L:D and different temperatures and salinities were tested, in a total of nine treatments for each isolate. Paralytic shellfish toxins were extracted with 0.05 M acetic acid, and analyzed by LC-FLD. For detection of *N*-sulfocarbamoyl toxins, a fraction of the acid extract was hydrolyzed. Less polar toxins SPX and GYM, were extracted with 100% methanol and LC coupled to high resolution mass spectrometry (LC-HRMS) was used. Analysis showed PSP toxins in Chilean and Baltic isolates, but no detectable amounts in the Mediterranean strain; PSP toxin profiles were similar in both strains but the Chilean isolate had a greater toxin content than the Baltic strain (4.0 ± 1.9 pg $cell^{-1}$). These results showed a significant correlation of toxin content and cell biovolume; as stated in previous studies with *A. catenella* from Chile, *A. ostenfeldii* showed higher toxin values when cultured at lower temperature (10°C), also at this temperature the cell volume was larger. In both isolates, detected toxins were GTX2/3 and STX. Trace amounts of dcSTX were found in all treatments except at 10°C and at a salinity of 32, where toxin content and cell biovolume were highest. Toxin profile and proportions did not change significantly among treatments (Salgado et al., 2015). Only the Mediterranean isolate produced detectable amounts of SPX, and only the Baltic isolate produced GYM toxins. Treatments affected mostly cell growth and toxin content, but no changes in the toxin profile were observed. The significantly high PSP toxin content detected in the Chilean isolate (max. 279.8 pg $cell^{-1}$) suggested that this species could be more toxic than isolates previously reported, and should be further analyzed given the natural conditions of the region, where water temperatures of 10°C and salinity of 32 are common (Almandoz et al., 2014). However, the three isolates showed great variations in toxin profiles in all treatments, suggesting this species has a great capability of adaptation, as reported for *A. catenella*. So far, information on this species complex in LAm is still scarce and there is a great need for more studies (Salgado et al., 2015).

Alexandrium tamiyavanichi

Only one study on *A. tamiyavanichi* toxin analysis has been reported in LAm. Toxins were extracted with 0.03 M acetic acid, and analyzed with LC-FLD. Analogs STX, NEO, GTX1-5, and dcGTX2/3 were searched for, but *N*-sulfocarbamoyl toxins were not analyzed. Reported toxins were STX, NEO, and GTX4 as the main components, followed by GTX3 and dcGTX2/3 (Menezes et al., 2010). In their study, despite general morphological characters agreed with those reported for *A. tamiyavanichi*, they detected important variability that could also match with the morphology of *A. cohorticula*, suggesting the possibility of being a conspecific species, pointing out the need

of genetic studies on wild and cultured populations to confirm their taxonomic identity, given that the use of morphological characters as the only tool for identification has caused previous misidentifications. Their phylogenetic analysis supported their identification as *A. tamiyavanichi*, even when the species has not been reported in the Atlantic coasts of South America. Previously *A. tamiyavanichi* has been reported in Asian countries (see Menezes et al., 2010 and references therein), and the possible explanation for this finding, given the lack of previous records of the species, could be that it has been part of the cryptic flora and that this zone has been part of their natural biogeography. The report of this toxin-producing species account to four PSTs producing dinoflagellates in the Brazilian coasts, where more studies are needed (Menezes et al., 2010).

Molecular Studies

Molecular markers have been particularly useful for discerning *Alexandrium* species when morphological traits are not sufficient, to know the genetic diversity among populations, or to determine phylogenetic relationships among species geographically distant. In LAm the few genetic studies in *Alexandrium* species have allowed to corroborate taxonomic questions, biogeographic history, and changes in distribution patterns. A classic study related with molecular phylogeny of *Alexandrium* genus in North America was published by Scholin et al. (1995). *Alexandrium catenella* and *A. tamarense* belong to the species complex *A. catenella*/*tamarense*/*fundyense*; this group has reached relevance in South America due to its impact on fishing and aquaculture activities. The molecular markers developed by these authors are still used to answer current taxonomic and phylogenetic issues (Scholin et al., 1995).

The first attempt to obtain sequences of LSUrDNA of *A. catenella* was carried out in Chile. Sequences showed at least two different strains of the species that bloom in this region, with a proximity to the North American ribotype and distant proximity from the Asian ribotype (Córdova and Müller, 2002). Six years later, Uribe et al. (2008), constructed cDNA libraries from axenic strains from the AYSR in Chile, obtaining 10,850 expressed sequence tags (ESTs), where the most expressed genes corresponded to proteins coding for bioluminescence, carbohydrates, and those associated with photosynthesis (Uribe et al., 2008). They also carried out a detailed analysis of bioluminescent proteins: luciferin-binding protein and luciferase, additionally two unigenes presented 100% identity with a toxic strain-specific sequence of *A. tamarense*. Moreover, ESTs of *A. catenella* were closely related to *A. tamarense*. Taking into account that bioluminescence proteins were among the most expressed genes in *A. catenella*, the authors suggested that probably bioluminescence could be related with physiological responses such as predation avoidance and cell communication (Uribe et al., 2008).

Aguilera-Belmonte et al. (2011) compared physiological and genetic variability in populations of *A. catenella* from southern Chile isolated from the same toxic event. A remarkable variability at the genetic and physiological level among strains was observed. Chilean strains showed a higher genetic diversity (3%) than other strains of the world, and differences on PSTs analogs were detected between strains. Intraregional diversity of this species, partial sequences of the LSU gene, and ITS regions of rDNA, as well as toxicological and morphological analysis, have been evaluated. The analysis of rDNA sequences of Chilean strains were separated as part of the Clade I (North American) of the *A. tamarense* species complex (Varela et al., 2012). However, a significant genetic diversity was observed between Chilean strains with ITS sequences. Although morphological variations within and between strains were observed, some features were absent, such as a ventral pore in the 1' plate, which was a distinctive characteristic in Chilean strains. These approaches indicate a significant intraregional variability, however this genetic diversity does not agree with the supposed northward expansion along the west coast of South America.

Sequences from the ITS1, 5.8S rDNA, ITS2, and D1-D5 LSUrDNA regions, were used to identify strains of the morphospecies *A. catenella* as *A. tamarense*. Species-specific primers designed for real time PCR were a good molecular tool to detect this dinoflagellate in bivalves such as *M. edulis* (Jedlicki et al., 2012).

Persich et al. (2006) investigated the probable origin of *A. tamarense* in Brazil, and found a close relationship between this species with *A. tamarense, A. fundyense* and *A. catenella* from North America, northern Europe and northern Asia (Scholin et al., 1994; Medlin et al., 1998). Interestingly, they did not find any close relationship to any of the *A. tamarense* complex from the southern hemisphere. According to the molecular information, they proposed that the isolates used for this study were probably transported from Uruguayan waters during coastal fronts in Argentina and Uruguay; they considered the possibility of further outbreaks from transported cells or resting cysts in Brazilian coasts (Persich et al., 2006). Even though the origin of the so-called "North American type" *Alexandrium* is not clear, DNA results in this study support the hypothesis of an early establishment of these populations during a period of cooler global oceans, when natural boundaries did not exist and transport across hemispheres was possible. This hypothesis was proposed since the LSUrDNA sequences from South American isolates have enough differences from the North American modern populations that may indicate that these populations have been separated for a long time, and evolutionary processes have occurred (Persich et al., 2006).

Recently, Fabro et al. (2017) carried out morphological and genetic analyses of three morphospecies of *Alexandrium* from the Argentinean Sea. Their results showed some variations in morphological characteristics, but were consistent with classical descriptions of *A. tamarense* and *A. ostenfeldii*, however cells of *A. minutum* had morphological traits that did not agree completely with the description from Balech (Balech, 1995; Fabro et al., 2017). Using qPCR method, it was possible to corroborate the presence of *A. minutum* and *A. ostenfeldii* in NT samples. While partial sequences of the LSUrRNA of the *A. tamarense* complex clustered within the *Alexandrium* ribotype Group I, and were consistent with *A. catenella*. This was the first report of *A. minutum* and *A. ostenfeldii* on this area of Argentinean Sea (Fabro et al., 2017).

Cruzat et al. (2018) determined genetic variability of *A. catenella* analyzing ITS1-5.8S-ITS2 sequences of ribosomal DNA of environmental samples from the Southern Austral ecosystem. The authors found 33 haplotypes, three of these highly frequent, increasing the genetic diversity from 2.8 to 3.1%, for this species in the study area. All sequences agreed with the morphological identification for *A. catenella*, with sub-clades that correspond to haplotypes from distinct geographic regions (Cruzat et al., 2018).

Toxin profiles, morphology and phylogeny were also investigated by Salgado et al. (2015) in three strains of *A. ostenfeldii* from the Baltic, Mediterranean, and southern Chile. The phylogenetic reconstruction was performed with LSUrRNA sequences, showing a geographic distribution congruent with the selected strains. Strain AOA32 from Chile only produced PSTs, and had a close phylogenetic relationship with a strain from Callao, Peru (Salgado et al., 2015).

Using morphological traits and LSU and ITS sequences Menezes et al. (2010), identified *A. tamiyavanichi* and general morphological characters agreed with those reported previously; however, they detected important variability that could also match with the morphology of *A. cohorticula.* The most important feature to identify cells of this species is the anterior sulcal plate, while less than 10% of the analyzed specimens did not agree with the morphology of *A. tamiyavanichi*. Therefore, they suggested the possibility of them being conspecific species, and pointed out the need of genetic studies on wild and cultured populations to confirm their taxonomic identity, given that the use of morphological characters as an only tool for identification has caused misidentifications. Phylogenetic reconstruction showed a monophyletic clade, which included Brazilian and Asiatic strains, with enough genetic distance between them. Moreover, based on their results they propose a *fraterculus* group (*A. tamiyavanichi/tropicale/fraterculus)* as a sister group of *A. tamarense* species complex (Menezes et al., 2010). New morphological and molecular data (28S and ITS rDNA) for *Alexandrium* species from Brazil, confirm a close phylogenetic relationship among *A. tamiyavanichi* and *A. fraterculus* (Menezes et al., 2018), while *A. catenella* sequences were grouped with con-specifics from North America, Chile and Japan, moreover a genetic variability within *A. tamutum* clade was found, including a new record of this species for Brazil. Two strains isolated from Guanabara Bay formed a monophyletic clade with both molecular markers; suggesting it could be a new species of *Alexandrium* closely related to *A. minutum* and *A. tamutum*.

In the Mexican Pacific coast *A. tamiyavanichii* was found and described less than a decade ago (Esqueda-Lara and Hernández-Becerril, 2010), due to the difficulty for identifying and quantify this species Hernández-Becerril et al. (2018) analyzed ITS2 rDNA sequences obtained by real-time quantitative PCR (qPCR), managing to detect cell abundances of <30 cells L^{-1} in field samples. Differences in cell densities and their distribution in water column were observed, and two explanations were proposed: *A. tamiyavanichii* could be transported off the coast due to physical forces such as winds and upwelling, or when environmental conditions are favorable, the physiological characteristics of this species allows it to maintain high cell abundances.

Associations With Bacteria

Dinoflagellates are characterized by different ecological relationships. A relationship that has acquired a great interest is the one established between bacteria and dinoflagellates. This interest relies since bacteria are capable of regulating the different HAB phases, in addition that they are considered to have a role in cell toxicity, growth, and other physiological aspects of dinoflagellates (Hold et al., 2001; Uribe and Espejo, 2003; Croft et al., 2005; Maas et al., 2007).

Alexandrium strains from the southern coast of Chile grow in association with heterotrophic bacteria that mostly affect its growth by the synthesis of algicidal substances that promote cell lysis (Vasquez et al., 2001; Córdova et al., 2002; Uribe and Espejo, 2003). The proliferation of *A. catenella* in the decade of 1980 in Chile, allowed to report the presence of intracellular bacteria in vegetative cells, and to describe the process of bacterial phagocytosis (Córdova et al., 2002). Also, this was the first time that bacteria of the genus *Moraxella* sp. were isolated from the cell surface of the dinoflagellate, and related with the production of small amounts of STX (Guzmán et al., 1975; Silva, 1982; Kodama et al., 1990; Orr et al., 2013).

The genera *Cytophaga, Pseudoalteromonas*, and *Ruegeria* released alga-lytic compounds among other compounds when grown in absence of phytoplankton cells, causing high mortality (>50%) in *A. catenella*. When the same bacteria, free of organic nutrients, were returned to the algal culture they displayed no detrimental effects on the dinoflagellate and recovered their symbiotic characteristics. Thus, bacterial-derived lytic activities are expressed only in the presence of high-nutrient media and it is likely that *in situ* environmental conditions modulate their expression (Amaro et al., 2005). Another aspect to consider is the geographical distribution of strains, since despite isolating the bacteria from different regions, the associated bacteria community is similar, recording the presence of the genera *Psychrobacter, Sulfitobacter, Aeromonas, Flavobacterium, Pseudomonas, Proteus* and *Moraxella* in isolates of *A. catenella* and *A. fundyense* from different regions of Chile and the Bay of Fundy, respectively (Ferrier et al., 2002).

Bacterial phagocytosis by *Alexandrium* has also been documented (Silva, 1982). In total absence of bacteria, cell toxicity of *A. catenella* decreased five times as the majority of the STX analogs decreased significantly, with the exception of NEO. Also, as the culture of the dinoflagellate reached the stationary phase, the cell abundance proportion of bacteria:dinoflagellate remained constant (3,000 bacteria $cell^{-1}$ of *Alexandrium*), however, bacterial size tended to increase, suggesting they could be saprophytic bacteria that feed on dinoflagellate by-products (Schut et al., 1993; Dantzer and Levin, 1997; Uribe and Espejo, 2003).

CONCLUSION

In LAm, the three main marine dinoflagellate genera that produce PSTs are widely distributed and represent an important

risk for human health, economic and ecological reasons. PSTs have been also related to epizoic events, fish and aquaculture losses, though most of the times the impacts have not been calculated in economic terms. Despite these problems and risks, few countries have established and maintained monitoring programs, and losses due to PSTs are becoming an important public concern. HABs research efforts have been concentrated in North America (*G. catenatum* in Mexico) and in South America (*A. catenella, A. tamarense* mainly in Chile and Argentina). Few studies regarding *P. bahamense* exist, probably because this species is found mainly in regions with low resources for research, which could explain partially why this is the PST species that has caused greater human impacts.

Studies related to bloom dynamics, autoecology and toxins of *G. catenatum* are mostly from Mexico, however, this species has been reported in several countries, but in some regions, the species identification remains to be confirmed. Blooms have been associated with a narrow temperature window (18–25°C), high irradiance, transitional hydrographic conditions, El Niño events, and nutrient increase, mostly from river runoffs and coastal upwelling (**Table 7**). Even though natural plankton samples have a low toxicity, and a profile dominated by the less toxic sulfocarbamoyl analogs, this profile can change in mollusks tissues due to their digestive metabolism and become highly toxic for consumers. Interestingly, autoecology studies have demonstrated that the cell toxin content is higher under *in vitro* conditions than in the natural environment, which can be partly explained by the higher nutrient concentrations used in culture media. It has also been proposed that the toxin composition is not a conservative feature in *G. catenatum*, however some populations appear to be pretty consistent in their toxin profile, such as strains from LAm, but strain studies from other regions of LAm need to be carried out to confirm this. It has also been demonstrated that under culture conditions, *G. catenatum* tolerates a wide range of temperature, salinity, irradiance and N:P ratios, which probably explains its wide distribution along the Mexican Pacific coast. Nevertheless, some variations exist, which have been related to strain origin, temperature, and culture age.

The distribution of *P. bahamense* is restricted to tropical latitudes. Blooms have been related to warm waters (27–31°C), heavy rains, strong winds, and to El Niño events (**Table 7**). This species is found in a broad salinity range from 7 to 35. It has been suggested that it could be an invasive species in the northern boundary of its distribution (GOLCA region). Data regarding toxicity and toxin profile of natural phytoplankton samples are scarce and contradictory, carbamoyl or sulfocarbamoyl analogs can dominate. Toxin profiles in mollusks is also quite variable. It is clear that more research and close monitoring is needed for the understanding of the bloom ecology on this species.

HABs of *Alexandrium* toxic species are mostly reported in South America, being the main responsible species *A. catenella* (Chile) and *A. tamarense* (Uruguay-Argentina), which have had severe social impacts, and for this reason research has been concentrated on these two species. In general, *Alexandrium* species bloom at colder temperatures (5–14°C) than *G. catenatum* and *P. bahamense*. HABs of *A. catenella* are related to high irradiances, water stratification, and extreme climatic conditions such as a higher SST, decreased salinity and precipitation, higher nutrients and El Niño events (**Table 7**). Deep water upwelling also trigger blooms and cyst beds play an important role in the initiation of blooms. Also, an important expansion in the northern limits of these species has been registered, but the reason for this remains to be understood. Seasonal differences in cell toxicity and in toxin analogs in *A. catenella* have been found in environmental samples, and contrary to *G. catenatum* cell toxin content is higher in environmental samples than cultured strains, indicating that toxin metabolism in both species is regulated by different factors.

Regarding toxin analysis in PSTs producers, an important factor to be considered is the different techniques used to extract and analyze toxins which have led to different and confusing results in some cases samples from the same species and region. Different studies have used two acids (hydrochloric acid or acetic acid) in various concentrations, sometimes even using thermic processing of samples. Post-column and pre-column oxidations methods are used, with different drawbacks such as lack of separation of some analogs or the inability to detect some others, and even the appearance of phantom peaks that could be mistaken for toxin analogs. Also, GC toxins are not yet considered in normal analysis and their presence is often neglected due to the longer retention times needed to elute them, and to the lack of commercial standards. It is clear that only a few countries in LAm have the sufficient technological and technical capacity to analyze paralytic toxins a factor that needs to be considered to support both research and monitoring programs in this region.

Other studies such as molecular biology, *in silico* analyses in order to assess toxicity, nutrient assimilation, trophic interactions, and bacterial community studies are still incipient in LAm, but given the great importance of PSTs producing species in LAm, undoubtedly they will continue to increase in the next few years.

This review evidences that studies have been concentrated in relatively few countries, species and topics. And in some regions studies are only supported for a few years by specific research groups. In general, there is equipment and technical limitation in the different regions of LAm. A regional HAB research program is needed in order to have a more complete understanding of the environmental conditions that favor the PST blooms in this region.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

ACKNOWLEDGMENTS

This research was aided by institutional projects of CIBNOR (PPAyC, Planeación Ambiental y Conservación) and from the Instituto Politécnico Nacional (IPN grants SIP 2018-0662). Consejo Nacional de Ciencia y Tecnología (FORDECyT

grant 260040, grant A1-S-14968), and Red Temática sobre Florecimientos Algales Nocivos, CONACyT (RedFAN). Authors thank Dr. J. Vinuesa for providing historical HABs epizootic data from Argentina and MF Sánchez-Bernal for the graphic design.

REFERENCES

Aguilera-Belmonte, A., Inostroza, I., Carrillo, K. S., Franco, J. M., Riobo, P., and Gómez, P. I. (2013). The combined effect of salinity and temperature on the growth and toxin content of four Chilean strains of *Alexandrium catenella* (Whedon and Kofoid) Balech 1985 (Dinophyceae) isolated from an outbreak occurring in southern Chile in 2009. *Harmful Algae* 23, 55–59. doi: 10.1016/j.hal.2012.12.006

Aguilera-Belmonte, A., Inostroza, I., Franco, J. M., Riobó, P., and Gómez, P. I. (2011). The growth, toxicity and genetic characterization of seven strains of *Alexandrium catenella* (Whedon and Kofoid) Balech 1985 (Dinophyceae) isolated during the 2009 summer outbreak in southern Chile. *Harmful Algae* 12, 105–112. doi: 10.1016/j.hal.2011.09.006

Aké-Castillo, J. A., and Poot-Delgado, C. A. (2016). "FAN en el Golfo de México: panorama general sobre eventos y especies," in *Florecimientos Algales Nocivos en México*, eds E. García-Mendoza, S. I. Quijano-Scheggia, A. Olivos-Ortíz, E. J. Núñez-Vázquez (Ensenada: CICESE), 298–307.

Akselman, R., Carreto, J., and Montoya, N. (1998). "*Gymnodinium catenatum* and autumn toxicity in northern shelf waters of Argentina," in *Harmful Algae*, eds B. Reguera, J. Blanco, M. L. Fernández, and T. Wyatt, (Vigo: Xunta de Galicia, IOC-UNESCO), 122–123.

Almandoz, G. O., Hernando, M. P., Ferreyra, G. A., Schloss, I. R., and Ferrario, M. E. (2011). Seasonal phytoplankton dynamics in extreme southern South America (Beagle Channel, Argentina). *J. Sea Res.* 66, 47–57. doi: 10.1016/j.seares.2011.03.005

Almandoz, G. O., Montoya, N. G., Hernando, M. P., Benavides, H. R., Carignan, M. O., and Ferrario, M. E. (2014). Toxic strains of the *Alexandrium ostenfeldii* complex in southern South America (Beagle Channel, Argentina). *Harmful Algae* 37, 100–109. doi: 10.1016/j.hal.2014.05.011

Alonso-Rodríguez, R., Gárate-Lizárraga, I., Luckas, B., Reinhardt, K., and Bustillos-Guzmán, J. (2004b). "Mortalidad de larvas de camarón en cultivo en Sinaloa, México, asociado a mareas rojas de *Gymnodinium catenatum,*" in *Book of abstracts XIII Reunión Nacional de la Sociedad Mexicana de Planctología* (Nuevo Vallarta), 54–55.

Alonso-Rodríguez, R., Mendoza-Amézquita, E., Velásquez-López, S. A., Seim, J. A., and Martínez-Rodríguez, V. M. (2015). Florecimientos algales nocivos producidos por *Pyrodinium bahamense* en Oaxaca, México (2009-2010). *Sal Pub Mex* 57, 343–351. doi: 10.21149/spm.v57i4.7578

Alonso-Rodríguez, R., and Páez-Osuna, F. (2003). Nutrients, phytoplankton and harmful algal blooms in shrimp ponds: a review with special reference to the situation in the Gulf of California. *Aquaculture* 219, 317–336. doi: 10.1016/S0044-8486(02)00509-4

Alonso-Rodríguez, R., Páez-Osuna, F., and Gárate-Lizárraga, I. (2004a). *El fitoplancton en la Camaronicultura y Larvicultura: Importancia de un Buen Manejo*. Mexico, Inst Cienc Mar Limnol-UNAM/CESASIN.

Alvarado, Y., Espinoza, J., and Amaya, O. (2014). First report of *Pyrodinium bahamense* cysts in marine sediments from the Gulf of Fonseca, El Salvador. *Harmful Algae News* 40, 10–11.

Alvarado, Y., Quintanilla, R., and Amaya, O. (2015). First report of *Alexandrium globosum* on the Pacific coast of Central America. *Harmful Algae News* 51:3.

Álvarez, G., Uribe, E., Vidal, A., Ávalos, P., González, F., Mariño, C., et al. (2009). Paralytic shellfish toxins in *Argopecten purpuratus* and *Semimytilus algosus* from northern Chile. *Aquat. Living Resour.* 22, 341–347. doi: 10.1051/alr/2009028

Amaro, A. M., Fuentes, M. S., Ogalde, S. R., Venegas, J. A., and Suárez-Isla, B. A. (2005). Identification and characterization of potentially algal-lytic marine bacteria strongly associated with the toxic dinoflagellate *Alexandrium catenella*. *J. Eukaryot. Microbiol.* 52, 191–200. doi: 10.1111/j.1550-7408.2005.00031.x

Amaya, O., Dechraoui-Bottein, M. Y., Leighfield, T., and Ruiz, G. (2017). "Five years of application of the Receptor Binding Assay (RBA) on seafood products and threatened turtles during outbreaks HABs in El Salvador," in *Marine and Fresh-Water Harmful Algae, Proceedings 17th International Conference on Harmful Algae*, eds L. A. O. Proença and G. M. Hallegraeff (Florianópolis: ISSHA, IOC-UNESCO), 130–132.

Amaya, O., Quintanilla, R., Stacy, B. A., Dechraoui-Bottein, M.-Y., Flewelling, L., Hardy, R., et al. (2018). Large-scale sea turtle mortality events in El Salvador attributed to paralytic shellfish toxin-producing algae blooms. *Front. Mar. Sci.* 5:411. doi: 10.3389/fmars.2018.00411

Amaya, O., Ruiz, G., Espinoza, J., and Rivera, W. (2014). *Saxitoxin Analyses With a Receptor Binding Assay (RBA) Suggest PSP Intoxication of Sea Turtles in El Salvador*. Harmful Algal News IOC-UNESCO.

Anderson, D. M., Alpermann, T. J., Cembella, A. D., Collos, Y., Masseret, E., and Montresor, M. (2012). The globally distributed genus *Alexandrium*: multifaceted roles in marine ecosystems and impacts on human health. *Harmful Algae* 14, 10–35. doi: 10.1016/j.hal.2011.10.012

Arzul, G., Seguel, M., Guzman, L., and Erard-Le Denn, E. (1999). Comparison of allelopathic properties in three toxic *Alexandrium* species. *J. Exp. Mar. Biol. Ecol.* 232, 285–295. doi: 10.1016/S0022-0981(98)00120-8

Ávila, M., de Zarate, C., Clement, A., Carbonell, P., and Pérez, F. (2015). Efecto de factores abióticos en el crecimiento vegetativo de *Alexandrium catenella* proveniente de quistes en laboratorio. *Rev. Biol. Mar. Oceanog.* 50, 177–185. doi: 10.4067/S0718-19572015000200004

Balech, E. (1964). *El plancton de Mar del Plata Durante el Período 1961-1962* Buenos Aires: Boletín del Instituto de Biología Marina.

Balech, E. (1995). *The Genus Alexandrium Halim (Dinoflagellata).* County Cork: Sherkin Island Marine Station.

Band-Schmidt, C., Bustillos-Guzmán, J., Morquecho, L., Gárate-Lizárraga, I., Alonso-Rodríguez, R., Reyes-Salinas, A., et al. (2006). Variations of PSP toxin profiles during different growth phases in *Gymnodinium catenatum* (Dinophyceae) strains isolated from three locations in the Gulf of California, Mexico. *J. Phycol.* 42, 757–768. doi: 10.1111/j.1529-8817.2006.00234.x

Band-Schmidt, C. J., Bustillos-Guzmán, J., Gárate-Lizárraga, I., Lechuga-Devéze, C., Reinhardt, K., and Luckas, B. (2005). Paralytic shellfish toxin profile in strains of the dinoflagellate *Gymnodinium catenatum* Graham and the scallop *Argopecten ventricosus* GB Sowerby II from Bahía Concepción, Gulf of California, Mexico. *Harmful Algae* 4, 21–31. doi: 10.1016/j.hal.2003.10.004

Band-Schmidt, C. J., Bustillos-Guzmán, J. J., Durán-Riveroll, L. M., López-Cortés, D. J., Hernández-Sandoval, F. E., and Núñez-Vázquez, E. J. (2016). "Autoecología de microalgas nocivas aisladas del Golfo de California," in *Florecimientos Algales Nocivos en México.*, eds E. García-Mendoza, S. I. Quijano-Scheggia, A. Olivos-Ortíz, E. J. Núñez-Vázquez (Ensenada: CICESE), 57–71.

Band-Schmidt, C. J., Bustillos-Guzmán, J. J., Hernández-Sandoval, F. E., Núñez-Vázquez, E. J., and López-Cortés, D. J. (2014). Effect of temperature on growth and paralytic toxin profiles in isolates of *Gymnodinium catenatum* (Dinophyceae) from the Pacific coast of Mexico. *Toxicon* 90, 199–212. doi: 10.1016/j.toxicon.2014.08.002

Band-Schmidt, C. J., Bustillos-Guzmán, J. J., López-Cortés, D. J., Gárate-Lizárraga, I., Núñez-Vázquez, E. J., and Hernández-Sandoval, F. E. (2010). Ecological and physiological studies of *Gymnodinium catenatum* in the Mexican Pacific: a review. *Mar. Drugs* 8, 1935–1961. doi: 10.3390/md8061935

Band-Schmidt, C. J., Bustillos-Guzmán, J. J., López-Cortés, D. J., Núñez-Vázquez, E. J., and Hernández-Sandoval, F. E. (2011). El estado actual del estudio de florecimientos algales nocivos en México. *Hidrobiólogica* 21, 381–412.

Band-Schmidt, C. J., Fernández-Herrera, L. J., Ramírez-Rodríguez, D. V., Zumaya-Higuera, M. G., Hernández-Sandoval, F. E., Núñez-Vázquez, E. J., et al. (2017). "Effect of different taxonomic groups on the growth and toxin content in *Gymnodinium catenatum* cultures from the Pacific coast of Mexico," in *Proceedings 17th International Conference on Harmful Algae*, eds L. A. O. Proença and G. M. Hallegraeff (Florianópolis: IOC-UNESCO), 54–57.

Band-Schmidt, C. J., Morquecho, M. L., Lechuga-Devéze, C. H., and Anderson, D. M. (2004). Effects of growth medium, temperature, salinity and seawater

source on the growth of *Gymnodinium catenatum* (Dinophyceae) from Bahía Concepción, Gulf of California, Mexico. *J. Plankton. Res.* 26, 1459–1470. doi: 10.1093/plankt/fbh133

Band-Schmidt, C. J., Rojas-Posadas, D. I., Morquecho, L., and Hernández-Saavedra, N. Y. (2008). Heterogeneity of LSU rDNA sequences and morphology of *Gymnodinium catenatum* dinoflagellate strains in Bahía Concepción, Gulf of California, Mexico. *J. Plankton Res.* 30, 755–763. doi: 10.1093/plankt/fbn035

Barraza, J. E. (2009). Food poisoning due to consumption of the marine gastropod *Plicopurpura collumellaris* in El Salvador. *Toxicon* 54, 895–896. doi: 10.1016/j.toxicon.2009.06.026

Baylón, M., Sánchez, S., Bárcena, V., López, J., and Mamani, E. (2015). Primer reporte del dinoflagelado potencialmente tóxico *Alexandrium minutum* Halim 1960 en el litoral peruano. *Rev. Peru Biol.* 22, 113–118. doi: 10.15381/rpb.v22i1.11129

Benavides, H., Prado, L., Días, S., and Carreto, J. (1995). "An exceptional bloom of *Alexandrium catenella* in the Beagle Channel, Argentina," in *Harmful Marine Algal Blooms*, eds P. Lassus, G. Arzul, E. Erard, P. Gentien, and C. Marcaillou (Paris: Technique et Documentation-Lavoisier), 113–119.

Blanco-Blanco, M., Aguilar-Olguín, S., and Morales-Blake, A. (1999). "Caracterización de una marea roja en la Bahía de Manzanillo, Colima, México," in *Proceedings VIII Congreso Latinoamericano sobre Ciencias del Mar, COLACMAR,* eds A. E. Tresierra-Aguilar and Z. G. Culquichicón-Malpica (Trujillo), 338–339

Bolch, C., Negri, A., and Blackburn, S. (2001). "Life cycle variation in PST content and cell toxicity in PST-producing dinoflagellates," in *Proceedings of the LIFEHAB: Life Histories of Harmful Algal Bloom Species European Union Workshop*, eds E. Garcés, A. Zingone, M. Montresor, and B. Reguera (Calviá), 37–42.

Bolch, C. J., Blackburn, S. I., Hallegraeff, G. M., and Vaillancourt, R. E. (1999). Genetic variation among strains of the toxic dinoflagellate *Gymnodinium catenatum* (Dinophyceae). *J. Phycol.* 35, 356–367. doi: 10.1046/j.1529-8817.1999.3520356.x

Brooijmans, N., and Kuntz, I. D. (2003). Molecular recognition and docking algorithms. *Annu. Rev. Biophys. Biomol. Struct.* 32, 335–373. doi: 10.1146/annurev.biophys.32.110601.142532

Bustillos-Guzmán, J., Band-Schmidt, C. J., López-Cortés, D., Gárate-Lizárraga, I., Núñez-Vázquez, E., and Hernández-Sandoval, F. E. (2012). Variaciones en el crecimiento y toxicidad en *Gymnodinium catenatum* Graham del Golfo de California bajo diferentes proporciones de nitrógeno y fósforo. *Cienc Mar.* 38, 101–117. doi: 10.7773/cm.v38i1A.1916

Bustillos-Guzmán, J., Vale, P., and Band-Schmidt, C. J. (2011). Presence of benzoate type toxins in *Gymnodinium catenatum* Graham isolated from the Mexican Pacific. *Toxicon* 57, 922–926. doi: 10.1016/j.toxicon.2011.02.023

Bustillos-Guzmán, J. J., Band-Schmidt, C. J., Durán-Riveroll, L. M., Hernández-Sandoval, F. E., López-Cortés, D. J., Núñez-Vázquez, E. J., et al. (2015). Paralytic toxin profile of the marine dinoflagellate *Gymnodinium catenatum* Graham from the Mexican Pacific as revealed by LC-MS/MS. *Food Addit. Contam A.* 32, 381–394. doi: 10.1080/19440049.2014.1000978

Bustillos-Guzmán, J. J., Band-Schmidt, C. J., López-Cortés, D. J., Hernández-Sandoval, F. E., Núñez-Vázquez, E., and Gárate-Lizárraga, I. (2013). Grazing of the dinoflagellate *Noctiluca scintillans* on the paralytic toxin-producing dinoflagellate *Gymnodinium catenatum*: Does grazing eliminate cells during a bloom? *Cienc Mar.* 39, 291–302. doi: 10.7773/cm.v39i3.2242

Bustillos-Guzmán, J. J., Leyva-Valencia, I., Hernández-Sandoval, F. E., Band-Schmidt, C. J., López-Cortés, D. J., and Núñez-Vázquez, E. J. (2016). "Ficotoxinas en aguas del Golfo de California: Una revisión," in *Florecimientos Algales nocivos en México*, eds E. García-Mendoza, S. I. Quijano-Scheggia, A. Olivos-Ortíz, and E. J. Núñez-Vázquez (Ensenada: CICESE), 38–55.

Callejas, L., Darce, A. C. M., Amador, J. J., Conklin, L., Gaffga, N., Rogers, H. S., et al. (2015). Paralytic shellfish poisonings resulting from an algal bloom in Nicaragua. *BMC Res Notes.* 8:74. doi: 10.1186/s13104-015-1012-4

Carreto, J., Elbusto, C., Sancho, H., Carignan, M., Yasumoto, T., and Oshima, Y. (1996). Comparative studies on paralytic shellfish toxin profiles of marine snails, mussels and an *Alexandrium tamarense* isolate from the Mar del Plata coast (Argentina). *Rev. Invest. Des. Pesq.* 10, 101–107.

Carreto, J. I., Akselman, R., Montoya, N. G., Negri, R. M., Benavides, H. R., Carignan, M. O., et al. (1998a). "*Alexandrium tamarense* bloom dynamics and Mytilus edulis toxicity in the coastal waters off Mar del Plata (Argentina)," in *Harmful Algae*, eds B. Reguera, J. Blanco, M. L. Fernández, T. Wyatt (Vigo: Xunta de Galicia), 135–138.

Carreto, J. I., Montoya, N. G., Akselman, R., Negri, R. M., Carignan, M. O., Cucchi-Colleoni, A. D., et al. (2004). "Differences in the PSP toxin profiles of *Mytilus edulis* during spring and autumn blooms of *Alexandrium tamarense* off Mar del Plata coast, Argentina," in *Proceedings Xth Harmful Algal Blooms Conference*, eds K. A. Stedinger, J. H. Lansberg, R. T. Tomar, and G. A. Vargo (St. Petersburg, FL: Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovermental Oceanographic Commission of UNESCO), 100–102.

Carreto, J. I., Montoya, N. G., Colleoni, A. D., and Akselman, R. (1998b). "*Alexandrium tamarense* blooms and shellfish toxicity in the Argentine sea: a retrospective view," in *Harmful Algae*, eds B. Reguera, J. Blanco, M. L. Fernández, and T. Wyatt (Vigo: IOC-UNESCO), 131–134.

Carreto, J. I., Negri, R. M., Benavides, H. R., and Akselman, R. (1985). "Toxic dinoflagellate blooms in the Argentine Sea," in *Toxic Dinoflagellates*, eds D. M. Anderson, A. W. White, and D. G. Baden (New York, NY: Elsevier Science Publishing Co.), 147–152.

Cassis, D., Muñoz, P., and Avaria, S. (2002). Variación temporal del fitoplancton entre 1993 y 1998 en una estación fija del seno Aysén, Chile (45°26'S 73°00'W). *Rev. Mar. Oceanog.* 37, 43–65. doi: 10.4067/S0718-19572002000100007

Castillo-Barrera, E., and García-Murillo, A. (2007). "Relación de organismos planctónicos presentes en florecimientos algales nocivos en bahía de Matachén, San Blas, Nayarit," in *Recursos y Medio Ambiente: Proceedings XIV Congreso Nacional de Ciencia y Tecnología* (Nuevo Vallarta).

Cembella, A. (1998). "Ecophysiology and metabolism of paralytic shellfish toxins in marine microalgae," in *Physiological Ecology of Harmful Algal Blooms*, eds D. M. Anderson, A. D. Cembella, G. M. Hallegraeff (Springer-Verlag; Heidelberg: NATO-Advanced Study Institute Series), 381–404.

Cembella, A., and Band-Schmidt, C. J. (2018). "*Gymnodinium catenatum*. Harmful algal species fact sheets," in *Harmful Algal Blooms: A Compendium Desk Reference*, eds S. E. Shumway, J. M. Burkholder, and S. L. Morton (Holboken, NJ: Wiley Blackwell), 605–611.

Chow, N., Vammen, K., and Reguera, B. (2010). First report of PSP on Pacific coast of Nicaragua associated with *Pyrodinium bahamense*. *Harmful Algae News* 41, 6–7.

Clément, A., Seguel, M., Arzul, G., Guzmán, L., and Alarcon, C. (2001). "A widespread outbreak of a haemolytic, ichthyotoxic *Gymnodinium* sp. in southern Chile," in *Harmful Algal Blooms* 2000, eds G. M. Hallegraeff, S. I. Blackburn, C. J. Bolch, R. J. Lewis (Paris: IOC-UNESCO), 66–69.

Córdova, J. L., Cárdenas, L., Cárdenas, L., and Yudelevich, A. (2002). Multiple bacterial infection of *Alexandrium catenella* (Dinophyceae). *J Plankton Res.* 24, 1–8. doi: 10.1093/plankt/24.1.1

Córdova, J. L., and Müller, I. (2002). Use of PCR and partial sequencing of the large-subunit rRNA gene to identify *Alexandrium catenella* (Dinophyceae) from the South of Chile. *Harmful Algae* 1, 343–350. doi: 10.1016/S1568-9883(02)00066-5

Cortés-Altamirano, R. (1987). Observaciones de mareas rojas en la Bahía de Mazatlán, Sinaloa, México. *Cienc Mar.* 13, 1–19. doi: 10.7773/cm.v13 i4.557

Cortés-Altamirano, R. (2002). "Mareas rojas: Biodiversidad de microbios que pintan el mar," in *Atlas de Biodiversidad de Sinaloa*, eds J. L. Cifuentes and J. Gaxiola-López (Colegio de Sinaloa), 442.

Cortés-Altamirano, R., and Alonso-Rodríguez, R. (1997). Mareas rojas durante 1997 en la Bahía de Mazatlán, Sinaloa, México. *Cien Mar. UAS.* 15, 31–37.

Cortés-Altamirano, R., Lavín, M., Sierra-Beltrán, A., and Cortés-Lara, M. C. (2006). Hipótesis sobre el transporte de microalgas invasoras desde el Pacífico oeste tropical hasta el Golfo de California por las corrientes marinas. *Ciencias del. Mar. U. A. S.* 18, 19–26.

Cortés-Altamirano, R., Licea, D. S., and Gómez-Aguirre, S. (1999b). "Evidencias de aumento de microalgas nocivas en la bahía de Mazatlán, Sin., México," in *VII Congreso Latinoamericano sobre Ciencias del Mar, 17 -21 de octubre de 1999*, eds A. E. Tresierra and Z. G. Culquichicón Malpica (Trujillo), 343–345.

Cortés-Altamirano, R., Manrique, F. A., and Luna-Soria, R. (1995). Presencia de mareas rojas en el costa este del Golfo de California. *Rev. Lat-Amer. Microbiol.* 37, 337–342.

Cortés-Altamirano, R., Muñoz-Cabrera, L., and Sotomayor-Navarro, O. (1993). Envenamiento paralítico por mariscos (PSP), causado por el dinoflagelado

Pyrodinium bahamense var. *compressum* en la costa suroeste de México. *An. Inst. Cienc. Mar. Limnol.* 1010, 43–54.

Cortés-Altamirano, R., and Núñez-Pasten, A. (1992). Doce años (1979-1990) de registros de mareas rojas en la Bahía de Mazatlán, Sinaloa, México. *An. Inst. Cienc. Mar. Limnol.* 19, 113–121.

Cortés-Altamirano, R., Núñez-Pasten, A., and Pasten-Miranda, N. (1999a). Abundancia anual de *Gymnodinium catenatum* Graham dinoflagelado tóxico de la costa este del Golfo de California. *Ciencia y Mar. Universidad del Mar.* 3, 50–56.

Costa, P. R., Pereira, P., Guilherme, S., Barata, M., Nicolau, L., Santos, M. A., et al. (2012). Biotransformation modulation and genotoxicity in white seabream upon exposure to paralytic shellfish toxins produced by *Gymnodinium catenatum*. *Aquat. Toxicol.* 106, 42–47. doi: 10.1016/j.aquatox.2011.08.023

Croft, M. T., Lawrence, A. D., Raux-Deery, E., Warren, M. J., and Smith, A. G. (2005). Algae acquire vitamin B 12 through a symbiotic relationship with bacteria. *Nature* 438:90. doi: 10.1038/nature04056

Cruzat, F. A., Muñoz, C., González-Saldía, R. R., Inostroza, A., and Andree, K. B. (2018). High genetic variability of *Alexandrium catenella* directly detected in environmental samples from the Southern Austral Ecosystem of Chile. *Mar. Poll. Bull.* 127, 437–444. doi: 10.1016/j.marpolbul.2017.12.022

Cusick, K. D., Wilhelm, S. W., Hargraves, P. E., and Sayler, G. S. (2016). Single-cell PCR of the luciferase conserved catalytic domain reveals a unique cluster in the toxic bioluminescent dinoflagellate *Pyrodinium bahamense*. *Aquat. Biol.* 25, 139–150. doi: 10.3354/ab00664

Dantzer, W. R., and Levin, R. (1997). Bacterial influence on the production of paralytic shellfish toxins by dinoflagellated algae. *J. Appl. Microbiol.* 83, 464–469. doi: 10.1046/j.1365-2672.1997.00246.x

de la Garza-Aguilar, J. (1983). Intoxicación alimentaria por ingestión de mariscos contaminados. *Sal. Pub. Mex.* 25, 145–150.

Deeb, O., Rosales-Hernández, M. C., Gómez-Castro, C., Garduño-Juárez, R., and Correa-Basurto, J. (2010). Exploration of human serum albumin binding sites by docking and molecular dynamics flexible ligand–protein interactions. *Biopolymers* 93, 161–170. doi: 10.1002/bip.21314

Deeds, J. R., White, K. D., Etheridge, S. M., and Landsberg, J. H. (2008). Concentrations of saxitoxin and tetrodotoxin in three species of puffers from the Indian River Lagoon, Florida, the location for multiple cases of saxitoxin puffer poisoning from 2002 to 2004. *T. Am. Fish. Soc.* 137, 1317–1326. doi: 10.1577/T07-204.1

Díaz, P. A., Molinet, C., Seguel, M., Díaz, M., Labra, G., and Figueroa, R. I. (2014). Coupling planktonic and benthic shifts during a bloom of *Alexandrium catenella* in southern Chile: Implications for bloom dynamics and recurrence. *Harmful Algae* 40, 9–22. doi: 10.1016/j.hal.2014.10.001

Durán-Riveroll, L., Krock, B., Cembella, A., Peralta-Cruz, J., Bustillos-Guzmán, J. J., and Band-Schmidt, C. J. (2017). Characterization of benzoyl saxitoxin analogs from the toxigenic marine dinoflagellate *Gymnodinium catenatum* by hydrophilic interaction liquid ion-chromatography-tandem mass spectrometry. *Nat. Prod. Chem. Res.* 5:275. doi: 10.4172/2329-6836.1000275

Durán-Riveroll, L. M., and Cembella, A. D. (2017). Guanidinium toxins and their interactions with voltage-gated sodium ion channels. *Mar. Drugs* 15:303. doi: 10.3390/md15100303

Durán-Riveroll, L. M., Cembella, A. D., Band-Schmidt, C. J., Bustillos-Guzmán, J. J., and Correa-Basurto, J. (2016). Docking simulation of the binding interactions of saxitoxin analogs produced by the marine dinoflagellate *Gymnodinium catenatum* to the voltage-gated sodium channel $Na_V1.4$. *Toxins* 8:129. doi: 10.3390/toxins8050129

Durán-Riveroll, L. M., Peralta-Cruz, J., Bustillos-Guzmán, J. J., and Band-Schmidt, C. J. (2013). Presencia de toxinas tipo benzoato en una cepa de *Gymnodinium catenatum* (Dinophyceae) aislada de Manzanillo, Colima, México. *Hidrobiológica* 23, 169–175.

Enrique Barraza, J., Armero-Guardado, J., and Valencia de Toledo, Z. M. (2004). The red tide event in El Salvador, August 2001-January 2002. *Rev. Biol. Trop.* 52(Suppl. 1):1–4.

Esqueda-Lara, K., and Hernández-Becerril, D. U. (2010). *Dinoflagelados Microplanctónicos Marinos del Pacífico Central de México (Isla Isabel, Nayarit y Costas de Jalisco y Colima)*. Mexico City: Universidad Nacional Autónoma de México. 206.

Esteves, J. L., Santinelli, N., Sastre, V., Díaz, R., and Rivas, O. (1992). A toxic dinoflagellate bloom and PSP production associated with upwelling in Golfo Nuevo, Patagonia, Argentina. *Hydrobiologia* 242, 115–122. doi: 10.1007/BF00018067

Estrada, N. A., Lagos, N., García, C., Maeda-Martínez, A. N., and Ascencio, F. (2007). Effects of the toxic dinoflagellate *Gymnodinium catenatum* on uptake and fate of paralytic shellfish poisons in the Pacific giant lions-paw scallop *Nodipecten subnodosus*. *Mar. Biol.* 151, 1205–1214. doi: 10.1007/s00227-006-0568-x

Fabro, E., Almandoz, G. O., Ferrario, M., John, U., Tillmann, U., Toebe, K., et al. (2017). Morphological, molecular, and toxin analysis of field populations of *Alexandrium* genus from the Argentine Sea. *J. Phycol.* 53, 1206–1222. doi: 10.1111/jpy.12574

FAO (2004). *Marine Biotoxins. Food and Agriculture Organization of the United Nations*. Rome: FAO Food and Nutrition paper 80.

Fernández-Herrera, L. J., Band-Schmidt, C. J., López-Cortés, D. J., Hernández-Guerrero, C. J., Bustillos-Guzmán, J. J., and Núñez-Vázquez, E. (2016). Allelopathic effect of *Chattonella marina* var. *marina* (Raphidophyceae) on *Gymnodinium catenatum* (Dinophycea). *Harmful Algae* 51, 1–9. doi: 10.1016/j.hal.2015.10.009

Ferrari, G. (2001). "HABs in the southwestern atlantic ocean," in *Proceedings 2nd International Conference on Harmful Algae Management and Mitigation* (November 2001, Qingdao, China), eds M. Zhu, Y. Zou, L. Cheong, S. Hall, (Paris: IOC-UNESCO), 34–35.

Ferrier, M., Martin, J., and Rooney-Varga, J. (2002). Stimulation of *Alexandrium fundyense* growth by bacterial assemblages from the Bay of Fundy. *J. Appl. Microbiol.* 92, 706–716. doi: 10.1046/j.1365-2672.2002.01576.x

Frangópulos, M., Guisande, C., and Maneiro, I. (2004). Toxin production and competitive abilities under phosphorus limitation of *Alexandrium* species. *Harmful Algae* 3, 131–139. doi: 10.1016/S1568-9883(03)00061-1

Fuentealba, R., Lozic, J., and Zegpi, A. (1981). Observaciones clínicas de una intoxicación masiva con veneno paralítico de los mariscos (Puerto Natales, Magallanes, Chile). *Ans. Inst. Patag. Pta. Arenas*. 12, 289–293.

Fuentes, C., Clement, A., and Aguilera, A. (2008). "Summer *Alexandrium catenella* bloom and the impact on fish farming, in the XI Aysén Region, Chile," in *Proceedings 12th International Conference on Harmful Algae*, eds Ø. D. Moestrup, G. Doucette, H. Enevoldsen, A. Godhe, G. Hallegraeff, and B. Luckas (Copenhaguen: IOC-UNESCO), 183–186.

Gárate-Lizárraga, I., Bustillos-Guzmán, J., Erler, K., Muñetón-Gómez, M., Luckas, B., and Tripp-Quezada, A. (2004). Paralytic shellfish toxins in the chocolata clam, *Megapitaria squalida* (Bivalvia: Veneridae), in Bahía de La Paz, Gulf of California. *Rev. Biol. Trop.* 52, 133–142.

Gárate-Lizárraga, I., Bustillos-Guzmán, J., Morquecho, L., Band-Schmidt, C. J., Alonso-Rodríguez, R., Erler, K., et al. (2005). Comparative paralytic shellfish toxin profiles in the strains of *Gymnodinium catenatum* Graham from the Gulf of California, Mexico. *Mar. Poll. Bull.* 50, 208–236. doi: 10.1016/j.marpolbul.2004.11.034

Gárate-Lizárraga, I., Bustillos-Guzmán, J. J., López-Cortés, D. J., Hernández-Sandoval, F., Erler, K., and Luckas, B. (2006). Paralytic shellfish toxin profiles in net phytoplankton samples from Bahía Concepción, Gulf of California, Mexico. *Mar. Poll. Bull.* 52, 800–815. doi: 10.1016/j.marpolbul.2006.03.003

Gárate-Lizárraga, I., Díaz-Ortíz, J., Pérez-Cruz, B., Alarcón-Tacuba, M., Torres-Jaramillo, A., Alarcón-Romero, M., et al. (2009). *Cochlodinium polykrikoides* and *Gymnodinium catenatum* in Bahía de Acapulco, Mexico (2005-2008). *Harmful Algae News* 40, 8–9.

Gárate-Lizárraga, I., Díaz-Ortíz, J. A., Pérez-Cruz, B., Alarcón-Romero, M. A., Chávez-Almazán, L. A., García-Barbosa, J. L., et al. (2011). A multi-species dinoflagellate bloom and shellfish toxicity in Costa Grande, Guerrero, Mexico (December, 2010). *CICIMAR Oceánides* 26, 67–71.

Gárate-Lizárraga, I., and González-Armas, R. (2011). Occurrence of *Pyrodinium bahamense* var. *compressum* along the southern coast of the Baja California Peninsula. *Mar. Poll. Bull.* 62, 626–630. doi: 10.1016/j.marpolbul.2011.01.009

Gárate-Lizárraga, I., Pérez-Cruz, B., Díaz-Ortíz, J., Alarcón-Tacuba, M., Chávez-Almazán, L., Alarcón-Romero, M., et al. (2012). Toxicity and paralytic toxin profile in *Pyrodinium bahamense* var. *compressum* and violet oyster in Bahía de Acapulco. *Harmful Algae News* 45, 2–3.

Gárate-Lizárraga, I., Pérez-Cruz, B., Díaz-Ortiz, J. A., Alarcón-Tacuba, M. A., Alarcón-Romero, M. A., Chávez-Almazán, L. A., et al. (2013). Blooms of

Pyrodinium bahamense var. *compressum* and rock oyster toxicity in Costa Chica, Guerrero, Mexico. *CICIMAR Océanides* 28, 37–42.

Gárate-Lizárraga, I., Pérez-Cruz, B., Díaz-Ortíz, J. A., and Band-Schmidt, C. J. (2008). Microalgas y biotoxinas marinas en las costas mexicanas. *Conversus* 9, 22–26.

Gárate-Lizárraga, I., Verdugo-Díaz, G., and Okolodkov, Y. B. (2016). "Florecimientos algales nocivos en la costa occidental de Baja California Sur," in *Florecimientos Algales Nocivos en México*, eds E. García-Mendoza, S. I. Quijano-Scheggia, A. Olivos-Ortíz, and E. J. Núñez-Vázquez (Ensenada: CICESE), 44–59.

García, C., del Carmen Bravo, M., Lagos, M., and Lagos, N. (2004). Paralytic shellfish poisoning: post-mortem analysis of tissue and body fluid samples from human victims in the Patagonia fjords. *Toxicon* 43, 149–158. doi: 10.1016/j.toxicon.2003.11.018

García, C., Pérez, F., Contreras, C., Figueroa, D., Barriga, A., López-Rivera, A., et al. (2015). Saxitoxins and okadaic acid group: accumulation and distribution in invertebrate marine vectors from Southern Chile. *Food Addit. Contam. A*. 32, 984–1002. doi: 10.1080/19440049.2015.1028107

García-Hansen, I., Cortés-Altamirano, R., and Sierra-Beltrán, A. (2004). La marea roja causada por el dinoflagelado *Alexandrium tamarense* en la costa Pacífica colombiana (2001). *Rev. Biol. Trop.* 52(Suppl. 1): 59–68.

García-Lagunas, N., Romero-Geraldo, R., and Hernández-Saavedra, N. Y. (2013). Genomics study of the exposure effect of *Gymnodinium catenatum*, a paralyzing toxin producer, on *Crassostrea gigas* defense system and detoxification genes. *PLoS ONE* 8:e72323. doi: 10.1371/journal.pone.0072323

García-Mendoza, E., Medina, J., Rivas, D., Ruiz, M. C., Bustillos-Guzmán, J., Núñez-Vázquez, E. J., et al. (2016). "Paralytic shellfish toxins cause seabirds and marine mammals massive mortalities in the upper gulf of California," in *17th International Conference on Harmful Algae Brazil, 9–14 October,* (Florianópolis), 83.

Garrido, C., Frangópulos M., and Varela, D. (2012). Efecto de diferentes proporciones de nitrógeno/fósforo en el crecimiento y toxicidad de *Alexandrium catenella* (Dinoflagellata). *An. Inst. Patagon.* 40, 113–123. doi: 10.4067/S0718-686X2012000200010

Gayoso, A. M. (2001). Observations on *Alexandrium tamarense* (Lebour) Balech and other dinoflagellate populations in golfo Nuevo, Patagonia (Argentina). *J. Plank Res.* 23, 463–468. doi: 10.1093/plankt/23.5.463

Gayoso, A. M., and Fulco, V. K. (2006). Occurrence patterns of *Alexandrium tamarense* (Lebour) Balech populations in the Golfo Nuevo (Patagonia, Argentina), with observations on ventral pore occurrence in natural and cultured cells. *Harmful Algae.* 5, 233–241. doi: 10.1016/j.hal.2004.12.010

Glibert, P. M., Landsberg, J. H., Evans, J. J., Al-Sarawi, M. A., Faraj, M., Al-Jarallah, M. A., et al. (2002). A fish kill of massive proportion in Kuwait Bay, Arabian Gulf, 2001: the roles of bacterial disease, harmful algae, and eutrophication. *Harmful Algae* 1, 215–231. doi: 10.1016/S1568-9883(02)00013-6

Gómez-Aguirre, S. (1998). First record of *Pyrodinium bahamense* (Dinoflagellata) in brackish waters of the Mexican Caribbean coast. *An. Inst. Biol.* 69, 121–123.

Gómez-Villarreal, M. C., Martínez-Gaxiola, M. D., and Peña-Manjarrez, J. L. (2008). Proliferaciones algales 2000-2001 en Bahía de Banderas, México según el sensor SeaWiFS. *Rev. Biol. Trop.* 56, 1653–1664.

González-Chan, R. B., Hernández-Silva, L., Navarro-Ornelas, J. R., and Blanco Padilla, M. A. (2007). "Proliferación algal nociva en las Bahías del puerto de Manzanillo, Colima (marzo-mayo 2007)," in *Recursos y Medio Ambiente: Memorias del XIV Congreso Nacional de Ciencia y Tecnología, 29–31 october 2007* (Nuevo Vallarta)

Gordon, D., Chen, R., and Chung, S.-H. (2013). Computational methods of studying the binding of toxins from venomous animals to biological ion channels: theory and applications. *Physiol. Rev.* 93, 767–802. doi: 10.1152/physrev.00035.2012

Graham, H. W. (1943). *Gymnodinium catenatum*, a new dinoflagellate from the Gulf of California. *T. Am. Microsc. Soc.* 62, 259–261. doi: 10.2307/3223028

Guinder, V. A., Tillmann, U., Krock, B., Delgado, A., Krohn, T., Garzón Cardona, J., et al. (2018). Plankton multiproxy analyses in the Northern Patagonian Shelf, Argentina: community structure, phycotoxins and characterization of *Alexandrium* strains. *Front. Mar. Sci.* 5:394. doi: 10.3389/fmars.2018.00394

Guzmán, L., Campodónico, I., and Hermosilla, J. (1975). Estudios sobre un florecimiento tóxico causado por *Gonyaulax catenella* en Magallanes. III. Fitoplancton asociado. *An. Inst. Patagonia* 6, 173–183.

Guzmán, L., Pacheco, H., Pizarro, G., and Alarcón, C. (2002). "*Alexandrium catenella* y veneno paralizante de los mariscos en Chile," in *Floraciones Algales Nocivas en el Cono sur Americano*, eds E. A. Sar, M. E. Ferrario, B. Reguera (Madrid: Instituto Español de Oceanografía), 235–255.

Guzmán, L., Vivanco, X., Vidal, G., Pizarro, G., Hernández, C., Tocornal, M. A., et al. (2010). "Spatial and temporal variability of *Alexandrium catenella* and PSP in southern Chile (438–558S) (May 2006–July 2010)," in *Proceedings 14th International Conference on Harmful Algae* Pagou, eds P. Hallegraeff and G. Crete (Greece), 69–71.

Hallegraeff, G. M. (1993). A review of harmful algal blooms and their apparent global increase. *Phycologia* 32, 79–99. doi: 10.2216/i0031-8884-32-2-79.1

Hallegraeff, G. M. (1995). "Harmful algal blooms: a global overview," in *Manual on Harmful Marine Microalgae,* 2nd Edition, eds G. M. Hallegraeff, D. M. Anderson, A. D. Cembella (Paris: UNESCO), 25–50.

Hallegraeff, G. M., Blackburn, S., Doblin, M., and Bolch, C. (2012). Global toxicology, ecophysiology and population relationships of the chainforming PST dinoflagellate *Gymnodinium catenatum*. *Harmful Algae* 14, 130–143. doi: 10.1016/j.hal.2011.10.018

Halperin, I., Ma, B., Wolfson, H., and Nussinov, R. (2002). Principles of docking: an overview of search algorithms and a guide to scoring functions. *Proteins* 47, 409–443. doi: 10.1002/prot.10115

Häussermann, V., Gutstein, C. S., Bedington, M., Cassis, D., Olavarria, C., Dale, A. C., et al. (2017). Largest baleen whale mass mortality during strong El Niño event is likely related to harmful toxic algal bloom. *PeerJ* 5:e3123. doi: 10.7717/peerj.3123

Heileman, L., and Siung-Chang, A. (1990). An analysis of fish kills in coastal and inland waters of Trinidad and Tobago, West Indies, 1976-1990. *Carib. Mar. Stud.* 1, 126–136.

Hernández, C., Díaz, P. A., Molinet, C., and Seguel, M. (2016). Exceptional climate anomalies and northwards expansion of Paralytic Shellfish Poisoning outbreaks in Southern Chile. *Harmful Algae News* 54, 1–2.

Hernández-Becerril, D. U., Alonso-Rodríguez, R., Álvarez-Góngora, C., Baron-Campis, S. A., Ceballos-Corona, G., Herrera-Silveira, J., et al. (2007). Toxic and harmful marine phytoplankton and microalgae (HABs) in Mexican Coasts. *J. Environ. Sci. Heal. A*. 42, 1349–1363. doi: 10.1080/10934520701480219

Hernández-Becerril, D. U., Lau, W. L. S., Hii, K. S., Leaw, C. P., Varona-Cordero, F., and Lim, P. T. (2018). Abundance and distribution of the potentially toxic thecate dinoflagellate *Alexandrium tamiyavanichii* (Dinophyceae) in the Central Mexican Pacific, using the quantitative PCR method. *Front. Mar. Sci.* 5:366. doi: 10.3389/fmars.2018.00366

Hernández-Sandoval, F. E., López-Cortés, D. J., Band-Schmidt, C. J., Gárate-Lizárraga, I., Núñez-Vázquez, E. J., and Bustillos-Guzmán, J. J. (2009). Toxinas paralizantes en moluscos bivalvos durante una proliferación de *Gymnodinium catenatum* Graham en la Bahía de La Paz, México. *Hidrobiologica* 19, 245–256.

Hold, G. L., Smith, E. A., Rappë, M. S., Maas, E. W., Moore, E. R., Stroempl, C., et al. (2001). Characterisation of bacterial communities associated with toxic and non-toxic dinoflagellates: *Alexandrium* spp. and *Scrippsiella trochoidea*. *FEMS Microbiol. Ecol.* 37, 161–173. doi: 10.1111/j.1574-6941.2001.tb00864.x

IPCS (1984). "Environmental Health Criteria 37. Aquatic (Marine and Freshwater) Biotoxins," in *International Programme on Chemical Safety, World Health Organization* (Geneva).

Jedlicki, A., Fernández, G., Astorga, M., Oyarzún, P., Toro, J. E., Navarro, J. M., et al. (2012). Molecular detection and species identification of *Alexandrium* (Dinophyceae) causing harmful algal blooms along the Chilean coastline. *AoB Plants* 2012:pls033. doi: 10.1093/aobpla/pls033

Kodama, M., Ogata, T., Sakamoto, S., Sato, S., Honda, T., and Miwatani, T. (1990). Production of paralytic shellfish toxins by a bacterium *Moraxella* sp. isolated from *Protogonyaulax tamarensis*. *Toxicon* 28, 707–714. doi: 10.1016/0041-0101(90)90259-A

Kremp, A., Tahvanainen, P., Litaker, W., Krock, B., Suikkanen, S., Leaw, C. P., et al. (2014). Phylogenetic relationships, morphological variation, and toxin patterns in the *Alexandrium ostenfeldii* (Dinophyceae) complex: implications for species boundaries and identities. *J. Phycol.* 50, 81–100. doi: 10.1111/jpy.12134

Krock, B., Borel, C. M., Barrera, F., Tillmann, U., Fabro, E., Almandoz, G. O., et al. (2015). Analysis of the hydrographic conditions and cyst beds in the San Jorge Gulf, Argentina, that favor dinoflagellate population development including toxigenic species and their toxins. *J. Mar. Syst.* 148, 86–100. doi: 10.1016/j.jmarsys.2015.01.006

Krock, B., Seguel, C. G., and Cembella, A. D. (2007). Toxin profile of *Alexandrium catenella* from the Chilean coast as determined by liquid chromatography with

fluorescence detection and liquid chromatography coupled with tandem mass spectrometry. *Harmful Algae* 6, 734–744. doi: 10.1016/j.hal.2007.02.005

La Barbera-Sánchez, A., Soler, F. J., Rojas de Astudillo, L., and Chang-Yen, I. (2004). Paralytic shellfish poisoning (PSP) in Margarita Island, Venezuela. *Rev. Biol. Trop*. 52(Suppl. 1), 89–98.

Lagos, N. (1998). Microalgal blooms: a global issue with negative impact in Chile. *Biol. Res*. 31, 375–386.

Lagos, N. (2003). Paralytic shellfish poisoning phycotoxins: occurrence in South America. *Comm. Toxicol*. 9, 1–19. doi: 10.1080/08865140302429

Lagos, N., Compagnon, D., Seguel, M., and Oshima, Y. (1996). "Paralytic shellfish toxin composition: a quantitative analysis in Chilean mussels and dinoflagellate," in *Harmful and Toxic Algal Blooms*, eds T. Yasumoto, T. Oshima, and Y. Fukuyo (Paris: IOC-UNESCO), 121–124.

Landsberg, J. H., Hall, S., Johannessen, J. N., White, K. D., Conrad, S. M., Abbott, J. P., et al. (2006). Saxitoxin puffer fish poisoning in the United States, with the first report of *Pyrodinium bahamense* as the putative toxin source. *Environ. Health Persp*. 114:1502. doi: 10.1289/ehp.8998

Leal, S., Delgado, G., and Nodas, F. (2001). Distribución y abundancia del fitoplancton en un área de la zona nororiental de Cuba. *Rev. Invest. Mar*. 23, 45–51.

Li, R. A., Ennis, I. L., French, R. J., Dudley, S. C., Tomaselli, G. F., and Marbán, E. (2001). Clockwise domain arrangement of the sodium channel revealed by μ-conotoxin (GIIIA) docking orientation. *J. Biol. Chem*. 276, 11072–11077. doi: 10.1074/jbc.M010862200

Licea, S., Navarrete, A., Castañeda, V., and Bustillos-Guzmán, J. J. (2012). "Monitoring program for harmful algal blooms in Salvadoran waters: report of *Pyrodinium bahamense* from November 2009 to June 2010," in *Proceedings of the 14th International Conference on Harmful Algae. International Society for the Study of Harmful Algae and Intergovernmental Oceanographic Commission of UNESCO 2013. ISSHA*, eds K. A. Pagou and G. M. Hallegraeff (Hersonissos-Crete: IOC-UNESCO).

Licea, S., Navarrete, A., Rodríguez, R., Bustillos-Guzmán, J., Martínez, B., and Ramírez, C. (2008). "Monitoring a bloom of *Pyrodinium bahamense* var. *compressum* in El Salvador and the southern coast of Mexico (November 2005–March 2006)," in *Proceedings 12th International Conference on Harmful Algae*, eds Ø. D. Moestrup, G. Doucette, H. Enevoldsen, A. Godhe, G. Hallegraeff and B. Luckas (Copenhague: IOC-UNESCO), 219–220.

Licea, S., Zamudio, M. E., Cortés-Altamirano, R., Luna, R., and Soto, P. J. (2013). "Distribution of known or presumed toxic dinoflagellates in the southern Gulf of Mexico, 1979-2008," in *Biological and Geological Perspectives of Dinoflagellates*, eds J. M. Lewis, F. Marret, L. R. Bradley (London: The Micropalaeontological Society), 155–160.

Licea, S., Zamudio, M. E., Luna, R., and Soto, J. (2004). Free-living dinoflagellates in the southern Gulf of Mexico: report of data (1979–2002). *Phycol. Res.* 52, 419–428. doi: 10.1111/j.1440-1835.2004.tb00351.x

Licea-Durán, S., Gómez-Aguirre, S., Cortés-Altamirano, R., and Gómez, S. (1999). "Notas sobre algunos florecimientos algales y la presencia de especies tóxicas en cinco localidades del Pacífico Mexicano (1996-1999)," in *VIII Congreso Latinoamericano Sobre Ciencias del Mar, octubre* 1999, eds A. E. Tresierra-Aguilar and Z. G. Culquichicón-Malpica (Trujillo), 335–337.

Limoges, A., de Vernal, A., and Ruiz-Fernández, A. C. (2015). Investigating the impact of land use and the potential for harmful algal blooms in a tropical lagoon of the Gulf of Mexico. *Estuar. Coast Shelf. S*. 167, 549–559. doi: 10.1016/j.ecss.2015.11.005

Llewellyn, L., Negri, A., and Robertson, A. (2006). Paralytic shellfish toxins in tropical oceans. *Toxin. Rev*. 25, 159–196. doi: 10.1080/15569540600599217

Llewellyn, L. E. (2006). Saxitoxin, a toxic marine natural product that targets a multitude of receptors. *Nat. Prod. Rep*. 23, 200–222. doi: 10.1039/b501296c

Maas, E. W., Latter, R. M., Thiele, J., Waite, A. M., and Brooks, H. J. (2007). Effect of multiple antibiotic treatments on a paralytic shellfish toxin-producing culture of the dinoflagellate *Alexandrium minutum*. *Aquat. Microb. Ecol*. 48, 255–260. doi: 10.3354/ame048255

MacKenzie, L., de Salas, M., Adamson, J., and Beuzenberg, V. (2004). The dinoflagellate genus *Alexandrium* (Halim) in New Zealand coastal waters: comparative morphology, toxicity and molecular genetics. *Harmful Algae* 3, 71–92. doi: 10.1016/j.hal.2003.09.001

Mafra-Junior, L. L., Fernandes, L. F., and Proença, L. A. O. (2006). Harmful algae and toxis in Paranaguá Bay, Brazil: bases for monitoring. *Braz. J. Oceanogr*. 54, 107–121. doi: 10.1590/S1679-87592006000200002

Manrique, F. A., and Molina, R. E. (1997). Presencia de mareas rojas en la Bahía de Bacochibampo, Guaymas, Sonora, México. *Hidrobiológica* 7, 81–84.

Mardones, J., Clément, A., Rojas, X., and Aparicio, C. (2010). *Alexandrium catenella* during 2009 in Chilean waters, and recent expansion to coastal ocean. *Harmful Algae News* 41, 8–9.

Mardones, J. I., Bolch, C., Guzmán, L., Paredes, J., Varela, D., and Hallegraeff, G. M. (2016). Role of resting cysts in Chilean *Alexandrium catenella* dinoflagellate blooms revisited. *Harmful Algae* 55, 238–249. doi: 10.1016/j.hal.2016.03.020

Mardones, J. I., Dorantes-Aranda, J. J., Nichols, P. N., and Hallegraeff, G. M. (2015). Fish gill damage by the dinoflagellate *Alexandrium catenella* from Chilean fjords: synergistic action of ROS and PUFA. *Harmful Algae* 49, 40–49. doi: 10.1016/j.hal.2015.09.001

Martínez-López, A., Ulloa-Pérez, A. E., and Escobedo-Urías, D. C. (2007). First record of vegetative cells of *Pyrodinium bahamense* (Gonyaulacales: Goniodomataceae) in the Gulf of California. *Pac. Sci*. 61, 289–293. doi: 10.2984/1534-6188(2007)61[289:FROVCO]2.0.CO;2

Mata, L., Abarca, G., Marranghello, L., and Víquez, R. (1990). Paralytic shellfish poisoning by *Spondylus calcifer* contaminated with *Pyrodinium bahamense*, Costa Rica, 1989-1990. *Rev. Biol. Trop*. 38, 129–136.

McLeod, C., Dowsett, N., Hallegraeff, G., Harwood, D. T., Hay, B., Ibbott, S., et al. (2017). Accumulation and depuration of paralytic shellfish toxins by Australian abalone *Haliotis rubra*: Conclusive association with *Gymnodinium catenatum* dinoflagellate blooms. *Food Control*. 73, 971–980. doi: 10.1016/j.foodcont.2016.10.012

Meave del Castillo, E., Rodríguez, S., and Vargas, M. (2006). "Blooms of *Pyrodinium bahamense* var. compressa along the Central America Pacific coast and south of Mexico," in *Proceedings 12th International Conference of Harmful Algae*, eds Ø. D. Moestrup, G. Doucette, H. Enevoldsen, A. Godhe, G. Hallegraeff, and B. Luckas (Copenhaguen: IOC–UNESCO), 239.

Medina-Elizalde, J., García-Mendoza, E., Turner, A. D., Sánchez-Bravo, Y. A., and Murillo-Martínez, R. (2018). Transformation and depuration of paralytic shellfish toxins in the geoduck clam *Panopea globosa* from the northern Gulf of California. *Front. Mar. Sci*. 5:335. doi: 10.3389/fmars.2018.00335

Medlin, L. K., Lange, M., Wellbrock, U., Donner, G., Elbrächter, M., Hummert, C., et al. (1998). Sequence comparisons link toxic European isolates of *Alexandrium tamarense* from the Orkney Islands to toxic North American stocks. *Eur. J. Protistol*. 34, 329–335. doi: 10.1016/S0932-4739(98)80060-6

Mee, L. D., Espinosa, M., and Díaz, G. (1986). Paralytic shellfish poisoning with a *Gymnodinium catenatum* red tide on the Pacific coast of Mexico. *Mar. Environ. Res*. 19, 77–92. doi: 10.1016/0141-1136(86)90040-1

Méndez, S., Anderson, D. M., and Kulis, D. M. (2001). "PSP toxin production of Uruguayan isolates of *Gymnodinium catenatum* and *Alexandrium tamarense*," in *Harmful Algal Blooms*, eds G. M. Hallegraeff, S. Blackburn, R. Lewis, C. Bolch (Paris: IOC-UNESCO), 352–355.

Méndez, S., Severov, D., Ferrari, G., and Mesones, C. (1996). "Early spring *Alexandrium tamarense* toxic blooms in Uruguayan waters," in *Harmful and Toxic Algal Blooms*, eds T. Yasumoto, Y. Oshima, and Y. Fukuyo (Paris: IOC-UNESCO), 113–116.

Méndez, S. M. (2006). "Impacto de las floraciones algales nocivas en Uruguay: origen, dispersión, monitoreo, control y mitigación," in *Bases Para la Conservación y el Manejo de la Costa Uruguaya*. eds. R. Menafra, F. Scarabino and D. Conde, 57–69.

Mendoza-Flores, A., Leyva-Valencia, I., Band-Schmidt, C. J., Galindo-Sánchez, C. E., and Bustillos-Guzmán, J. J. (2018). Identification of the Gene *sxtA* (domains *sxtA1* and *sxtA4*) in Mexican strains of *Gymnodinium catenatum* (Dinophyceae) and their evolution. *Front. Mar. Sci.* 5:289. doi: 10.3389/fmars.2018.00289

Menezes, M., Branco, S., Miotto, M. C., and Alves-de-Souza, C. (2018). The genus *Alexandrium* (Dinophyceae, Dinophyta) in Brazilian coastal waters. *Front. Mar. Sci*. 5:421. doi: 10.3389/fmars.2018.00421

Menezes, M., Proença, L. A. O., Branco, S., and Schramm, M. A. (2007). Bloom of *Alexandrium minutum* Halim on Rio de Janeiro coast: occurrence and toxicity. *Harmful Algal News*. 34, 7–9.

Menezes, M., Varela, D., de Oliveira Proença, L. A., da Silva Tamanaha, M., and Paredes, J. (2010). Identification of the toxic alga *Alexandrium tamiyavanichi* (Dinophyceae) from northeastern Brazil: a combined morphological and rDNA sequence (partial LSU and ITS) approach. *J. Phycol*. 46, 1239–1251. doi: 10.1111/j.1529-8817.2010.00918.x

Mertens, K. N., Wolny, J., Carbonell-Moore, C., Bogus, K., Ellegaard, M., Limoges, A., et al. (2015). Taxonomic re-examination of the toxic armored dinoflagellate *Pyrodinium bahamense* Plate 1906: can morphology or LSU sequencing separate *P. bahamense* var. *compressum* from var. *bahamense*? *Harmful Algae* 41, 1–24. doi: 10.1016/j.hal.2014.09.010

Molinet, C., Lafon, A., Lembeye, G., and Moreno, C. A. (2003). Patrones de distribución espacial y temporal de floraciones de *Alexandrium catenella* (Whedon & Kofoid) Balech 1985, en aguas interiores de la Patagonia noroccidental de Chile. *Rev. Chil. Hist. Nat*. 76, 681–698. doi: 10.4067/S0716-078X2003000400011

Montebruno, D. (1993). Paralytic shellfish poisoning in Chile. *Med. Sci. Law*. 33, 243–246. doi: 10.1177/002580249303300310

Montes, M. R., Rojas, X., Artacho, P., Tello, A., and Quiñones, R. A. (2018). Quantifying harmful algal bloom thresholds for farmed salmon in southern Chile. *Harmful Algae* 77, 55–65. doi: 10.1016/j.hal.2018.05.004

Montoya, N., Akselman, R., Carignan, M., and Carreto, J. (2006). Pigment profile and toxin composition during a red tide of *Gymnodinium catenatum* Graham and *Myrionecta rubra* (Lohman) Jankowski in coastal waters off Mar del Plata, Argentina. *Afr. J. Mar. Sci*. 28, 199–202. doi: 10.2989/18142320609504147

Montoya, N. G., and Carreto, J. I. (2007). *Informe Sobre Mortandad de Aves Marinas Ocurrida en las Costas de Chubut (Noviembre de 2006), Asociada a la Presencia de Toxinas Paralizantes de Moluscos*. Informe Técnico INIDEP No. 34/2017, Mar del Plata: INIDEP.

Montoya, N. G., Fulco, V. K., Carignan, M. O., and Carreto, J. I. (2010). Toxin variability in cultured and natural populations of *Alexandrium tamarense* from southern South America–Evidences of diversity and environmental regulation. *Toxicon* 56, 1408–1418. doi: 10.1016/j.toxicon.2010.08.006

Montoya, N. G., Reyero, M. I., Akselman, R., Franco, J. M., and Carreto, J. J. (1998). "Paralytic shellfish toxins in the anchovy *Engraulis anchoita* from the Argentinian coast," in *Harmful Algae, Proceedings VIII International Conference on Harmful Algae (June 1999, Vigo, Spain)*, eds B. Reguera, J. Blanco, M. Fernández, and T. Wyatt (Vigo: Xunta de Galicia, IOC-UNESCO), 72–73.

Morales-Blake, A., Hernández-Becerril, D. U., and Cavazos-Guerra, C. (2000). "Registros de mareas rojas en las Bahías de Manzanillo, Colima, México," in *Estudios Sobre Plancton en Mexico y el Caribe*, eds E. Ríos-Jara, E. Juárez-Carrillo, M. Pérez-Peña, E. López-Linares, E. G. Robles-Jarero, D. U. Hernández-Becerril, and M. Silva-Briano (Guadalajara: Laboratorio de Ecosistemas Marinos y Acuicultura, Departamento de Ecología, Centro Universitario de Ciencias Biológicas y Agropecuaria, Universidad de Guadalajara), 81–82.

Moreira-González, A. (2009). First record of *Gymnodinium* cf. *catenatum* and other potentially toxic plankton in southern Cuba. *Harmful Algae News* 40, 14–15.

Moreira-González, A., Betancourt, C., Toledo, L., Barcia, S., and Comas, A. (2013). Notas acerca del fitoplancton de la Laguna Guanaroca, Cienfuegos, Cuba. *Rev. Invest. Mar*. 33, 39–45.

Morey-Gaines, G. (1982). *Gymnodinium catenatum* Graham (Dinophyceae): morphology and affinities with armoured forms. *Phycologia* 21, 154–163. doi: 10.2216/i0031-8884-21-2-154.1

Morquecho, L. (2008). Morphology of *Pyrodinium bahamense* plate (Dinoflagellata) near Isla San José, Gulf of California, Mexico. *Harmful Algae* 7, 664–670. doi: 10.1016/j.hal.2008.02.003

Morquecho, L., and Lechuga-Devéze, C. H. (2004). Seasonal occurrence of planktonic dinoflagellates and cyst production in relationship to environmental variables in subtropical Bahía Concepción, Gulf of California. *Bot. Mar*. 47, 313–322. doi: 10.1515/BOT.2004.037

Muciño-Márquez, R. E. (2010). *Variación Estacional de la Comunidad Fitoplanctónica en Granjas Atuneras en la Bahía de La Paz, Baja California Sur*. Master thesis, Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas, 122.

Navarro, J., Munoz, M., and Contreras, A. (2006). Temperature as a factor regulating growth and toxin content in the dinoflagellate *Alexandrium catenella*. *Harmful Algae* 5, 762–769. doi: 10.1016/j.hal.2006.04.001

Negri, A., Bolch, C., Blackburn, S., Dickman, M., Llewellyn, L., and Méndez, S. (2001). "Paralytic shellfish toxins in *Gymnodinium catenatum* strains from six countries," in *Proceedings 9th International Conference-Harmful Algal Blooms 2000*, eds G. M. Hallegraeff, S. I. Blackburn, C. J. Bolch, and R. J. Lewis (Paris: IOC-UNESCO), 210–213.

Negri, A., Llewellyn, L., Doyle, J., Webster, N., Frampton, D., and Blackburn, S. (2003a). Paralytic shellfish toxins are restricted to few species among Australia's taxonomic diversity of cultured microalgae. *J. Phycol*. 39, 663–667. doi: 10.1046/j.1529-8817.2003.02131.x

Negri, A., Stirling, D., Quilliam, M., Blackburn, S., Bolch, C., Burton, I., et al. (2003b). Three novel hydroxybenzoate saxitoxin analogues isolated from the dinoflagellate *Gymnodinium catenatum*. *Chem. Res. Toxicol*. 16, 1029–1033. doi: 10.1021/tx034037j

Negri, A. P., Bolch, C. J., Geier, S., Green, D. H., Park, T.-G., and Blackburn, S. I. (2007). Widespread presence of hydrophobic paralytic shellfish toxins in *Gymnodinium catenatum*. *Harmful Algae* 6, 774–780. doi: 10.1016/j.hal.2007.04.001

Núñez-Vázquez, E. J., Band-Schmidt, C. J., Hernández-Sandoval, F. E., Bustillos-Guzmán, J. J., López-Cortés, D. J., Medina-Elizalde, J., et al. (2016). "Impactos de los FAN en la salud pública y animal (silvestres y de cultivo) en el Golfo de California," in *Florecimientos Algales Nocivos en México* (Ensenada: CICESE), 196–212.

Núñez-Vázquez, E. J., Bustillos-Guzmán, J. J., Ramírez-Camarena, C., and Hernández-Sandoval, F. (2007a). "Perfiles cromatográficos de toxinas paralizantes en moluscos bivalvos asociados a *Pyrodinium bahamense* var. *compressum* en el Pacífico Sur Mexicano," in *Proceedings of II Taller sobre Florecimientos Algales Nocivos (CICESE-CETMAR)* (Ensenada), 18.

Núñez-Vázquez, E. J., Cordero-Tapia, A., and Arnaud, G. (2007b). "Origen e impacto de las Biotoxinas Marinas en la Salud de Tortugas Marinas Salud Pública," in *1er Encuentro Internacional de Medicina de la Conservación, 18-21 July*, (Vitoria).

Núñez-Vázquez, E. J., Gárate-Lizárraga, I., Band-Schmidt, C. J., Cordero-Tapia, A., López-Cortés, D. J., Sandoval, F. E. H., et al. (2011). Impact of harmful algal blooms on wild and cultured animals in the Gulf of California. *J. Environ. Biol*. 32, 413–423.

Ochoa, J. L., Sierra-Beltrán, A., Alonso-Colmenares, G., Barrada-Sánchez, H., Cruz-Villacorta, A., Núñez-Vázquez, E., et al. (1998). "Biotoxins in the Pacific Coast of Mexico," in *Mycotoxins and Phycotoxins Developments in Chemistry, Toxicology and Food Safety, International Union Purity Analytical Chemistry (IUPAC). IX IUPAC International Symposium on Mycotoxins and Phycotoxins*, eds M. Miraglia, H. Van Egmond, C. Brera and J. Gilbert (Rome: Alaken, Inc. Fort Collins Co. U.S.A), 441–448.

Odebrecht, C., Azevedo, S. M. F. O., Garcia, V. M. T., Huszar, V. L. M., Magalhaes, V. F., Menezes, M., et al. (2002). "Floraciones de microalgas nocivas en Brasil: estado del arte y proyectos en curso," in *Floraciones Algales Nocivas en el Cono Sur Americano*, eds E. A. Sar, M. E. Ferrairo and B. Reguera (Vigo: Instituto Español de Oceanografía, IOC Harmful Algal Bloom Programme), 219–233.

Ogata, T., Kodama, M., and Ishimaru, T. (1987). Toxin production in the dinoflagellate *Protogonyaulax tamarensis*. *Toxicon* 25, 923–928. doi: 10.1016/0041-0101(87)90154-1

Ordás, M. C., Fraga, S., Franco, J. M., Ordás, A., and Figueras, A. (2004). Toxin and molecular analysis of *Gymnodinium catenatum* (Dinophyceae) strains from Galicia (NW Spain) and Andalucia (S Spain). *J. Plankton Res*. 26, 341–349. doi: 10.1093/plankt/fbh037

Orellana-Cepeda, E., Martínez-Romero, E., Muñoz-Cabrera, L., López-Ramírez, P., Cabrera-Mancilla, E., and Ramírez-Camarena, C. (1998). "Toxicity associated with blooms of *Pyrodinium bahamense* var. *compressum* in southwestern Mexico," in *Proceedings of the Harmful algae, VIII International Conference on Harmful Algae*, eds B. Reguera, J. Blanco, M. L. Fernández, and T. Wyatt (Vigo: Xunta de Galicia and IOC-UNESCO), 60.

Orlova, T. Y., Selina, M. S., Lilly, E. L., Kulis, D. M., and Anderson, D. M. (2007). Morphogenetic and toxin composition variability of *Alexandrium tamarense* (Dinophyceae) from the east coast of Russia. *Phycologia* 46, 534–548. doi: 10.2216/06-17.1

Orr, R. J., Stüken, A., Murray, S. A., and Jakobsen, K. S. (2013). Evolutionary acquisition and loss of saxitoxin biosynthesis in dinoflagellates: the second "core" gene-*sxtG*. *Appl. Environ. Microbiol.* 03279–03212. doi: 10.1128/AEM.03279-12

Oshima, Y. (1995). "Chemical and enzymatic transformation of paralytic shellfish toxins in marine organisms," in *Harmful Algal Blooms*, eds P. Lassus, G. Arzul, P. Gentien and C. Marcaillou (Paris: Technique et documentation-Lavoisier), 475–480.

Osorio-Tafall, B. (1943). El Mar de Cortés y la productividad fitoplanctónica de sus aguas. *An. Esc. Nal. Cienc. Biol.* 3, 73–118.

Oyaneder-Terrazas, J., Contreras, H. R., and García, C. (2017). Prevalence, variability and bioconcentration of saxitoxin-group in different marine species present in the food chain. *Toxins* 9:190. doi: 10.3390/toxins9060190

Palomares-García, R., Bustillos-Guzmán, J., Band-Schmidt, C., López-Cortés, D., and Luckas, B. (2006). Effect of the toxic dinoflagellate *Gymnodinium catenatum* on the grazing, egg production, and hatching success of the copepod *Acartia clausi*. *Cienc. Mar.* 32, 97–109. doi: 10.7773/cm.v32i12.1041

Paredes-Banda, P. E., Cruz-López, R., García-Mendoza, E., Santiago-Morales, I., Ruiz-de la Torre, M. C., Murillo-Martínez, R., et al. (2016). "Estudios de ecofisiología sobre especies fitoplanctónicas nocivas en Baja California," in *Florecimientos Algales Nocivos en México*, eds E. García-Mendoza, S. I. Quijano-Scheggia, A. Olivos-Ortíz and E. J. Núñez-Vázquez (Ensenada: CICESE), 78–91.

Parrilla-Cerrillo, M. C., Vázquez-Castellanos, J. L., Sáldate-Castañeda, O., and Nava-Fernández, L. M. (1993). Brotes de toxiinfecciones alimentarias de origen microbiano y parasitario. *Sal. Pub. Mex.* 35, 456–463.

Pérez-Linares, J., Ochoa, J., and Gago-Martínez, A. (2008). Effect of PSP toxins in white leg shrimp *Litopenaeus vannamei* Boone (1931). *J. Food. Sci.* 73, T69–T73. doi: 10.1111/j.1750-3841.2008.00710.x

Persich, G. R., Kulis, D. M., Lilly, E. L., Anderson, D. M., and García, V. M. (2006). Probable origin and toxin profile of *Alexandrium tamarense* (Lebour) Balech from southern Brazil. *Harmful Algae* 5, 36–44. doi: 10.1016/j.hal.2005.04.002

Pizarro, G., Paz, B., Alarcón, C., Toro, C., Frangópulos, M., Salgado, P., et al. (2018). Winter distribution of toxic, potentially toxic phytoplankton, and shellfish toxins in fjords and channels of the Aysén region, Chile. *Lat. Am. J. Aquat. Res.* 46, 120–139. doi: 10.3856/vol46-issue1-fulltext-13

Plate, L. (1906). Pyrodinium bahamense ng, n. sp., die Leucht-Peridinee des" Feuersees" von Nassau, Bahamas. *Arch. Protistenk.* 7, 411–428.

Poot-Delgado, C. A., Okolodkov, Y. B., Castillo, J. A. A., and Rendón-Von Osten, J. (2015). Annual cycle of phytoplankton with emphasis on potentially harmful species in oyster beds of Términos Lagoon, southeastern Gulf of Mexico. *Rev. Biol. Mar. Oceanog.* 50, 465–477. doi: 10.4067/S0718-19572015000400006

Proença, L. A. O., Tamanaha, M. S., and de Souza, N. P. (2001). The toxic dinoflagellate *Gymnodinium catenatum* Graham in southern Brazilian waters: occurrence, pigments and toxins. *Atlântica* 23, 59–65.

Quijano-Scheggia, S., Olivos-Ortíz, A., Bustillos-Guzmán, J. J., Garcés, E., Gaviño-Rodríguez, J. H., Galicia-Pérez, M. A., et al. (2012). Bloom of *Gymnodinium catenatum* in Bahía Santiago and Bahía Manzanillo, Colima, Mexico. *Rev. Biol. Trop.* 60, 173–186. doi: 10.15517/rbt.v60i1.2750

Quijano-Scheggia, S., Olivos-Ortíz, A., Pérez-Morales, A., Álvarez-García, C., Gaviño-Rodríguez, J. H., and Sosa-Avalos, R. (2016). "Registros de microalgas nocivas o toxicas formadoras de florecimientos algales en la Bahía de Manzanillo, Colima, México," in *Florecimientos Algales Nocivos en México*, eds E. García-Mendoza, S. I. Quijano-Scheggia, A. Olivos-Ortíz, E. J. Núñez-Vázquez (Ensenada: CICESE), 218–227.

Ramírez-Camarena, C., Muñoz-Cabrera, L., Cabrera-Mancilla, E., Castro-Ramos, R., López-Ramírez, P., and Orellana-Cepeda, E. (1996). "Identificación de la marea roja frente a la costa suroeste de México en Oct.-Dic. 1995," in *I Reunión Internacional de Planctología y VIII Reunión Nacional de la Sociedad Mexicana de Planctología* (Pátzcuaro), 47.

Ramírez-Camarena, C., Rojas-Crisóstomo, R., Muñoz-Cabrera, L., Sarniento-Nafate, S., and Juárez-Ruíz, N. O. (2002). "Mortandad de peces e intoxicaciones humanas en la Costa de Chiapas en el 2001," in IX *Congreso Nacional de Ciencia y Tecnología del Mar. Nuevo Vallarta* (Nayarit), 1–2.

Ramírez-Camarena, C. Cortés-Altamirano, R., and Muñoz-Cabrera, L. (1999). Mareas rojas provocadas por el dinoflagelado *Gymnodinium catenatum (Gymnodinales: Gymnodiniaceae)* en la Bahía de Mazatlán, Sin., México, en 1997. *Rev. Biol. Trop.* 47, 77–80.

Rodríguez, D. C., Etzel, R. A., Hall, S., de Porras, E., Velasquez, O. H., Tauxe, R. V., et al. (1990). Lethal paralytic shellfish poisoning in Guatemala. *Am. J. Trop. Med. Hyg.* 42, 267–271. doi: 10.4269/ajtmh.1990.42.267

Rodríguez-Salvador, R., and Meave del Castillo, M. E. (2007). "Monitoreo del dinoflagelado tóxico *Pyrodinium bahamense* var. *compressum* en las costas de Chiapas," in *II Taller sobre Florecimientos Algales Nocivos* (Ensenada), 6.

Rojas-Crisóstomo, R., Ramírez-García, H., Barón-Campis, S., and Ojeda-Salinas, H. (2007). "Presencia de *Pyrodinium bahamense* var. *compressum*, niveles de toxina en moluscos bivalvos e identificación del fitoplancton marino en las costas de La Colorada y La Ventosa, Oaxaca durante 2006," in *Resúmenes del II Taller sobre Florecimientos Algales Nocivos Ensenada, México, Noviembre 2007* (Ensenada: CICESE-CETMAR), 2007.

Rosales-Loessener, F. (1989). "The guatemala experience with red tides and paralytic shellfish poisoning," in *Biology, Epidemiology and Management of Pyrodinium Red Tides, ICLARM Conference Proceedings*, eds G. M. Hallegraeff and J. Maclean (Manila: Fisheries Department, Ministry of Development, Brunei Darussalam, and International Center for Living Aquatic Resources Management), 49–51.

Rosales-Loessener, F., Porras, E. D., and Dix, M. V. (1989). "Toxic shellfish poisoning in Guatemala," in *Red Tides, Biology, Environmental Science and Toxicology*, eds T. Okaichi, D. M. Anderson, T. Nemoto (New York, NY: Elsevier), 113–116.

Saldate-Castañeda, O., Vázquez-Castellanos, J., Galván, J., Sánchez-Anguiano, A., and Nazar, A. (1991). Poisoning from paralytic shellfish toxins in Oaxaca, Mexico. *Sal. Pub. Mex.* 33, 240–247.

Salgado, P., Riobó, P., Rodríguez, F., Franco, J. M., and Bravo, I. (2015). Differences in the toxin profiles of *Alexandrium ostenfeldii* (Dinophyceae) strains isolated from different geographic origins: evidence of paralytic toxin, spirolide, and gymnodimine. *Toxicon* 103, 85–98. doi: 10.1016/j.toxicon.2015.06.015

Sánchez-Cabeza, J. A., Ruiz-Fernandez, A. C., de Vernal, A., and Machain-Castillo, M. L. (2012). Reconstruction of *Pyrodinium* blooms in the tropical East Pacific (Mexico): are they related to ENSO? *Environ. Sci. Technol.* 46, 6830–6834. doi: 10.1021/es204376e

Sánchez-Flores, H. E. (2011). Envenenamiento paralizante severo por consume de moluscos. Reporte de un caso. *Arch. Med. Urgen. Mex.* 3, 30–33.

Sánchez-Suárez, I., and Troncone-Osorio, F. (1994). Diversidad y equitabilidad del fitoplancton del Golfo de Paria (Venezuela, junio 1984). *Acta Cient Venez.* 45, 296–306.

Scholin, C., Hallegraeff, G., and Anderson, D. (1995). Molecular evolution of the *Alexandrium tamarense* 'species complex'(Dinophyceae): dispersal in the North American and West Pacific regions. *Phycologia* 34, 472–485.

Scholin, C. A., Herzog, M., Sogin, M., and Anderson, D. M. (1994). Identification of group and strain specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). II Sequence analyisis of a fragment of the LSU rDNA gene. *J. Phycol.* 30, 999–1011. doi: 10.1111/j.0022-3646.1994.00999.x

Schut, F., de Vries, E. J., Gottschal, J. C., Robertson, B. R., Harder, W., Prins, R. A., et al. (1993). Isolation of typical marine bacteria by dilution culture: growth, maintenance, and characteristics of isolates under laboratory conditions. *Appl. Environ. Microbiol.* 59, 2150–2160.

Seok Jin, O., Matsuyama, Y., Yoon, Y. H., Miyamura, K., Choi, C. G., Yang, H. S., et al. (2010). Comparative analysis of paralytic shellfish toxin content and profile produced by dinoflagellate *Gymnodinium catenatum* isolated from Inokushi Bay, Japan. *J. Fac. Agr. Kyushu* 55, 47–54.

Shen, H., Zhou, Q., Pan, X., Li, Z., Wu, J., and Yan, N. (2017). Structure of a eukaryotic voltage-gated sodium channel at near-atomic resolution. *Science* 355:eaal4326. doi: 10.1126/science.aal4326

Sierra-Beltrán, A., Cruz, A., Núñez-Vázquez, E., Del Villar, L. M., Cerecero, J., and Ochoa, J. L. (1998). An overview of the marine food poisoning in Mexico. *Toxicon* 36, 1493–1502. doi: 10.1016/S0041-0101(98)00139-1

Silva, E. S. (1982). Relationship between dinoflagellates and intracellular bacteria. *Mar. Al. Pharmacol. Sci.* 2, 269–288.

Silva, N. J., Tang, K. W., and Lopes, R. M. (2013). Effects of microalgal exudates and intact cells on subtropical marine zooplankton. *J. Plankton. Res.* 35, 855–865. doi: 10.1093/plankt/fbt026

Siu, G. K., Young, M. L., and Chan, D. (1997). "Environmental and nutritional factors which regulate population dynamics and toxin production in the dinoflagellate *Alexandrium catenella*," in *Asia-Pacific Conference on Science and Management of Coastal Environment Developments in Hydrobiology*, eds Y.-S. Wong and N. F. Y. Tam (Dordrecht: Springer), 117–140.

Sotomayor-Navarro, O., and Domínguez-Cuellar, E. (1993). "Toxic red tide of *Pyrodinium bahamense* var. *compressum*, in the Tehuantepec Gulf, and the Central American Pacific System," in *6th International Conference on Toxic Marine Phytoplankton* (Nantes), 185.

Steidinger, K., Tester, L., and Taylor, F. J. R. (1980). A redescription of *Pyrodinium bahamense* var. *compressa* (Böhm) stat. nov. from Pacific red tides. *Phycologia* 19, 329–334. doi: 10.2216/i0031-8884-19-4-329.1

Su, Z., Sheets, M., Ishida, H., Li, F., and Barry, W. H. (2004). Saxitoxin blocks L-type ICa. *J. Pharmac. Exp. Therap*. 308, 324–329. doi: 10.1124/jpet.103.056564

Suárez-Isla, B., and Guzmán-Méndez, L. (1998). *Floraciones Algales Nocivas. Mareas Rojas y Toxinas Marinas*. Available online at http://www.ifop.cl/marearoja/wp-content/uploads/sites/2/2016/01/8_-FLORACIONES-DE-ALGAS-NOCIVAS-Mareas-Rojas-y-Toxinas-Marinas-Guzman-y-Suarez-1998.pdf

Sunesen, I., Lavigne, A., Goya, A., and Sar, E. A. (2014). Episodios de toxicidad en moluscos de aguas marinas costeras de la Provincia de Buenos Aires (Argentina) asociados a algas toxígenas (marzo de 2008-marzo de 2013). *Bol. Soc. Argent. Bot*. 49, 327–339.

Tillmann, U., Alpermann, T. L., da Purificação, R. C., Krock, B., and Cembella, A. (2009). Intra-population clonal variability in allelochemical potency of the toxigenic dinoflagellate *Alexandrium tamarense*. *Harmful Algae* 8, 759–769. doi: 10.1016/j.hal.2009.03.005

Torres, G. (2000). Mareas rojas durante 1989-1999, en aguas ecuatorianas. *Acta Ocean Pac*. 10, 127–136.

Torres-Chuquimarca, G. M. (2011). *Eventos de Mareas Rojas: Estrategias de Manejo Preventivas en Ecuador*. Master thesis: Universidad de Guayaquil. Available online at http://repositorio.ug.edu.ec/handle/redug/11942

Uribe, J. C., Oyarzún, S., and Latorre, V. (2010). *Alexandrium catenella* (Whedon & Kofoid) Balech, 1985, en aguas magallánicas, Chile. *An. Inst. Patagonia* 38, 103–110.

Uribe, P., and Espejo, R. T. (2003). Effects of associated bacteria on the growth and toxicity of *Alexandrium catenella*. *Appl. Environ. Microbiol*. 69, 659–662. doi: 10.1128/AEM.69.1.659-662.2003

Uribe, P., Fuentes, D., Valdés, J., Shmaryahu, A., Zúñiga, A., Holmes, D., et al. (2008). Preparation and analysis of an expressed sequence tag library from the toxic dinoflagellate *Alexandrium catenella*. *Mar. Biotechnol*. 10, 692–700. doi: 10.1007/s10126-008-9107-8

Usup, G., Ahmad, A., Matsuoka, K., Lim, P. T., and Leaw, C. P. (2012). Biology, ecology and bloom dynamics of the toxic marine dinoflagellate *Pyrodinium bahamense*. *Harmful Algae* 14, 301–312. doi: 10.1016/j.hal.2011.10.026

Vale, P. (2008). Complex profiles of hydrophobic paralytic shellfish poisoning compounds in *Gymnodinium catenatum* identified by liquid chromatography with fluorescence detection and mass spectrometry. *J. Chromatogr. A*. 1195, 85–93. doi: 10.1016/j.chroma.2008.04.073

Vale, P. (2010). New saxitoxin analogues in the marine environment: developments in toxin chemistry, detection and biotransformation during the 2000s. *Phytochem. Rev*. 9, 525–535. doi: 10.1007/s11101-010-9196-7

Varela, D., Paredes, J., Alves-de-Souza, C., Seguel, M., Sfeir, A., and Frangópulos, M. (2012). Intraregional variation among *Alexandrium catenella* (Dinophyceae) strains from southern Chile: morphological, toxicological and genetic diversity. *Harmful Algae* 15, 8–18. doi: 10.1016/j.hal.2011.10.029

Vargas-Montero, M., and Freer, E. (2004). "Paralytic Shellfish poisoning outbreaks in Costa Rica," in *Harmful Algae* 2002, eds. K. A. Steidinger, J. H. Landsberg, C. R. Tomas and G. A. Vargo (St. Petersburg Beach, FL: Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography. IOC-UNESCO), 482–484.

Vasquez, M., Gruttner, C., Gallacher, S., and Moore, E. (2001). Detection and characterization of toxigenic bacteria associated with *Alexandrium catenella* and *Aulacomya ater* contaminated with PSP. *J. Shellfish. Res*. 20, 1245–1249.

Vinuesa, J. H. (1993). "Marea Roja en el Canal de Beagle," in *Contribución Científico* (Ushuaia Tierra del Fuego: Centro Austral de Investigaciones Científicas), 1–34.

Vinuesa, J. H., and Labal, M. L. (1996). Marea roja y Mortalidad masiva en el canal de Beagle. *Nat. Patagón Cienc. Biol*. 4, 167–171.

Wang, J., Salata, J. J., and Bennett, P. B. (2003). Saxitoxin is a gating modifier of HERG K+ channels. *J. Gen. Physiol*. 121, 583–598. doi: 10.1085/jgp.200308812

Warren, G. L., Andrews, C. W., Capelli, A.-M., Clarke, B., LaLonde, J., Lambert, M. H., et al. (2006). A critical assessment of docking programs and scoring functions. *J. Med. Chem*. 49, 5912–5931. doi: 10.1021/jm050362n

Wiese, M., D'Agostino, P. M., Mihali, T. K., Moffitt, M. C., and Neilan, B. A. (2010). Neurotoxic alkaloids: saxitoxin and its analogs. *Mar. Drugs*. 8, 2185–2211. doi: 10.3390/md8072185

Yen, I. C., De Astudillo, L. R., Soler, J. F., and La Barbera-Sánchez, A. (2004). Paralytic shellfish poisoning toxins in green mussels (*Perna viridis*) from the Gulf of Paria, Trinidad. *Toxicon* 44, 743–747. doi: 10.1016/j.toxicon.2004.07.030

Zamudio, M. E., Licea, S., and Luna, R. (2013). "Relative abundance and distribution of unarmoured dinoflagellate species in the South Gulf of Mexico (2005-2010)," in *Biological and Geological Perspectives of Dinoflagellates*, eds J. M. Lewis, F. Marret, L. Bradley (London: The Micropalaeontological Society, Special Publications. Geological Society), 233–238.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Band-Schmidt, Durán-Riveroll, Bustillos-Guzmán, Leyva-Valencia, López-Cortés, Núñez-Vázquez, Hernández-Sandoval and Ramírez-Rodríguez. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Marine Science

BRIEF RESEARCH REPORT
published: 26 February 2019
doi: 10.3389/fmars.2019.00082

Ichthyotoxicity of the Dinoflagellate *Karlodinium veneficum* in Response to Changes in Seawater pH

Marius N. Müller[1], Juan José Dorantes-Aranda[2], Andreas Seger[2], Marina T. Botana[3], Frederico P. Brandini[3] and Gustaaf M. Hallegraeff[2]*

[1] Department of Oceanography, Federal University of Pernambuco, Recife, Brazil, [2] Institute for Marine and Antarctic Studies, Hobart, TAS, Australia, [3] Oceanographic Institute, University of São Paulo, São Paulo, Brazil

The ichthyotoxic dinoflagellate *Karlodinium veneficum* has a worldwide distribution and produces highly potent lytic toxins (karlotoxins) that have been associated with massive fish kill events in coastal environments. The capacity of *K. veneficum* to gain energy from photosynthesis as well as phagotrophy enables cellular maintenance, growth and dispersal under a broad range of environmental conditions. Coastal ecosystems are highly dynamic in light of the prevailing physicochemical conditions, such as seawater carbonate speciation (CO_2, HCO_3^-, and CO_3^{2-}) and pH. Here, we monitored the growth rate and ichthyotoxicity of *K. veneficum* in response to a seawater pH gradient. *K. veneficum* exhibited a significant linear reduction in growth rate with elevated seawater acidity [$pH_{(totalscale)}$ from 8.05 to 7.50]. Ichthyotoxicity was assessed by exposing fish gill cells to *K. veneficum* extracts and subsequent quantification of gill cell viability via resorufin fluorescence. Extracts of *K. veneficum* indicated increased toxicity when derived from elevated pH treatments. The variation in growth rate and toxin production per cell in regard to seawater pH implies that (1) future alteration of seawater carbonate speciation, due to anthropogenic ocean acidification, may negatively influence physiological performance and ecosystem interactions of *K. veneficum* and (2) elevated seawater pH values (>8.0) represent favorable conditions for *K. veneficum* growth and toxicity. This suggests that prey of *K. veneficum* may be exposed to increased karlotoxin concentrations at conditions when nutrients are scarce and seawater pH has been elevated due to high photosynthetic activity from prior autotrophic phytoplankton blooms.

Keywords: Harmful dinoflagellate, ocean acidification, seawater carbonate chemistry, toxin production, *Karlodinium veneficum*, ichthyotoxicity

OPEN ACCESS

Edited by:
Il-Nam Kim,
Incheon National University,
South Korea

Reviewed by:
Ju-Hyoung Kim,
Kunsan National University,
South Korea
Margarita Fernández Tejedor,
Institute of Agrifood Research and Technology (IRTA), Spain

****Correspondence:***
Marius N. Müller
mariusnmuller@gmail.com

Specialty section:
This article was submitted to Marine Biogeochemistry, a section of the journal Frontiers in Marine Science

Received: *22 October 2018*
Accepted: *13 February 2019*
Published: *26 February 2019*

Citation:
Müller MN, Dorantes-Aranda JJ, Seger A, Botana MT, Brandini FP and Hallegraeff GM (2019) Ichthyotoxicity of the Dinoflagellate Karlodinium veneficum in Response to Changes in Seawater pH. Front. Mar. Sci. 6:82. doi: 10.3389/fmars.2019.00082

INTRODUCTION

The accumulation of anthropogenic carbon dioxide in the atmosphere and its concomitant absorption by the ocean surface causes distinct chemical changes, known as ocean acidification (reduction of pH and increase of CO_2). Ocean acidification has been recorded over the past decades at long-term monitoring stations (Dore et al., 2009) and has the potential to affect phytoplankton physiology and community structure (Dutkiewicz et al., 2015). In comparison to the open ocean,

coastal areas are subject to multiple environmental drivers, such as riverine fresh water input, eutrophication, aquaculture fish farming, deep-water upwelling and high biological productivity. These factors result in highly fluctuating pH regimes with seasonal and daily changes of up to 0.6 pH units (Duarte et al., 2013; Melzner et al., 2013; Carstensen et al., 2018). For example, upwelling waters with pH values < 7.7 have been reported for the continental shelfs of California and Chile (Feely et al., 2008; Torres et al., 2011). Less buffered waters of marine aquaculture facilities can even reach acidic pH levels corresponding to pCO_2 values > 10,000 μatm, associated with high respiration rates and organismal densities (Ellis et al., 2017).

The marine dinoflagellate *Karlodinium veneficum* has a global distribution and produces powerful lytic karlotoxins that have been associated with extensive fish kills (Mooney et al., 2010; Place et al., 2012; Adolf et al., 2015; Escobar-Morales and Hernández-Becerril, 2015). Emerging evidence suggests that these toxins are primarily produced to facilitate feeding by prey immobilization (Sheng et al., 2010). Strain specific variability of *K. veneficum* has been demonstrated in regard to trophic growth mode (auto- and mixotrophy), toxin production and DNA content which suggests the existence of different ecotypes (Place et al., 2012). Nutrient availability and other environmental drivers, such as seawater carbonate chemistry, have the potential to alter growth and cellular toxicity of *K. veneficum* (Fu et al., 2010). Here, we present controlled laboratory experiments investigating the autotrophic growth and ichthyotoxicity of one *K. veneficum* strain in response to an extended seawater pH gradient.

MATERIALS AND METHODS

Culture Conditions

Monospecific cultures of *K. veneficum* (strain KVSR01, obtained from the Algae Culture Collection at the Institute for Marine and Antarctic Studies of the University of Tasmania, Australia) were maintained in filter-sterilized (0.2 μm) natural seawater with a salinity of 35, at 20°C under a light:dark cycle of 12:12 h with a photon flux density of 110 μmol photons m^{-2} s^{-1} provided by full spectrum cool white fluorescent tubes. Nutrients for autotrophic growth were added according to the GSe/2 medium recipe (Blackburn et al., 2001) and supplemented with 5 ml l^{-1} of soil extract, yielding in improved growth (Sweeney, 1951; Provasoli et al., 1957). Experimental cultures were kept in exponential growth by regular transfer to fresh media to avoid nutrient limitation.

Experimental Set-Up

Experimental incubations were carried out in duplicates under dilute batch culture conditions in 300 ml sterile polystyrene Falcon® culture flasks with minimal headspace. Carbonate chemistry speciation was adjusted by the addition of calculated amounts of HCl, NaOH, and $NaHCO_3$, resulting in a gradient of pCO_2 from 445 to 1703 μatm (**Table 1**). Exponentially growing cultures were acclimated to the experimental conditions for three generations (5 days). Acclimated cultures were transferred to the experimental treatments to match a starting density of 100 cells ml^{-1} and were allowed to grow exponentially for 5 to 6 generations over the course of the experiment, corresponding to a maximal dissolved inorganic carbon (C_T) consumption of 3%. The low biomass build up during the experiments ensured (1) nutrient replete conditions and (2) minor changes in the seawater carbonate chemistry due to biological activity (see also Riebesell et al., 2011). Samples for C_T and total alkalinity (A_T) were taken at the beginning and the end of the experimental incubations. Samples for cell density and ichthyotoxicity tests were taken at the end of the experiment.

Carbonate Chemistry

The carbonate system was monitored via triplicate C_T and A_T measurements applying the infrared detection method after Goyet and Snover (1993) with an Apollo SciTech DIC-Analyzer (Model AS-C3, Apollo SciTech, Newark, DE, United States) and the potentiometric titration method (Dickson et al., 2003), respectively. Data were corrected to repeated analyses of Certified Reference Materials (CRM, Scripps Institution of Oceanography, La Jolla, CA, United States) following the recommendations for ocean acidification research (Riebesell et al., 2011). Consecutive measurements of the CRM resulted in an average precision of >99.8% for both C_T and A_T. Carbonate system parameters, such as $pH_{(total\ scale)}$, were calculated from temperature, salinity, C_T and A_T using CO2SYS (version 2.1 by E. Lewis and D. W. R. Wallace), with the stoichiometric equilibrium constants for carbonic acid given in Roy et al. (1993).

Cell Density and Growth Rate

Samples for cell density were measured in triplicate directly after sampling using a Coulter Multisizer™ 4 (Beckman Coulter Life Sciences, Indianapolis, IN, United States) equipped with a 100 μm aperture. Prior to each measurement, samples of *K. veneficum* were incubated at 4°C for 10 min in order to reduce cellular metabolism, avoiding undesired swimming motions during analysis. Equipment settings were calibrated using standard latex particles (Beckman Coulter Life Sciences) with a nominal size of 10 μm. The mean cell densities were used to calculate the growth rate, μ ($days^{-1}$) as:

$$\mu = (lnc_1 - lnc_0)/(t_1 - t_0), \quad (1)$$

where c_0 and c_1 are the cell densities at the beginning (t_0) and end of the incubation period (t_1), expressed in days. Estimates of cell densities were associated with a random error of <3%, which was determined by repeated measurements of identical *K. veneficum* culture material ($n = 10$).

Ichthyotoxicity Assessment

Toxin Extraction

The remaining *K. veneficum* cultures were pooled for each treatment (total of 400 ml) and centrifuged at 2000 × *g* for 10 min (Sigma 3–16P). The supernatant was discarded

TABLE 1 | Carbonate chemistry speciation (±1 SD), averaged from the start and end values of the experimental incubations, with the corresponding growth rates (μ) of *Karlodinium veneficum* and the estimated LD_{50} values, representing the lethal dose of *K. veneficum* cells for 50% of gill cell mortality.

Treatment	C_T (μmol kg^{-1})	A_T	pCO_2 (μatm)	pH (total scale)	μ (days^{-1})	LD_{50} (cells ml^{-1})
1	2113 ± 7	2389 ± 59	445 ± 84	8.03 ± 0.08	0.47	31,793
2	2180 ± 12	2396 ± 49	603 ± 91	7.92 ± 0.07	0.46	38,458
3	2273 ± 22	2383 ± 53	1112 ± 170	7.68 ± 0.07	0.45	45,722
4	2320 ± 27	2386 ± 60	1515 ± 274	7.56 ± 0.09	0.43	145,594
5	2342 ± 34	2388 ± 29	1703 ± 83 x	7.51 ± 0.01	0.42	108,757

T = 20°C, salinity = 35, light intensity = 110 μmol photons m^{-2} s^{-1}.

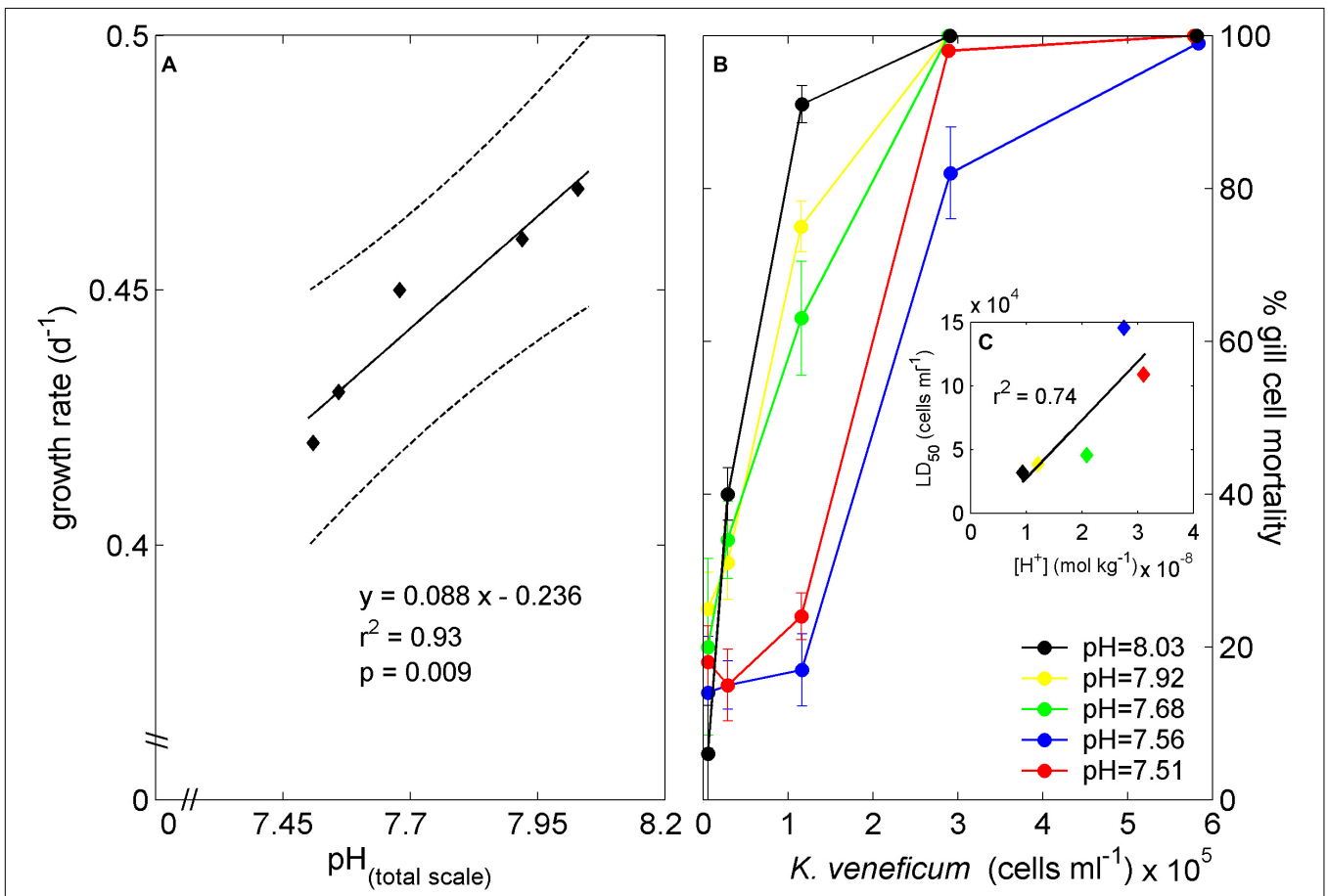


FIGURE 1 | Cellular growth rate response to changes in seawater pH **(A)** and gill cell mortality when exposed to dilution gradients of *K. veneficum* cell extracts with standard deviation derived from repeated fluorescent measurements **(B)**. Black line in **(A)** represents linear regression fit with 95% prediction intervals (dashed line). LD_{50} values (cells ml^{-1}) were estimated from idealized Michaelis-Menten-like fits to the treatment specific gill cell mortalities and plotted against seawater hydrogen concentration **(C)**. Linear regression fit in **(C)** resulted in $LD_{50} = 4.59 \times 10^{12}$ [H$^+$] – 1.86×10^4 ($r^2 = 0.74$, $p = 0.062$).

and cell pellets resuspended in methanol (0.3 – 0.5 ml; based on cell densities of each treatment) to yield a constant final extract concentration equivalent to 5.7×10^6 cells ml^{-1}. Resuspended pellets were sonicated with a probe type sonicator (Measuring and Scientific Equipment Ltd., London, United Kingdom) with tubes maintained in ice for 10 min at an amplitude of 7 μm peak to peak, which describes the longitudinally expansion and contraction of the tip. Samples were kept on ice to counteract potential heating due to prolonged (>1 min) sonication. The applied settings were tested and resulted in a 99% lysis of *K. veneficum* cells which was verified via Coulter Multisizer^{TM} 4 analyses. After sonication the sample was centrifuged at 1400 × *g* for 10 min, the resulting supernatant was collected and stored at −20°C for a maximum of 24 h before testing the toxicity on gill cell lines.

Gill Cell Line Assay

The fish gill cell line RTgill-W1 was maintained and used for the experiments following the protocol described by Dorantes-Aranda et al. (2011). Gill cells grown in L-15 medium (L1518, Sigma) were seeded into 96-well plates (Greiner 655180) at 2×10^5 cells ml^{-1} and allowed to attach for 48 h. Confluence of the adherent gill cell monolayer was confirmed by light microscopy, and L-15 medium was replaced with L-15/ex medium (Schirmer et al., 1997) 12 h before the experimental exposures. *K. veneficum* extracts were diluted to 0.1–10% in L-15/ex medium. The final methanol concentration for all experimental treatments, including the non-toxic control (L-15/ex), was 10%. Immediately prior to exposures, the L-15/ex medium was discarded and 100 μL of each treatment was added to quadruplicate wells, and plates were incubated for 2 h at experimental light conditions. After this period, individual plates were rinsed twice with 100 μL phosphate buffer saline to subsequently receive 100 μL of 5% resazurin in L-15/ex. Plates were incubated for a further 2 h in the dark. The gill cell metabolic reduction of resazurin to fluorescent resorufin was quantified using a microplate reader (Fluostar Optima, BMG Labtech), with excitation and emission wavelengths of 540 and 590 nm, respectively. Fluorescence values were blank corrected (resazurin only, no gill cells) and results were expressed as percentage of mortality compared to the non-toxic control.

RESULTS AND DISCUSSION

The experimental protocol applied to alter the seawater carbonate chemistry mimics the process of ocean acidification and resulted in elevated total dissolved inorganic carbon concentrations at constant total alkalinity (**Table 1**). Growth rates of *K. veneficum* were positively correlated with seawater pH (**Figure 1A**), resulting in a maximum observed growth rate of 0.47 $days^{-1}$ at a pH of 8.03. Certain variables of the seawater carbonate system (i.e., CO_2, HCO_3^-, CO_3^{2-}, and H^+) are directly associated with cellular processes, such as photosynthesis, growth and enzyme reactions. An increase of aquatic CO_2 and HCO_3^- concentrations at constant pH results commonly in enhanced growth and photosynthetic rates (McMinn et al., 2014, 2017). On the other hand, increased H^+ concentrations can disturb cellular homeostasis of cytosol pH (Suffrian et al., 2011) with generally negative consequences for phytoplankton growth (McMinn et al., 2014; Müller et al., 2015). These two general mechanisms jointly result in an optimum growth curve response over extended pH/CO_2 gradients and have been demonstrated for coccolithophore and dinoflagellate species (e.g., McMinn et al., 2014; Müller et al., 2017). The fertilizing effect of CO_2 on growth rates of the dinoflagellate *Alexandrium catenella* has been observed in laboratory experiments at pH levels between 8.7 and 8.1 (Mardones et al., 2017). A further pH reduction (<8.1) had the adverse effect and resulted in reduced growth of *A. catenella*, similar to the here observed growth response of *K. veneficum* (**Figure 1A**). The linear decrease in growth rate of *K. veneficum*, when pH is reduced from 8.03 to 7.51 (**Figure 1**), indicates that the optimum pH condition is located at a pH of ≥8.03. Indeed, autotrophic growth rates of *K. veneficum* have been reported with rates up to 0.53 $days^{-1}$ at ambient pH of ∼8.2 under similar temperature, salinity and light settings (Calbet et al., 2011).

Gill cell mortality was enhanced with exposure to increasing densities of *K. veneficum* in all tested pH treatments (**Figure 1B**), and reached mortality of 99% at densities $>5.7 \times 10^5$ cells ml^{-1}. Idealized Michaelis-Menten-like kinetics were fitted to the treatment specific gill cell mortalities and the number of *K. veneficum* cells responsible for a 50% lethal dose for gill cells (LD_{50}) indicated a positive linear trend with increasing seawater hydrogen concentrations ($r^2 = 0.74$, $p = 0.062$, **Figure 1C**). An increased toxin content and production may facilitate prey paralysation and ingestion (Sheng et al., 2007, 2010) and, as suggested by our results, can be partly related to seawater pH. The altered toxicity of *K. veneficum* (**Figure 1**) with varying pH levels can theoretically be induced by higher cellular toxin content or by elevated toxin potency under elevated pH. However, the applied gill cell protocol to measure the toxicity excludes the latter possibility because toxin effects were tested under identical pH conditions. Thus, the here observed mechanism of increased toxicity at elevated seawater pH represents elevated cellular karlotoxins content compared to low pH conditions. Fu et al. (2010) documented no change in *Karlodinium* toxicity (measured as saponin equivalent) within a pH range from 8.37 to 7.94 under nutrient replete culture conditions. This is in good agreement with our results that indicate no substantial change in toxicity between the 8.03 and the 7.92 treatments (**Figure 1**). Under phosphate limited growth, however, Fu et al. (2010) observed an increased toxicity with decreasing pH (from 8.37 to 7.94). Phosphate limitation reduces cellular division rates in phytoplankton to ensure accurate DNA syntheses while the biosynthesis of carbon and nitrogen rich compounds proceeds, leading to organic compound accumulations inside the cell (Müller et al., 2008; Li et al., 2016). This could explain the increased toxicity under phosphate limitation with increased CO_2 availability for biosynthesis (Fu et al., 2010), while, at the same time, $[H^+]$ concentration were not high enough to induce a negative effect on the cellular metabolism and toxin production. However, it should be stressed that *K. veneficum* demonstrates a high inter-strain physiological plasticity (Place et al., 2012) which hampers an overall generalization of results derived from laboratory experiments testing a single strain.

Aquaculture ponds with extensive farming activities can experience highly elevated pCO_2 values reaching concentrations >10,000 μatm (Ellis et al., 2017) and it certainly would be of interest to test the physiological performance and ichthyotoxicity of *K. veneficum* under these extreme conditions. The amplitudes of oscillating pH regimes in aquaculture ponds and coastal environments are projected to expand due to ocean acidification and the associated increasing Revelle factor (Schulz and Riebesell, 2013). In the coming years, it will be essential to monitor and register the seawater carbonate chemistry as a basic environmental and experimental parameter in line with

temperature, salinity, and nutrient concentrations to improve our understanding of HAB bloom dynamics.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the supplementary files.

AUTHOR CONTRIBUTIONS

MM, JD-A, AS, and MB conceived, designed, and performed the experiments and analysis. MM analyzed the data and wrote the manuscript with essential contributions from all authors.

FUNDING

This work was funded by the "Conselho Nacional de Desenvolvimento Científico e Tecnológico Brasil (CNPq, Processo: 405585/2013-6)," the University of Tasmania providing a Visiting Fellowship to MM and the Federal University of Pernambuco (Edital Propesq Professor Visitante n°. 01/2017).

ACKNOWLEDGMENTS

We are grateful for all reviewers' suggestions and comments which significantly improved the manuscript.

REFERENCES

Adolf, J. E., Bachvaroff, T. R., Deeds, J. R., and Place, A. R. (2015). Ichthyotoxic *Karlodinium veneficum* (Ballatine) J Larsen in the upper swan river estuary (Western Australia): ecological conditions leading to a fish kill. *Harmful Algae* 48, 83–93. doi: 10.1016/j.hal.2015.07.006

Blackburn, S. I., Bolch, C. J. S., Haskard, K. A., and Hallegraeff, G. M. (2001). Reproductive compatibility among four global populations of the toxic dinoflagellate *Gymnodinium catenatum* (Dinophyceae). *Phycologia* 40, 78–87. doi: 10.2216/i0031-8884-40-1-78.1

Calbet, A., Bertos, M., Fuentes-Grünewald, C., Alacid, E., Figueroa, R., Renom, B., et al. (2011). Intraspecific variability in *Karlodinium veneficum*: growth rates, mixotrophy, and lipid composition. *Harmful Algae* 10, 654–667. doi: 10.1016/j.hal.2011.05.001

Carstensen, J., Chierici, M., Gustafsson, B. G., and Gustafsson, E. (2018). Long-term and seasonal trends in estuarine and coastal carbonate systems. *Gob. Biogeochem. Cycles* 32, 497–513. doi: 10.1002/2017GB005781

Dickson, A. G., Afghan, J. D., and Anderson, G. C. (2003). Reference materials for oceanic CO2 analysis: a method for the certification of total alkalinity. *Mar. Chem.* 80, 185–197. doi: 10.1016/S0304-4203(02)00133-0

Dorantes-Aranda, J. J., Waite, T. D., Godrant, A., Rose, A. L., Tovar, C. D., Woods, G. M., et al. (2011). Novel application of a fish gill cell line assay to assess ichthyotoxicity of harmful marine microalgae. *Harmful Algae* 10, 366–373. doi: 10.1016/j.hal.2011.01.002

Dore, J. E., Lukas, R., Sadler, D. W., Church, M. J., and Karl, D. M. (2009). Physical and biogeochemical modulation of ocean acidification in the central North Pacific. *Proc. Natl. Acad. Sci. U.S.A.* 106, 12235–12240. doi: 10.1073/pnas.0906044106

Duarte, C. M., Hendriks, I. E., Moore, T. S., Olsen, Y. S., Steckbauer, A., Ramajo, L., et al. (2013). Is ocean acidification an open-ocean syndrome? Understanding anthropogenic impacts on seawater pH. *Estuaries Coasts* 36, 221–236. doi: 10.1007/s12237-013-9594-3

Dutkiewicz, S., Morris, J. J., Follows, M. J., Scott, J., Levitan, O., Dyhrman, S. T., et al. (2015). Impact of ocean acidification on the structure of future phytoplankton communities. *Nat. Clim. Chang.* 5, 1002–1006. doi: 10.1038/nclimate2722

Ellis, R. P., Urbina, M. A., and Wilson, R. W. (2017). Lessons from two high CO2 worlds – future oceans and intensive aquaculture. *Glob. Chang. Biol.* 23, 2141–2148. doi: 10.1111/gcb.13515

Escobar-Morales, S., and Hernández-Becerril, D. U. (2015). Free-living marine planktonic unarmoured dinoflagellates from the Gulf of Mexico and the Mexican Pacific. *Botanica Marina* 58, 9–22. doi: 10.1515/bot-2014-0049

Feely, R. A., Sabine, C. L., Hernandez-Ayon, J. M., Ianson, D., and Hales, B. (2008). Evidence for upwelling of corrosive "Acidified". Water onto the continental shelf. *Science* 320, 1490–1492. doi: 10.1126/science.1155676

Fu, F. X., Place, A. R., Garcia, N. S., and Hutchins, D. A. (2010). CO2 and phosphate availability control the toxicity of the harmful bloom dinoflagellate *Karlodinium veneficum*. *Aquat. Microb. Ecol.* 59, 55–65. doi: 10.3354/ame01396

Goyet, C., and Snover, A. K. (1993). High-accuracy measurements of total dissolved inorganic carbon in the ocean - comparison of alternate detection methods. *Mar. Chem.* 44, 235–242. doi: 10.1016/0304-4203(93)90205-3

Li, M., Shi, X., Guo, C., and Lin, S. (2016). Phosphorus deficiency inhibits cell division but not growth in the dinoflagellate *Amphidinium carterae*. *Front. Microbiol.* 7:826. doi: 10.3389/fmicb.2016.00826

Mardones, J. I., Müller, M. N., and Hallegraeff, G. M. (2017). Toxic dinoflagellate blooms of *Alexandrium catenella* in Chilean fjords: a resilient winner from climate change. *ICES J. Mar. Sci.* 74, 988–995. doi: 10.1093/icesjms/fsw164

McMinn, A., Müller, M. N., Martin, A., and Ryan, K. G. (2014). The response of Antarctic sea ice algae to changes in pH and CO2. *PLoS One* 9:e86984. doi: 10.1371/journal.pone.0086984

McMinn, A., Müller, M. N., Martin, A., Ugalde, S. C., Lee, S., Castrisios, K., et al. (2017). Effects of CO2 concentrations on a late summer surface sea ice community. *Mar. Biol.* 164:87. doi: 10.1007/s00227-017-3102-4

Melzner, F., Thomsen, J., Koeve, W., Oschlies, A., Gutowska, M. A., Bange, H. W., et al. (2013). Future ocean acidification will be amplified by hypoxia in coastal habitats. *Mar. Biol.* 160, 1875–1888. doi: 10.1007/s00227-012-1954-1

Mooney, B. D., Hallegraeff, G. M., and Place, A. R. (2010). Ichthyotoxicity of four species of gymnodinioid dinoflagellates (Kareniaceae, Dinophyta) and purified karlotoxins to larval sheepshead minnow. *Harmful Algae* 9, 557–562. doi: 10.1016/j.hal.2010.04.005

Müller, M. N., Antia, A. N., and LaRoche, J. (2008). Influence of cell cycle phase on calcification in the coccolithophore *Emiliania huxleyi*. *Limnol. Oceanogr.* 53, 506–512. doi: 10.4319/lo.2008.53.2.0506

Müller, M. N., Trull, T. W., and Hallegraeff, G. M. (2015). Differing responses of three Southern Ocean *Emiliania huxleyi* ecotypes to changing seawater carbonate chemistry. *Mar. Ecol. Progress Ser.* 531, 81–90. doi: 10.3354/meps11309

Müller, M. N., Trull, T. W., and Hallegraeff, G. M. (2017). Differing responses of three Southern Ocean *Emiliania huxleyi* ecotypes to changing seawater carbonate chemistry. *ISME J.* 11, 1777–1787. doi: 10.1038/ismej.2017.53

Place, A. R., Bowers, H. A., Bachvaroff, T. R., Adolf, J. E., Deeds, J. R., and Sheng, J. (2012). *Karlodinium veneficum* - The little dinoflagellate with a big bite. *Harmful Algae* 14, 179–195. doi: 10.1016/j.hal.2011.10.021

Provasoli, L., McLaughlin, J. J., and Droop, M. R. (1957). The development of artificial media for marine algae. *Arch. Mikrobiol.* 25, 392–428. doi: 10.1007/BF00446694

Riebesell, U., Fabry, V. J., Hansson, L., and Gattuso, J.-P. (eds) (2011). *Guide to Best Practices for Ocean Acidification Research and Data Reporting*. Luxembourg: Publications Office of the European Union, 258. doi: 10.2777/66906

Roy, R. N., Roy, L. N., Vogel, K. M., Porter-Moore, C., Pearson, T., Good, C. E., et al. (1993). The dissociation constants of carbonic acid in seawater at salinities 5 to 45 and temperatures 0 to 45°C. *Mar. Chem.* 44, 249–267. doi: 10.1016/0304-4203(93)90207-5

Schirmer, K., Chan, A. G. J., Greenberg, B. M., Dixon, D. G., and Bols, N. C. (1997). Methodology for demonstrating and measuring the photocytotoxicity of fluoranthene to fish cells in culture. *Toxicol. Vitro* 11, 107–119. doi: 10.1016/S0887-2333(97)00002-7

Schulz, K. G., and Riebesell, U. (2013). Diurnal changes in seawater carbonate chemistry speciation at increasing atmospheric carbon dioxide. *Mar. Biol.* 160, 1889–1899. doi: 10.1007/s00227-012-1965-y

Sheng, J., Malkiel, E., Katz, J., Adolf, J., Belas, R., and Place, A. R. (2007). Digital holographic microscopy reveals prey-induced changes in swimming behavior of predatory dinoflagellates. *Proc. Natl. Acad. Sci. U.S.A.* 104, 17512–17517. doi: 10.1073/pnas.0704658104

Sheng, J., Malkiel, E., Katz, J., Adolf, J., and Place, A. R. (2010). A dinoflagellate exploits toxins to immobilize prey prior to ingestion. *Proc. Natl. Acad. Sci. U.S.A.* 107, 2082–2087. doi: 10.1073/pnas.0912254107

Suffrian, K., Schulz, K. G., Gutowska, M. A., Riebesell, U., and Bleich, M. (2011). Cellular pH measurements in *Emiliania huxleyi* reveal pronounced membrane proton permeability. *New Phytologist* 190, 595–608. doi: 10.1111/j.1469-8137.2010.03633.x

Sweeney, B. M. (1951). Culture of the Dinoflagellate *Gymnodinium* with soil extract. *Am. J. Bot.* 38, 669–677. doi: 10.2307/2437913

Torres, R., Pantoja, S., Harada, N., González, H. E., Daneri, G., Frangopulos, M., et al. (2011). Air-sea CO2 fluxes along the coast of Chile: from CO2 outgassing in central northern upwelling waters to CO2 uptake in southern Patagonian fjords. *J. Geophys. Res.* 116, 1–17. doi: 10.1029/2010JC006344

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Müller, Dorantes-Aranda, Seger, Botana, Brandini and Hallegraeff. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Marine Science

ORIGINAL RESEARCH
published: 13 March 2019
doi: 10.3389/fmars.2019.00085

Massive Blooms of *Chattonella subsalsa* Biecheler (*Raphidophyceae*) in a Hypereutrophic, Tropical Estuary—Guanabara Bay, Brazil

Tatiana V. Viana[1,2†], Giovana O. Fistarol[1†], Michelle Amario[1,2], Rafael B. Menezes[1], Beatriz L. R. Carneiro[1], Daisyane M. Chaves[1], Paulo I. Hargreaves[1], Arthur W. Silva-Lima[1], Jean L. Valentin[3], Denise R. Tenenbaum[1], Edilson F. Arruda[4], Rodolfo Paranhos[5] and Paulo S. Salomon[1]*

[1] *Laboratório de Fitoplâncton Marinho, Departamento de Biologia Marinha, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil,* [2] *Programa de Pós-Graduação em Genética, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil,* [3] *Laboratório de Zooplâncton Marinho, Departamento de Biologia Marinha, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil,* [4] *Programa de Engenharia de Produção, Instituto Alberto Luiz Coimbra de Pós Graduação de Pesquisa de Engenharia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil,* [5] *Laboratório de Hidrobiologia, Departamento de Biologia Marinha, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil*

Cell concentrations of the potentially harmful raphidophyte *Chattonella subsalsa* Biecheler were quantified in surface waters of Guanabara Bay, a heavily eutrophicated estuarine system in tropical Brazil, from February 2014 to January 2018. Cells were imaged and quantified in live samples by means of an automated imaging system (FlowCam®). Bloom episodes ($>0.1 \times 10^6$ cells L^{-1}) were observed in 37 samples, mostly in a shallow (<10 m) area with extremely high nutrient and organic matter loads (average total $P = 19\,\mu M$ and total $N = 344\,\mu M$), intermediate salinity (average 24.5), and low water transparency (average Secchi depth = 0.54 m) due to continental runoff. Blooms in this area reached up to 13.3×10^6 cells L^{-1}. *C. subsalsa* cell concentration was correlated with parameters linked to eutrophication of the bay. On a monthly basis, *C. subsalsa* abundance was correlated with a period of positive Multivariated El Niño/Southern Oscilation Index (MEI) that lasted from the beginning of 2015 to mid-2016 (known as Godzilla El Niño), indicating a potential influence of regional climate on the occurrence of *C. subsalsa*. Notably, at least six fish kill episodes were reported in the Bay during this period which, added to the toxicity of *C. subsalsa* strains isolated from the bay to *Artemia* nauplia (48h-$LC_{50} = 7.3 \times 10^6$ cells L^{-1}), highlights the threat that this HAB species poses to the environment. This is the first report of recurrent, massive *C. subsalsa* blooms in Guanabara Bay. Regardless of the influence of climatic forcing in favoring *C. subsalsa* development, reducing nutrient loads would be the best strategy to mitigate blooms of this and other potentially harmful algae in Guanabara Bay.

Keywords: *Chattonella subsalsa*, Guanabara Bay, eutrophication, harmful algae, climate, MEI

OPEN ACCESS

Edited by:
Juan Jose Dorantes-Aranda, University of Tasmania, Australia

Reviewed by:
Tomoyuki Shikata, National Research Institute of Fisheries and Environment of Inland Sea (FEIS), Japan
Kathryn Coyne, University of Delaware, United States

***Correspondence:**
Paulo S. Salomon
pssalomon@gmail.com

† These authors have equally contribution to this work

Specialty section:
This article was submitted to Marine Ecosystem Ecology, a section of the journal Frontiers in Marine Science

Received: *03 September 2018*
Accepted: *13 February 2019*
Published: *13 March 2019*

Citation:
Viana TV, Fistarol GO, Amario M, Menezes RB, Carneiro BLR, Chaves DM, Hargreaves PI, Silva-Lima AW, Valentin JL, Tenenbaum DR, Arruda EF, Paranhos R and Salomon PS (2019) Massive Blooms of Chattonella subsalsa Biecheler (Raphidophyceae) in a Hypereutrophic, Tropical Estuary—Guanabara Bay, Brazil. Front. Mar. Sci. 6:85. doi: 10.3389/fmars.2019.00085

INTRODUCTION

Members of the genus *Chattonella* (Class *Raphidophyceae*) are successful, cosmopolitan microalgae commonly found in the plankton of coastal marine areas such as estuarine ecosystems. This group of photosynthetic protists has various characteristics (such as phenotypic plasticity, tolerance to a wide range of temperatures, capacity for diel vertical migrations, and mixotrophy) that allow them to thrive in eutrophicated coastal waters despite the changeable conditions of these environments (Paranhos and Mayr, 1993; Marshall and Hallegraeff, 1999; Chenna et al., 2003; Band-Schmidt et al., 2004; Handy et al., 2005; Hosoi-Tanabe et al., 2006; Imai and Yamaguchi, 2012; Klöpper et al., 2013). Besides, some species of *Chattonella* form toxic blooms with harmful effects to other marine organisms, especially fish. These traits make species of this genus a common target during monitoring studies in coastal marine areas (Khan et al., 1995; Imai and Yamaguchi, 2012).

Chattonella spp., as other raphidophytes, are unicellular, bi-flagellated protists, typically with gold-brown coloration due to the production of fucoxantin (Hallegraeff and Hara, 2003; Klöpper et al., 2013). Their morphological diversity, even among the same species, has made the use of molecular markers almost a requirement for its precise identification. The occurrence of mixotrophy has been described for this genus, which may confer a competitive advantage during nutrient shortage (Jeong et al., 2010). Furthermore, the species *Chattonella antiqua*, *C. marina*, *C. ovata*, and *C. subsalsa* have been described to form harmful algal blooms (HABs), and cause massive fish kills (Hiroishi et al., 2005; Imai et al., 2006; Imai and Yamaguchi, 2012). The mechanism of the fish kills is not yet fully understood. Studies have demonstrated the production of neurotoxins (brevetoxins), hemolytic substance, and also of reactive oxygen species (ROS) (Hallegraeff et al., 2004; Imai and Yamaguchi, 2012). Nevertheless, it seems that suffocation by physically clogging the gills is the ultimate cause of fish death, with production of ROS playing an important role (Marshall et al., 2003; Imai and Yamaguchi, 2012). Massive fish kills caused by *Chattonella* spp., with associated economic losses, have been described around the world (e.g., in Japan, India, Australia, USA) (Hallegraeff et al., 1998; Edvardsen and Imai, 2006; Imai et al., 2006; Lewitus et al., 2008; Imai and Yamaguchi, 2012).

Even though the genus *Chattonella* is found worldwide, its growth is affected by environmental factors such as temperature, salinity, nutrient concentrations, as well as by biotic factors such as competition, parasitism, and grazing. Different strains of *Chattonella* were shown to have different optimal temperature and salinity growth conditions (Nakamura and Watanabe, 1983; Nakamura et al., 1989; Marshall and Hallegraeff, 1999; Imai and Yamaguchi, 2012), although they can be found outside these optimal conditions in natural environments. It has been shown that *Chattonella* can form cysts, and, in temperate regions with wide seasonal variations in temperature and salinity, encystment may be used as a survival strategy (Nakamura et al., 1990; Edvardsen and Imai, 2006). Encystment may also allow bloom formation during the return of favorable conditions (Imai, 1989; Portune et al., 2009).

Guanabara Bay is a large, highly-eutrophicated bay on the southeast coast of Brazil. It is surrounded by eleven municipalities, including the worldwide known city of Rio de Janeiro. Along the years, the anthropogenic impacts in this environment have caused severe disturbances and changes in its waters and on the habitats around the bay. One of the most critical impacts is the increase in nutrient concentrations, which leads to high chlorophyll levels (e.g., it has been registered chlorophyll *a* concentrations as high as 483 mg m^{-3}) (Paranhos et al., 2001). As a probable consequence of eutrophication, it has also been observed changes in the composition of the plankton community, with a shift toward a community dominated by more heterotrophic/mixotrophic organisms, and an increase in the frequency of HABs (Valentin et al., 1999; Santos et al., 2007; Fistarol et al., 2015; Odebrecht et al., 2018). Among the potentially harmful microalgae detected in Guanabara Bay are members of the genus *Chattonella* (Villac and Tenenbaum, 2010; Fistarol et al., 2015).

Although the occurrence of *Chattonella* spp. has been previously described in Brazil (Hallegraeff and Hara, 2003; Villac and Tenenbaum, 2010; Fistarol et al., 2015), including its presence as a member of the planktonic community in Guanabara Bay several decades ago (Valentin et al., 1999), there is a lack of systematic studies on the occurrence of blooms of these raphidophytes in the region. Due to its wide distribution and tolerance to different environmental conditions, added to the aforementioned records of the presence of *Chattonella* spp. in Brazilian waters, it is reasonable to assume that blooms of these raphidophytes in Guanabara Bay are more common than what has been hitherto documented in the literature.

In this study we present the results of an intense, 4-year monitoring program in Guanabara Bay that unveiled the occurrence of massive blooms of the raphidophyte *Chattonella subsalsa* in this environment. The taxonomic identity, tolerance to salinity and toxic potential of *C. subsalsa* was assessed by means of cultures established from cells isolated from the bay. The environmental drivers that favor the occurrence of *C. subsalsa* in Guanabara Bay are discussed.

MATERIALS AND METHODS

Sampling

Plankton samples for quantification of *C. subsalsa* in Guanabara Bay were collected over a 4-year period, from January, 2014 to January, 2018 along four sampling series (A to D) (**Figure 1**). Each sampling series had a particular timespan, number of sampling locations and sampling frequency: (i) monthly samplings at two fixed locations (A1 and A2) from January, 2014 to December, 2015 and bimonthly samplings at the same locations from January, 2016 to January, 2018; (ii) monthly samplings at five fixed locations (B1 to B5) from October, 2014 to January, 2018; (iii) monthly samplings at 6 fixed locations (C1 to C6) from October, 2016 to January, 2018; (iv) weekly samplings at 4 fixed locations (D1 to D4) from March, 2017 to January, 2018. Samples for cell counts and chemical analyses were collected at the water surface with a bucket or a Niskin bottle. Immediately after sampling, aliquots of water were sieved through a 200 μm

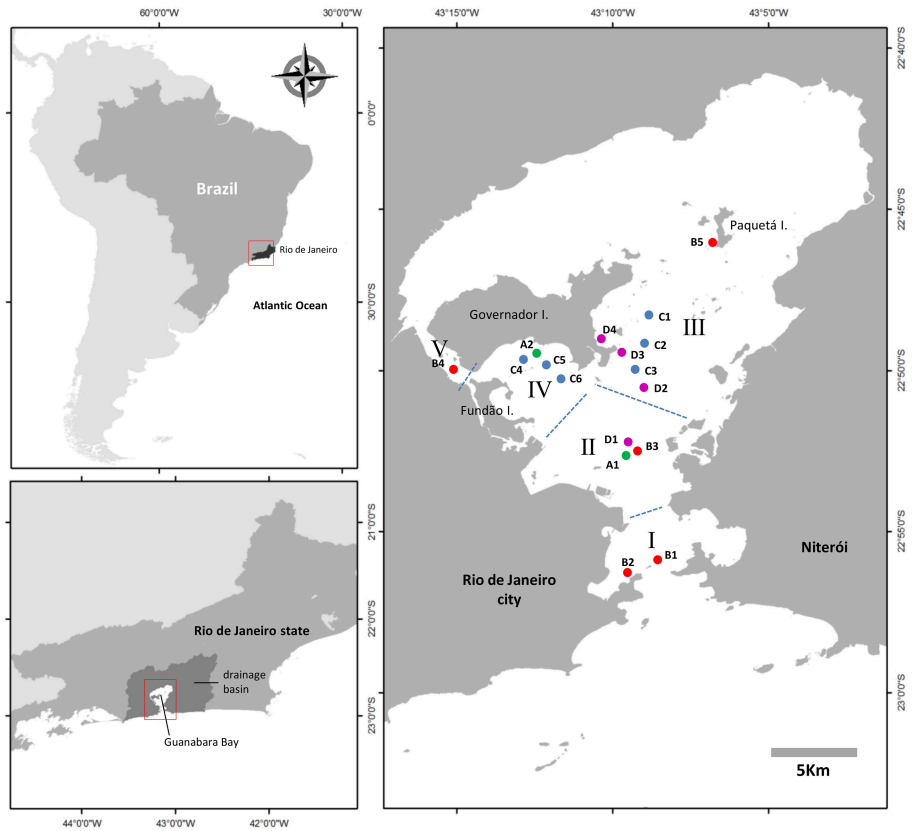


FIGURE 1 | Map of Guanabara Bay showing the locations sampled in this study. Four sampling series (series A: green dots; series B: red dots; series C: blue dots; series D: magenta dots) were conducted from February, 2014 to January, 2018 to monitor *C. subsalsa* cell concentration in surface waters of the bay. Samples collected in series B were also analyzed for physical and chemical water parameters. Roman numbers represent zones within an eutrophication gradient from I (less impacted) to V (more impacted). See text for details on each sampling series.

nylon mesh to remove large predators and stored cool in 2 to 5L plastic bottles until arrival to the laboratory. Samples for inorganic nutrient, total N and P, and chlorophyll *a* were stored in plastic bottles and kept on ice until being processed in the same day in the laboratory. Opportunity samples for cell counts only were collected off the fixed sampling locations when blooms were detected by discoloration of the water surface.

Chattonella subsalsa Cell Counts and Morphology

Cell counts were done on live samples by means of an automated flow imaging microscopy system, FlowCam® (Fluid Imaging Technologies, Inc.), within 3 h after sampling. Samples brought from the field were further sieved through a 100 μm nylon mesh to remove large particles, thus preventing blockage of the capillary lines of the equipment. The FlowCam® was fitted with a 90 μm field-of-view flow-cell and a 10X magnification objective. Sample flow rate was set to 0.1 mL min^{-1}. Samples were run for 20 min and imaged at 20 frames per second in auto-image mode with the threshold for acquisition set on an ESD (equivalent spherical diameter) value of 5 μm. Classification of *C. subsalsa* cells was done with the VisualSpreadsheeth® particle analysis software provided by the manufacturer of the FowCam® (Fluid Imaging Technologies, Inc.) based on a library of ca. five thousand *C. subsalsa* cells images selected from the Guanabara Bay database. A visual inspection was then performed by a human operator to correct for false positive and false negative errors by the software. *C. subsalsa* cell morphology was studied in live material following samplings in Guanabara Bay. A total of 1,776 sharp images from the FlowCam® displaying the cells from the dorso-ventral view were selected for measurements of cell length and width using the program VisualSpreadsheeth®. In all samples with detectable numbers of *Chattonella* cells they were also observed alive in the same day of sampling using an inverted microscope (Olympus IX70) equipped with a digital camera (Olympus XC-50). Morphology-based taxonomic identification was done based on the observations of live material in the microscope following Throndsen (1997) and Tomas et al. (2002).

Physical, Chemical, and Biological Water Parameters

Physical, chemical, and biological properties of the bay's water were assessed using standard oceanographic methods (Grasshoff et al., 1983; Parsons et al., 1984). Water temperature was measured with a YSI multiparameter sonde. Salinity was estimated chemically from chlorinity measurements. Water

transparency was measured with a Secchi disk. Inorganic nutrients were analyzed as follow: ammoniacal nitrogen (the sum of $N\text{-}NH_3 + N\text{-}NH_4^+$, referred to as ammonia) by indophenol; nitrite by diazotation; nitrate by reduction in a Cd-Cu column followed by diazotation; orthophosphate by reaction with ascorbic acid; silicate by reaction with molybdate. Total nitrogen was analyzed by nitrate determination after digestion with potassium persulfate, whereas total phosphorous was analyzed as phosphate after acid digestion. All nutrient analyses were performed in a Seal model AA3 AutoAnalyzer. Chlorophyll *a* analyses were performed after vacuum filtration (<25 cm of Hg) of water samples aliquots (minimum 250 mL) onto mixed cellulose esters membranes (Millipore HAWP 0.45 μm). Chlorophyll was extracted overnight in 90% acetone at 4°C and analyzed in a UV-VIS Perkin Elmer Lambda 20 spectrophotometer (Perkin Elmer, USA).

Climatological Data

Air temperature, wind speed, and pluviosity were obtained from a meteorological station located nearby sampling station B4 (**Figure 1**). Multivariated El Niño/Southern Oscillation (ENSO) Index, MEI (3-month running mean values) was obtained from the Earth System Research Laboratory database (National Oceanic and Atmospheric Administration—NOAA, https://www.esrl.noaa.gov/psd/data/climateindices/). MEI is determined as the first seasonally varying principal component of six atmospheric and oceanographic parameters (sea level air pressure, zonal, and meridional components of the surface wind, sea surface temperature, surface air temperature and cloudiness) in the tropical Pacific Ocean basin using data from the ICOADS (International Comprehensive Ocean-Atmosphere Data Set) (Wolter and Timlin, 1993, 1998). Negative values of the MEI represent the cold ENSO phase (La Niña) whereas positive MEI values represent the warm ENSO phase (El Niño).

Establishment of *Chattonella subsalsa* Cultures

Cultures of *C. subsalsa* were established from cells collected in Guanabara Bay in 2014 on sampling stations B4 and B5. Cells were picked with a capillary glass tube in an inverted microscope, transferred to sterile f/2 medium (Guillard, 1975) prepared with Guanabara Bay water with salinity adjusted to 20 and kept at 26°C, a photon flux of 50 μmoles photons m^{-2} s^{-1} (cool white fluorescent tubes) with a 14:10 ligh:dark cycle. After ca. 1 month, clonal cultures were established by single-cell sorting in a MoFlo® (Beckman-Coulter) flow cytometer (Fistarol et al., 2018). Four cultures established by this method were incorporated in the Culture Collection of Microorganisms at UFRJ (CCMR) under the collection codes CCMR0024, CCMR0025, CCMR0026, and CCMR0028. Cultures have since been maintained at the temperature and light regime as above by successive transfers to fresh medium on a monthly basis.

Growth Rates Estimates

C. subsalsa strains were grown in batch mode in f/2 medium prepared with Guanabara Bay water. Temperature and light regimes were as above. Strains were maintained in medium with salinity 20. Prior to the tests the salinity was gradually (1 salinity unit per day) adjusted toward salinity 10 and 30. None of the strains sustained growth during the salinity decrease. Strains that grew at salinity 30 were acclimated for 3 generations. Growth rates of each strain were then measured at salinity 20 and 30 in triplicate 500-mL florence flat-bottom borosilicate glass flasks containing 250 mL of f/2 medium prepared with Guanabara Bay water. Volumes of 5 mL were harvested from each flask every second day, fixed with a mixture of 1% paraformaldehyde, 1% glutaraldehyde, 30 mM HEPES and an amount of sucrose to match the osmotic pressure of the medium (Katano et al., 2009), and counted in the microscope at 20X magnification using Palmer-Maloney counting chambers. A minimum of 400 cells were counted in each sample. Specific growth rates were calculated as the slope of a least-squares regression line between the natural logarithm of cell concentration and time, with time as the abscissa, during the exponential growth phase (Guillard, 1973).

Toxicity Assays

The toxicity of two *C. subsalsa* strains (CCMR0024 and CCMR0026) kept in culture were accessed by exposing *Artemia salina* nauplia to live raphidophyte cells. Dried *A. salina* cysts were hatched in seawater with the salinity adjusted to 20. *Artemia salina* nauplia with 48 h of life after hatching were used in the tests. *C. subsalsa* strains were grown in 250 mL batch cultures under the conditions described above. Dilution series of *C. subsalsa* cells were prepared with cells harvested at late exponential phase by addition of fresh f/2 medium to achieve five concentrations ranging from 624 to 40,000 cells mL^{-1}. Aliquots of 10 mL from each *C. subsalsa* cell dilution were added to quadruplicate vials containing 20 *A. salina* nauplia each, resulting in five concentrations ranging from 312 to 20,000 cells mL^{-1}. The vials were incubated at the same conditions as the *C. subsalsa* cultures used for the tests. *Artemia salina* nauplia were observed after 48 h and scored as alive (motile) or dead (non-motile).

Molecular Characterization of *Chattonella subsalsa*

Cultures of *C. subsalsa* established from single cells collected in the bay were characterized by 18S rDNA sequencing. DNA extraction was done on a cell pellet collected from 500 μL of culture by CTAB and chloroform method (Rogers and Bendich, 1994). The small ribosomal subunit (SSU) was amplified by PCR with primers 1F (5'-AACCTGGTTGATCCTGCCAGT-3') and 1528R (5'-TGATCCTTCTGCAGGTTCACCTAC-3') (Klöpper et al., 2013). Amplification was performed in a Gene Amp 9,700 thermocycler in 25 μL reaction volumes containing 2.5 μL of a 10 μM solution of each primer, 1 μL of template DNA (50 ng), 12.5 μL of GoTaq® G2 Green Master Mix, and 6.5 μL of ddH_2O. The thermal cycle consisted of an initial denaturation step at 95°C for 2 min., followed by 30 cycles of 95°C for 1.5 min., 55°C for 1 min., 72°C for 2 min., and a final extension at 72°C for 7 min. Aliquots of 5 μL of the PCR products were purified with 2 μL of Exosap-IT™ for 15 min. at 37°C and then 15 min. at 80°C. Sequencing was done using dye-terminator chemicals (BigDye® Terminator v3.1 Cycle Sequencing Kit)

following the manufacturer's instructions and read in an ABI PRISM® 3500 Genetic Analyzer (Applied Biosystems). Sequencing primers were 528F (5′-GCGGTAATTCCAGCTCC AA-3′), 1055F (5′-GGTGGTGCATGGCCGTTCTT-3′), 536R (5′-AATTACCGCGGCKGCTGGCA-3′) and 1055R (5′-ACGGCCATGCACCACCACCCAT-3′) (Scholin et al., 1994; Klöpper et al., 2013). The sequences obtained were analyzed with the software Kodon v. 2.04 (Applied Maths). Their similarity with other published sequences was obtained using the BLASTn option in the GenBank database (https://www.ncbi.nlm.nih.gov). A phylogenetic reconstruction was done based on a sequence alignment conducted in Clustal (Higgins and Sharp, 1988; Chenna et al., 2003) including four *C. subsalsa* sequences obtained in this study and sequences from other 16 raphidophytes of the genera *Chattonella* and *Heterosigma*, the latter used as an outgroup. The evolutionary model was determined with ModelTest. The GTR+I+G was chosen and implemented in the phylogenetic reconstruction that was done by the Maximum-Likelihood method with 1,000 bootstrap replications using Mega7 (Kumar et al., 2016).

Statistical Analysis

A principal component analysis (PCA) was performed based on a correlation matrix with data from the 5 locations of the sampling series B (B1 to B5) using *C. subsalsa* cell concentration, chlorophyll *a* concentration, water temperature, salinity and transparency (Secchi depth), inorganic nutrient concentrations (orthophosphate, nitrite, nitrate, ammonia, and silicate), and total phosphorus and nitrogen concentrations as variables. Another PCA was conducted based on a correlation matrix using maximum monthly *C. subsalsa* cell concentrations for the whole bay, the Multivariated ENSO Index (MEI), average monthly air temperature, accumulated monthly pluviosity, and maximum monthly wind speed as variables. A multiple regression analysis was performed to test the correlation between maximum monthly *C. subsalsa* cell concentration over the whole bay and the climatological variables MEI, average monthly air temperature, accumulated monthly pluviosity, and maximum monthly wind speed.

For the laboratory growth experiments, an analysis of variance (ANOVA) followed by a Tukey HSD (honestly significant difference) test was done to check for differences among average *C. subsalsa* strains growth rates cultivated at different salinities. For the toxicity tests, the concentration of *C. subsalsa* cells causing 50% *Artemia salina* nauplia mortality within 48 h of exposure (48 h-LC_{50}) was estimated by the Trimmed Spearman-Karber method with 95% confidence intervals (Hamilton et al., 1977).

RESULTS

Physical and Chemical Characteristics of the Environment

Water quality parameters were measured during the monthly monitoring that took place from October 2014 to January 2018 at five fixed locations (sampling series B). Average salinity for this period was above 30 in the stations close to the bay's entrance and along the bays main channel, whereas in the shallow embayment, such as at station B4 (**Figure 1**), salinity was lower, averaging 25 with wide variation toward lower salinities (minimum of 14.6) (**Figure 2A**). Water temperature, on the other hand, was on average higher on station B4 (average 25°C) compared to the other stations (**Figure 2B**). Transparency, measured as Secchi disc depth, showed a strong variation across the bays axis, from ca. 3 m at the bay's entrance to < 1 m at station B4 (**Figure 2C**). Silicate increased from the entrance (stations B1 and B2) toward the inner parts of the bay, with the highest values (average 24 μM) at station B4 (**Figure 2D**). Average nitrate concentration was above 2 μM, except in station B4 (average 0.7 μM) with maxima in excess of 9.8 μM (**Figure 2E**). Nitrite concentrations ranged from 1.6 to 2.2 μM, increasing from the bay's entrance toward its inner parts (**Figure 2F**). Ammonia (**Figure 2G**) and orthophosphate (**Figure 2H**) were much higher at station B4 (>200 and >10 μM, respectively) that in other parts of the bay, as also observed for organic nitrogen (**Figure 2I**) and organic phosphorus (**Figure 2J**). Average chlorophyll *a* concentrations were high across the bay, increasing from the entrance to the inner parts, with extremely high values in excess of 500 μg L^{-1} at station B4 (average 110 μg L^{-1}) (**Figure 2K**).

Climatology

The Multivariated El Niño Index (MEI) during the 4 years of the monitoring in Guanabara Bay (February 2014 to January 2018) showed a strong, positive phase that lasted from the end of 2014 to the first quarter of 2016, with the strongest positive period (MEI above +1) during the austral spring and summer of 2015–2016 (**Figure 3B**). Local monthly average air temperature (**Figure 3C**) ranged between 23°C during winters to ca. 29°C in the summers. Average maxima were close to 40°C in the summers whereas minima were ca. 16°C in winters. Average monthly rainfall (**Figure 3D**) was higher from December to March with peaks of 100 to 150 mm with exception of the summer 2015–2016 that showed levels up to 220 mm, coinciding with the strong positive MEI period.

Chattonella Subsalsa Blooms

The analysis of 563 samples collected within the 4 sampling series (A to D) during the survey in Guanabara Bay from February 2014 to January 2018 showed a consistent occurrence of *C. subsalsa* cells above the detection limit of our technique using the FlowCam® (i.e., 0.00087 × 10^6 cells L^{-1}). Cell densities in excess of 13 × 10^6 cell L^{-1} were observed during massive blooms (**Figure 2L**, **3A** and **Table 1**). A total of 37 samples had >0.1 × 10^6 cell L^{-1} with 24 of those above 0.5 × 10^6 cell L^{-1} (**Table 1**). Most of these high cell density events were observed within the zones IV and V (see map in **Figure 1**) that encompass stations A2, B4, C4, to C6 in the shallow arears south and west of the Governador Island. Station B4 alone (zone V) accounted for 42% (10 out of 24) of all records of *C. subsalsa* above 0.5 × 10^6 cell L^{-1} (**Table 1**). The number of blooms and the maximum concentrations of *C. subsalsa* decreased consistently toward sampling stations within zones I to II, located on or near the bay's main channel (**Figure 2L**, **Table 1**). Fish-kills were reported in the local media at least on six occasions from April,

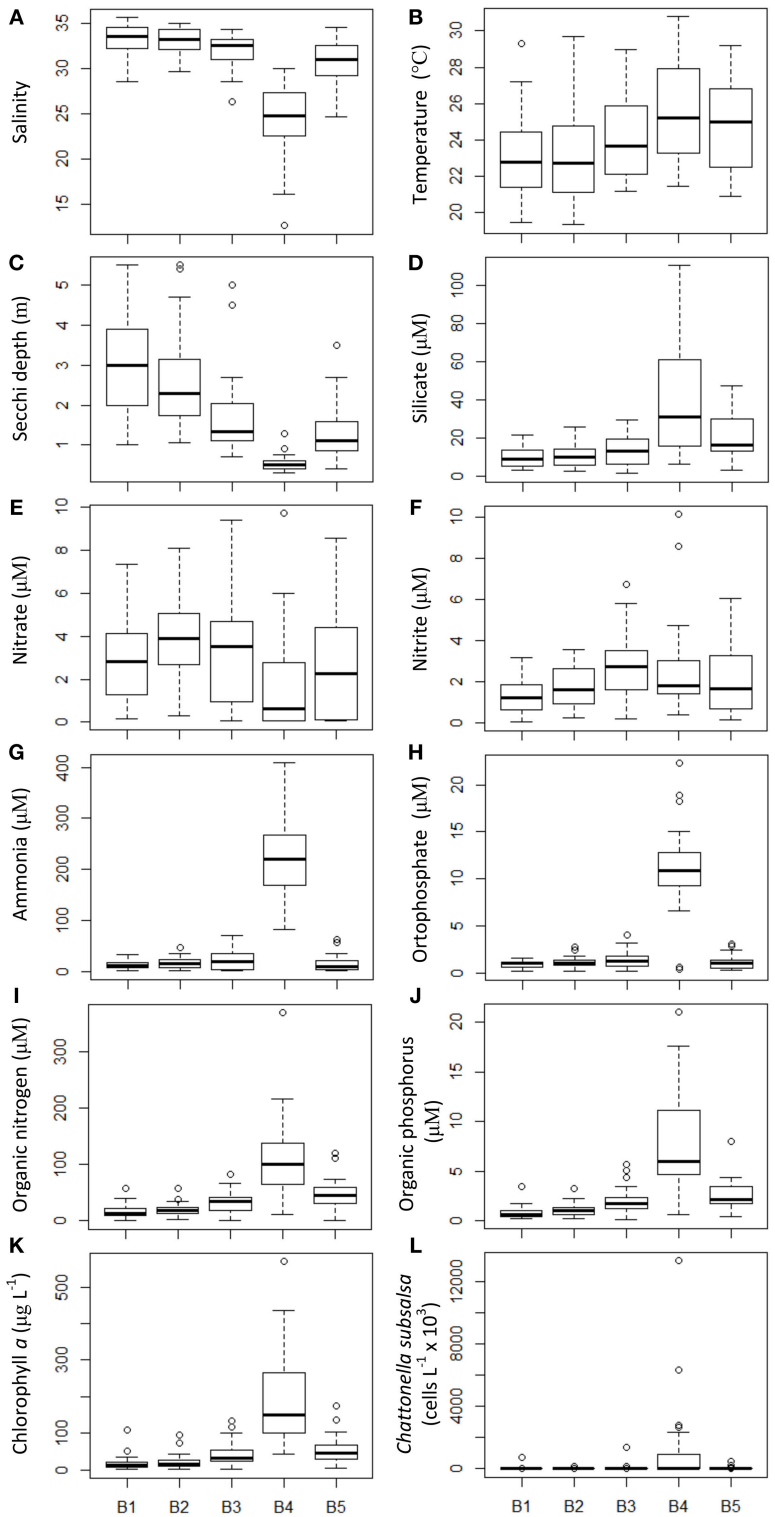


FIGURE 2 | Physical and chemical water parameters and *C. subsalsa* cell concentration in five locations (B1 to B5, see map in **Figure 1**) in Guanabara Bay sampled on a monthly basis from October 2014 to January 2018. **(A)** salinity; **(B)** water temperature; **(C)** Secchi depth; **(D)** silicate; **(E)** nitrate; **(F)** nitrite; **(G)** ammonia; **(H)** orthophosphate; **(I)** organic nitrogen; **(J)** organic phosphorus; **(K)** chlorophyll *a*; **(L)** *C. subsalsa* cell concentration. The box plot shows mean (thick line inside the box), first and third quartiles (lower and upper box limits), standard deviation (whiskers) and outliers (open circles beyond the whiskers).

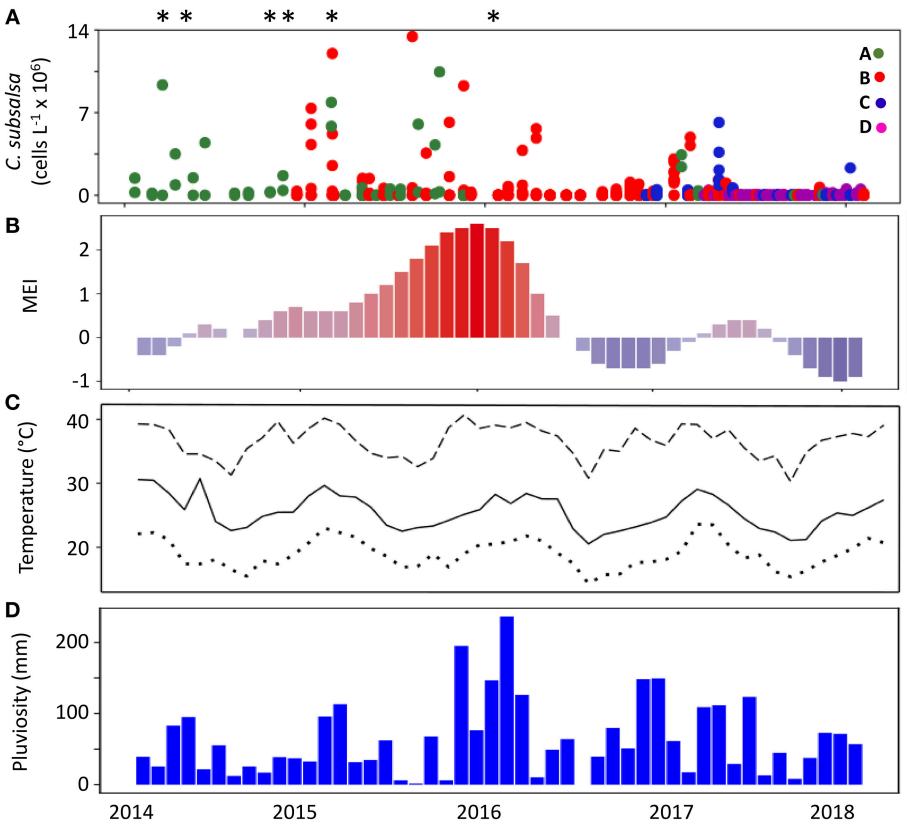


FIGURE 3 | Concentration of *C. subsalsa* cells in Guanabara Bay and climatological parameters over a 4-year period (February, 2014 to January, 2018). **(A)** *C. subsalsa* cell concentration detected in samples from four monitoring series (**A–D**, see map in **Figure 1** for details), *Fish-kills in Guanabara Bay reported in the local media; **(B)** Multivariate El Niño Index; **(C)** average (solid line), maximum (dashed line), and minimum (dotted line) monthly air temperature; **(D)** accumulated monthly pluviosity.

TABLE 1 | Number of *C. subsalsa* blooms with >0.1 × 10^6 cells L^{-1} and >0.5 × 10^6 cells L^{-1} in five zones of Guanabara Bay as shown in the map of **Figure 1**.

Sampling zone	Number of samplings with >0.1 × 10^6 cells L^{-1}	Number of samplings with >0.5 × 10^6 cells L^{-1}	Highest cell concentration × 10^6 L^{-1}
I	2	1	0.7
II	4	2	2.5
III	5	1	0.5
IV	14	10	8.0
V	12	10	13.3
All	*37*	*24*	*13.3*

The highest cell concentration recorded for the period in each zone is also shown.

TABLE 2 | Linear dimensions of *C. subsalsa* from Guanabara Bay based on 1,776 images of live cells obtained with the FlowCam®.

	Length (μm)	Width (μm)
Mean (SD)	34.9 (4.5)	20.1 (3.1)
Range	23.0–56.4	12.2–32.1

SD, standard deviation.

2014 to mid-January, 2016 (**Figure 3A**), mostly in the inner parts of the bay.

Chattonella Morphology and Molecular Characterization

Live *Chattonella* cells from Guanabara Bay were analyzed in the microscope and in the FlowCam® in all samples where cells were detectable. A set of 1,776 cells measured in the images from the FlowCam® were 34.9 ± 4.5 μm (mean ± SD) long and 20.1 ± 3.1 μm (mean ± SD) wide (**Table 2**). Cells appeared pyriform, often elongated, with roundish, golden to brownish chloroplasts densely packed filling up nearly the whole cell (**Figure 4**). Cell surface often appeared lumpy with chloroplasts protruding to form an irregular surface. Two flagella emerging from a subapical groove were visible in the microscope. A hyaline, tail-like protrusion at the posterior end of the cells, one of the hallmarks of *C. subsalsa* Biecheler (Tomas et al., 2002), was often observed both in the microscope and in the FlowCam® images (**Figure 4**).

Sequencing of the 18S rDNA gene from the four *Chattonella* isolates produced 701 nt. long, identical sequences. The sequences are deposited in the GenBank database (National Center for Biotechnology Information, NCBI) under accession numbers MK217469, MK217470, MK217471, and MK217472. A

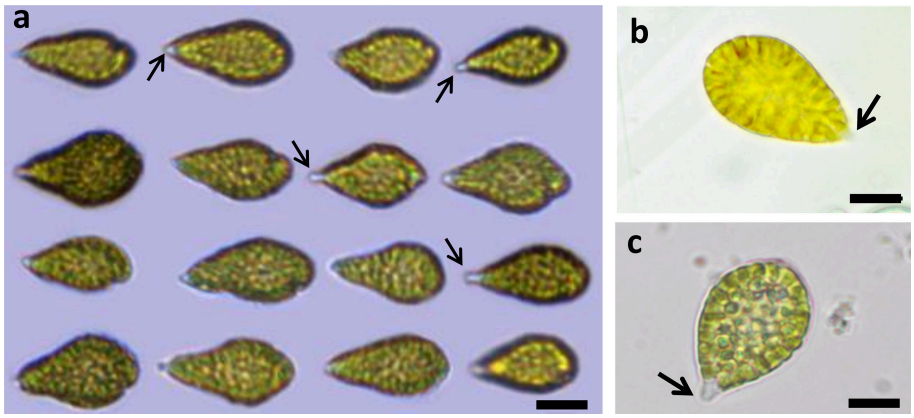


FIGURE 4 | Live *C. subsalsa* cells from plankton samples collected in Guanabara Bay. **(a)** Live cells imaged by the FlowCam®; **(b,c)** Live cells observed in an inverted mircroscope at 40X magnification. The hyaline, tail-like protrusion at the posterior end of the cells typical of *C.subsalsa* can be seen (arrows). Scale: a: 10 μm, b and c: 20 μm.

BLASTn search showed that the sequences were 100% similar to sequences of *C. subsalsa*. In a phylogenetic reconstruction (**Figure 5**) with other raphidophytes, 18S rDNA sequences from the four Guanabara Bay isolates clustered together with other *C. subsalsa* sequences with 97% bootstrap support, as a sister group to a cluster containing *C. marina* var. *antiqua* and *C. minima*. The *Chattonella* genus cluster was separated from the other raphytophyte genus, *Heterosigma*, with 99% bootstrap support.

Growth Rates of *C. subsalsa* Cultures

The four *C. subsalsa* strains isolated from Guanabara Bay grew well when inoculated in f/2 medium with salinity 20 and 30 (**Figure 6**). Attempts to grow the strains in salinity 10 and in freshwater failed. Growth rates (μ) ranged from 0.23 ± 0.06 d^{-1} (mean ± SD) for strain CCMR0025 up to 0.81 ± 0.04 d^{-1} (mean ± SD) for strain CCMR0026, at salinity 20. Average growth rate for all four strains was 0.50 ± 0.23 d^{-1} (mean ± SD) when growing in salinity 20 and 0.54 ± 0.10 d^{-1} (mean ± SD) in salinity 30. Strain CCMR0025 grew significantly faster at salinity 30, whereas strain CCMR0026 grew significantly faster at salinity 20. The other two strains grew equally fast at both salinities (**Figure 6**; **Table S1**).

Toxicity of *C. subsalsa* Cultures

The *C. subsalsa* strains CCMR0024 and CCMR0026 were both equally toxic to *Artemia salina* nauplia. The LC_{50}-48h was 7.5×10^6 cells L^{-1} (95% CI: 4.7×10^6 to 11.9×10^6 cells L^{-1}) for strain CCMR0024 and 7.3×10^6 cells L^{-1} (95% CI: 4.5 to $11.7 \times 10^6 \times 10^6$ cells L^{-1}) for strain CCMR0026.

Influence of Water Parameters and Climatology on *C. subsalsa* Cell Concentrations

A total of 163 samples collected during the survey in Guanabara Bay (sampling series B) were used in a PCA to check the influence of local environmental variables on the occurrence of *C. subsalsa* (**Figures 7A,B**; **Table S2**). The first and second components explained 71% of the variance. The first component was negatively correlated (factor-variable correlation <-0.5) with *C. subsalsa* cell concentration (−0.53), chlorophyll (−0.88), phosphate (−0.87), organic phosphorus (−0.86), total phosphorus (−0.97), ammonium (−0.86), organic nitrogen (−0.85), total nitrogen (−0.94), and silicate (−0.67), and positively correlated (factor-variable correlation >0.5) with salinity (0.83) and water transparency (0.66). The second component was negatively correlated with nitrite (−0.84) and nitrate (−0.79) concentrations and positively correlated with water temperature (0.56). Samples collected at station B4 (shallow enclosed area of the bay) clustered together separated from the others (main channel of the bay) (**Figure 7B**). A second PCA was done to check the influence of climatological variables on *C. subsalsa* cell concentrations (**Figures 7C,D**; **Table S3**). The two first components explained 67% of the variance. In this case, the first component was negatively correlated (factor-variable correlation <-0.5) with average monthly air temperature (−0.88) and maximum monthly wind speed (−0.82). The second component was negatively correlated with maximum monthly *C. subsalsa* cell concentrations (−0.56) and the MEI (−0.88) (**Figure 7C**; **Table S3**). Accumulated monthly pluviosity, despite being closely associated with MEI and *C. subsalsa* in the diagram of the two first components (**Figure 7C**), was only weakly correlated to the first (−0.38) and the second (−0.31) components (**Table S3**). Samples collected in summer and spring formed a cluster apart from samples taken in autumn and winter, influenced by the variables average monthly air temperature and maximum wind speed of component 1 (**Figure 7D**). The multiple regression analysis between monthly maximum *C. subsalsa* cell concentrations and climatological variables showed significant correlation only between *C. subsalsa* concentration and MEI (Pearson's $r = 0.36$; $p = 0.007$) (**Table S4**).

DISCUSSION

Frequency and magnitude of harmful algal blooms in aquatic environments result from a combination of factors. Cultural eutrophication and climate-induced changes in physical and

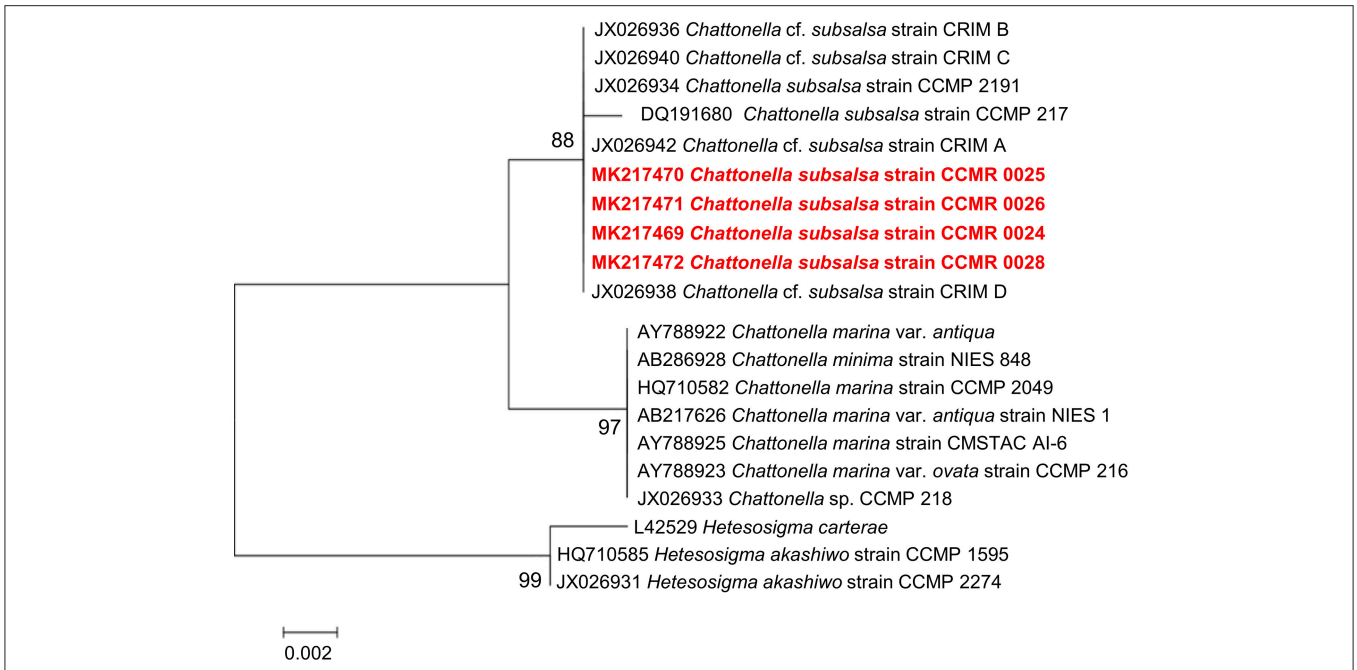


FIGURE 5 | Phylogenetic reconstruction of *C. subsalsa* strains isolated from Guanabara Bay (red, boldface font) and other raphidophytes based on 18S rDNA sequences. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 0.1000)]. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 20 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 699 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

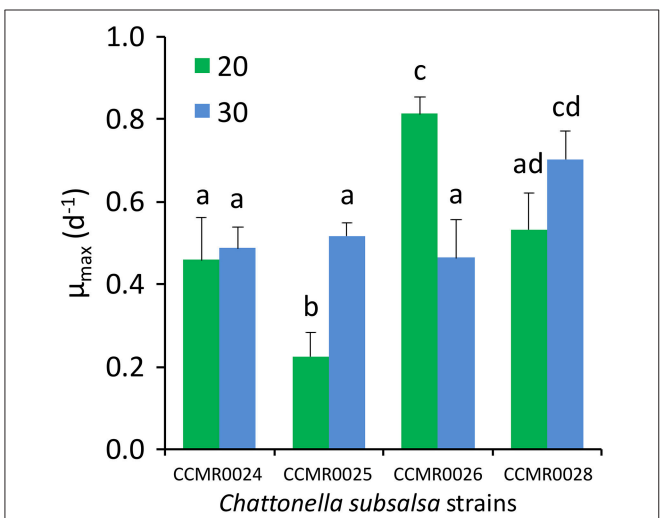


FIGURE 6 | Maximum growth rate of four *C. subsalsa* strains isolated from Guanabara Bay cultivated in two salinities (20 and 30). Values are average of triplicate cultures for each salinity. Different letters above the bars indicate significant differences in growth rate according to an ANOVA with Tukey's HSD *post-hoc* test ($p < 0.05$). Whiskers: 1SD.

chemical properties of coastal ecosystems are major drivers of a global increase of such blooming events (Burkholder et al., 2006; Heisler et al., 2008). Raphidophytes such as the genus *Chattonella* thrive in nutrient-enriched waters during warm periods (Onitsuka et al., 2015). Their ability to perform mixotrophy by the uptake of dissolved organic compounds as well as particles such as bacteria make them good competitors as eutrophication increases (Burkholder et al., 2008; Jeong et al., 2010). Our four-year monitoring unveiled a consistent presence of *C. subsalsa* in the surface waters of Guanabara Bay, with recurrent blooming events at very high concentrations. Although the genus *Chattonella* has been previously reported as a component of the bay's phytoplankton community (Valentin et al., 1999; Villac and Tenenbaum, 2010; Odebrecht et al., 2018) even in bloom conditions (Sevrin-Reyssac et al., 1979), this is the first systematic, long-term survey that shows its regular presence and blooming frequency in this important and heavily impacted estuarine system.

Guanabara Bay's poor water quality and high pollution levels, especially due to high loads of untreated urban waste from its drainage basin is well-known and acknowledged as one of the main environmental issues in the highly populated metropolitan area of Rio de Janeiro city (Paranhos et al., 1998; Fistarol et al., 2015). High chlorophyll levels normally found in the bay are evidence of its high primary productivity and the occurrence of algal blooms (Fistarol et al., 2015; Oliveira et al., 2016). During our survey of series B (five stations), chlorophyll concentrations in excess of 100 μgL^{-1} were registered several times. Remarkably, little is known of the identity of the causative microalgae blooming in the bay's waters. The nearby coastal area of the Atlantic Ocean and other estuarine systems north and southward from the Guanabara Bay entrance, notably the

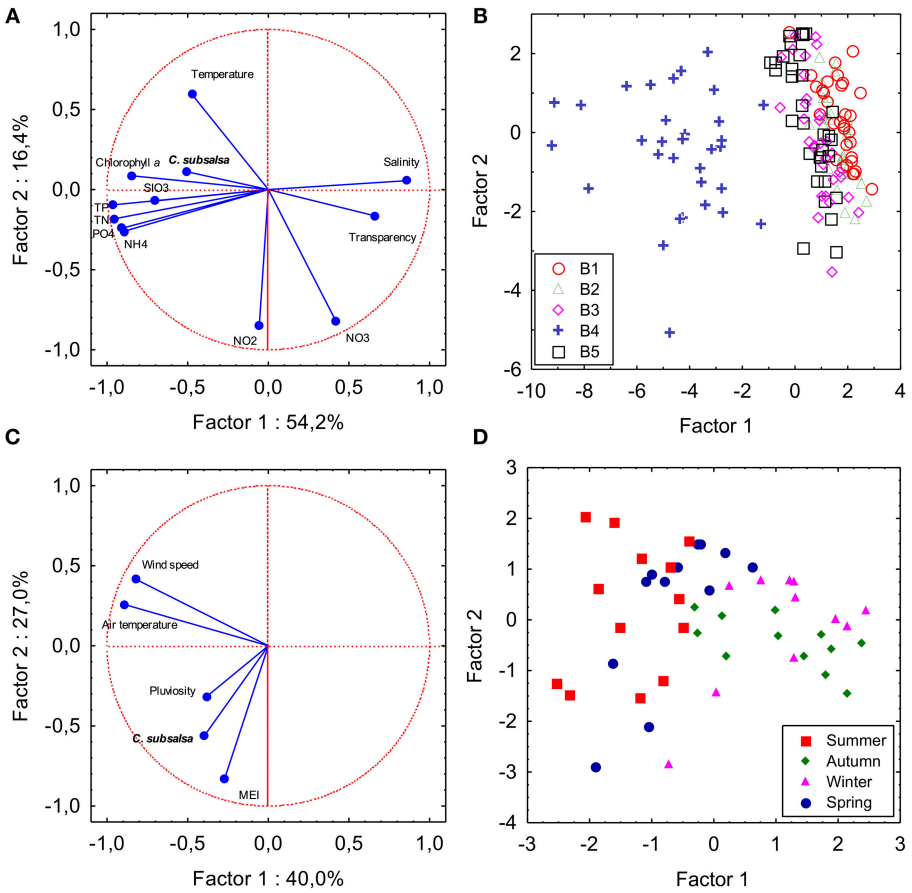


FIGURE 7 | Principal component analysis. Plots showing variables and samples plotted on the two first components axes. **(A,B)** PCA based on a correlation matrix of *C. subsalsa* cell concentration and water physical and chemical variables of 163 samples collected in five locations in Guanabara Bay (series B). SIO3: silicate; PO4: orthophosphate; NH4: ammonia; NO2: nitrite; NO3: nitrate; TP: total phosphorus; TN: total nitrogen. **(C,D)** PCA based on a correlation matrix of monthly *C. subsalsa* maximum cell concentration and climatological variables from February, 2014 to January, 2018. MEI: Multivariate El Niño Index. Sampling season is indicated in the samples coordinates plot.

Rodrigo de Freitas Lagoon, characterized by high nutrient levels and very limited water exchange with the ocean, have recurrent blooms of harmful species, including the fish-killer raphidophyte *Heterosigma akashiwo*, with multiple records of massive fish-kills (Branco et al., 2014; Castro et al., 2016).

The time-span, spatial coverage and high number of samplings (>500 in total) conducted in this study was crucial to construct a clear picture of the main areas of the bay where *C. subsalsa* is more abundant, i.e., the shallow enclosed areas west of the main channel nearby Governador Island (zones IV and V, **Figure 1**, **Table 1**). This was also the area with the highest levels of nutrients, as well as organic P and N, and chlorophyll *a* concentrations, and the lowest water transparency, and therefore this region is classified as hypereutrophic (Ryding and Rast, 1989; Fistarol et al., 2015). Autotrophic biomass production is directly linked to the availability of light (Cadée and Hegeman, 1979; Joint and Pomroy, 1981; Pennock and Sharp, 1986). The contribution of nutrients and organic matter tends to decrease water transparency in coastal areas (Cloern, 1987; Gameiro et al., 2011). Light is likely a limiting factor for autotrophic growth in the shallow areas of Guanabara Bay around zone IV and V as Secchi depth was often <1 m, mainly during the rainy season (spring and summer). During periods of light limitation, *C. subsalsa* may utilize mixotrophy as this nutritional strategy may be triggered not only by nutrients limitation, but also by light shortage (Nygaard and Tobiesen, 1993; Jones, 1994, 2000; Jeong et al., 2010). The PCA with *C. subsalsa* concentration and water quality properties corroborates this observation as *C. subsalsa* was correlated with the component that represents eutrophication caused by freshwater discharges into the bay (low salinity, low transparency, high P, and N organic loads, high phosphate and ammonia). The occurrence of *C. subsalsa* in other sampling areas along the main axis of the central channel (zones I, II and III) was much lower and is probably the result of transport of cells from the shallow areas by tidal currents that penetrate the bay from the entrance in the south and reach up to the area around station B5, nearby Paquetá Island, in the north portion of the bay (Kjerfve et al., 1997). The area around station B4 is, therefore, a source of *C. subsalsa* that, on occasions, might be detected in bloom concentrations as far as the bay entrance (zone

I). Similarly, the shallow areas north of the Governador Island that are also nutrient-rich (Mayr et al., 1989; Kjerfve et al., 1997; Fistarol et al., 2015) might also be suitable for *C. subsalsa* blooms formation. As this area was not covered by our monitoring, this remains as a hypothesis to be investigated in future studies.

Members of the genus *Chattonella* are known to be euryahaline (Khan et al., 1995; Strom et al., 2013) which is well in line with our observation of growth by the four *C. subsalsa* strains in both salinity 20 and 30. This phenotypical plasticity of *C. subsalsa* is an important trait for its success in a highly variable environment influenced by the tide as the Guanabara Bay (Paranhos and Mayr, 1993). The fact that *C. subsalsa* isolated from the bay grew well in both conditions, albeit significant differences in two of the four strains, indicates that salinity is not the limiting factor for the occurrence of the species and that low hydrodynamics and excess of nutrient and organic matter in the enclosed areas offer a suitable niche for the species to bloom. Moreover, the relatively high growth rates of most strains tested in this study highlights the potential of *C. subsalsa* to rapidly develop blooms when environmental conditions are suitable. An average specific growth rate of 0.5 d^{-1}, as measured for our strains, equals a doubling time of 1.3 d, which is fast enough to overcome flushing by the tides, since it takes ca. 11 days to renew 50% of the water in the bay (Kjerfve et al., 1997). Also, this half-residence time is an average for the whole bay. Water renewal in areas of more restricted circulation, as around station B4, is likely to be longer.

Perhaps one of the most striking features of the *C. subsalsa* concentration time-series produced here is the observation that blooms were more frequent during the positive MEI period, which also coincided with a slightly high rainfall period in the bay area. Accordingly, the PCA based on climatological data associated *C. subsalsa* abundance with positive MEI, whereas local temperature and wind speed had less contribution in explaining the blooms. Average monthly pluviosity, although correlated with the same principal component as *C. subsalsa* and MEI, indicating that these variables may change together, was not significantly correlated to monthly maximum *C. subsalsa* cell concentrations in the multiple regression analysis. Nevertheless, the PCA performed with individual sampling locations water parameters indicated that *C. subsalsa* concentration is higher in areas of the bay with low salinity and high nutrient loads. Thus, it was reasonable to expect some positive effect of periods of higher pluviosity on the occurrence of blooms due to an increase in riverine discharge that carries more nutrients into the bay. Such effect may not have been fully captured in our analysis due to the temporal and spatial scales of pluviosity and *C. subsalsa* cell concentrations of our dataset. Also, a possible time lag in the response of the raphidophyte growth to precipitation might have contributed to this lack of correlation. A more comprehensive time series analysis of pluviosity over the entire bay's drainage basin and *C. subsalsa* concentrations is necessary to clarify such causal relationship. Periods of El Niño (the positive phase of the ENSO phenomenon, thus positive MEI) are marked by an increase in average precipitation and also in the frequency and intensity of extreme rainfall events in the south and southeastern regions of South America, including the borderline of the tropical belt (22.79°S) where Guanabara Bay is located (Grimm and Tedeschi, 2009). Conditions for *C. subsalsa* bloom development are strongly linked to nutrient discharged into the bay, which is driven by factors that influence pluviosity, e.g., regional climatic setups that are governed by cyclic, global climatic phenomenon with rather irregular decadal frequency such as the ENSO. The influence of climate-induced changes in coastal ecosystems as promoter of more frequent and intense HABs is well-acknowledged (Edwards et al., 2006; Onitsuka et al., 2015). Extreme El Niño events are expected to occur twice as often in a scenario of global temperature increase of 1.5°C (Wang, 2017), which will likely increase even more the frequency of *C. subsalsa* blooms in Guanabara Bay.

The occurrence of *Chattonella* species in coastal waters is a nuisance mostly due to their high fish-killing potential (Marshall et al., 2003). *C. subsalsa* strains isolated from Guanabara Bay were lethally toxic to brine shrimp nauplia at cell concentrations of 7.4×10^6 cells L^{-1} which is less than the highest natural levels found in this environment. Harmful effects of *Chattonella* on fish can be elicited at cell densities as low as 0.1 $x10^6$ cells L^{-1} (Imai et al., 2006). Mortality to brine shrimp, although not a direct measure of ichthyotoxicity, demonstrates a toxic potential by Guanabara Bay's *C. subsalsa* cultures. Brine shrimp is commonly used as test organism to screen the toxic potential of microalgae and cyanobacteria (Lincoln et al., 2008) and also respond to the presence of ichthyotoxic species as shown during blooms of *Prymnesium parvum* in a brackish lake in Finland (Lindholm et al., 1999). The toxic potential of our strains, added to repeated reports of fish-kills during the period when *C. subsalsa* blooms were more frequent and intense in the bay, highlight the threat posed by this microalga to the bay's fish communities. *Brevoortia aurea*, known as Brazilian menhaden, is the main fish species affected during mass mortalities observed in the bay (Fistarol et al., 2015). A more robust causal relationship between the occurrence of *C. subsalsa* blooms and fish-kills in this environment awaits further investigation as the occurrence of harmful algae during fish kills does not necessarily reflect a cause-and-effect relationship (Lewitus et al., 2003). Water samples might be collected too long after fish kills to assess whether mortality is associated with phycotoxins, or a more general effect e.g. oxygen depletion and suffocation. The scale of sampling might also be inadequate to register the exact conditions of the bloom at the onset or during fish-kills. Also, it is important to notice that this hypereutrophic estuary harbors other potential fish-killing microalga e.g., the dinoflagellate *Karlodinium venificum* and the raphidophyte *Heterosigma akashiwo* (Fistarol et al., 2015; Higashi et al., 2017). However, during the four-year monitoring neither genera were found as a major phytoplankter in our samples. Likewise, *Chattonella* cell morphology detected throughout our monitoring was consistent with *C. subsalsa* (Throndsen, 1997; Tomas et al., 2002) across sampling points and over time. Moreover, our molecular characterization corroborates the morphology of the raphidophyte as *C. subsalsa.* We thus conclude that although other raphidophytes do occur in Guanabara Bay (Higashi et al., 2017) it seems that the dominant member of the group in this estuary is *C. subsalsa*.

A recent and exhaustive review on the occurrence of HABs along the Brazilian coast from 2006 to 2016 (Castro et al., 2016) did not report blooms in Guanabara Bay, despite the extremely high chlorophyll biomass normally found in its waters (Mayr et al., 1989; Kjerfve et al., 1997; Oliveira et al., 2016). Our monitoring focusing on a single HAB species unveiled several blooms over a relatively short period of time. Recurrent, massive HAB events seem to be a neglected subject in Guanabara Bay research programs. Potentially toxic blooms of *C. subsalsa* in Guanabara Bay are nourished by a heavy load of nutrients of riverine origin. Global climatic drivers such as the ENSO and its influence on regional climate result in periods of more favorable conditions for bloom formation. As seen in other coastal areas worldwide (e.g., Imai et al., 2006) reducing organic matter and inorganic nutrient inputs to Guanabara Bay is paramount to mitigate *C. subsalsa* blooms and its harmful effects.

AUTHOR CONTRIBUTIONS

PS designed and coordinated the study. TV, GF, and PS wrote the manuscript. DT and AS-L revised the paper. RM did the field sampling. RM, MA, and TV processed field samples for cell counts. BC and DC performed laboratory experiments. PH was responsible for DNA sequencing. TV, GF, AS-L, JV, and EA contributed to data analyses. RP contributed with data and logistic support for field sampling.

ACKNOWLEDGMENTS

We thank the Brazilian research council Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq (grants 477429/2012-2 to PS, 441215/2017-3 to JV, and 311075/2018-5 to EA), and the Rio de Janeiro state research council, FAPERJ (grants E26/202.911/2018 to PS, E26/111.584/2014 to JV, and E-26/202.789/2015 to EA) for funding. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brasil (CAPES)-Finance Code 001. TV thanks CNPq and CAPES for PhD scholarships. GF thanks CNPq for the postdoctoral scholarship (150206/2018-6).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2019.00085/full#supplementary-material

REFERENCES

Band-Schmidt, C. J., Morquecho, L., Hernández-Becerril, D. U., Reyes-Salinas, A., and Bravo-Sierra, E. (2004). Raphidophyceans on the coasts of Mexico. *Hydrobiologia* 515, 79–89. doi: 10.1023/B:HYDR.0000027320.00977.8b

Branco, S., Menezes, M., Alves-de-Souza, C., Domingos, P., Schramm, M. A., and Proença, L. A. O. (2014). Recurrent blooms of *Heterosigma akashiwo* (*Raphidophyceae*) in the Piraquê Channel, Rodrigo de Freitas Lagoon, Southeast Brazil. *Braz. J. Biol.* 74, 529–537. doi: 10.1590/bjb.2014.0074

Burkholder, J. M., Dickey, D. A., Kinder, C. A., Reed, R. E., Mallin, M. A., McIver, M. R., et al. (2006). Comprehensive trend analysis of nutrients and related variables in a large eutrophic estuary: a decadal study of anthropogenic and climatic influences. *Limnol.Oceanogr.* 51, 463–487. doi: 10.4319/lo.2006.51.1_part_2.0463

Burkholder, J. M., Glibert, M. P., and Skelton, H. M. (2008). Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. *Harmful Algae* 8, 77–93. doi: 10.1016/j.hal.2008.08.010

Cadée, G. C., and Hegeman, J. (1979). Phytoplankton primary production, chlorophyll and composition in an inlet of the western Wadden Sea (Marsdiep). *Neth. J. Sea Res.*, 13, 224–241. doi: 10.1016/0077-7579(79)90004-8

Castro, N. O., Domingos, P., and Moser, G. A. O. (2016). National and international public policies for the management of harmful algal bloom events. A case study on the Brazilian coastal zone. *Ocean Coast. Manage.* 128, 40–51. doi: 10.1016/j.ocecoaman.2016.04.016

Chenna, R., Sugawara, H., Koike, T., Lopez, R., Gibson, T. J., Higgins, D. G., et al. (2003). Multiple sequence alignment with the Clustal series of programs. *Nucl. Acids Res.* 31, 3497–3500. doi: 10.1093/nar/gkg500

Cloern, J. E. (1987). Turbidity as a control on phytoplankton biomass and productivity in estuaries. *Cont. Shelf Res.* 7, 1367–1381. doi: 10.1016/0278-4343(87)90042-2

Edvardsen, B., and Imai, I. (2006). "The ecology of harmful flagellates within *Prymnesiophyceae* and *Raphidophyceae*," in *Ecology of Harmful Algae*, ed E. Granéli (Berlin: Springer Verlag), 67–79.

Edwards, M., Johns, D. G., Leterme, S. C., Svendsen, E., and Richardon, A. J. (2006). Regional climate change and harmful algal blooms in the northeast Atlantic. *Limnol. Oceanogr.* 51, 820–829. doi: 10.4319/lo.2006.51.2.0820

Fistarol, G. O., Coutinho, F. H., Moreira, A. P. B., Venas, T., Cánovas, A., de Paula, S. E. M. Jr., et al. (2015). Environmental and Sanitary Conditions of Guanabara Bay, Rio de Janeiro. *Front. Microbiol.* 6:1232. doi: 10.3389/fmicb.2015.01232

Fistarol, G. O., Hargreaves, P. I., Walter, J. M., Viana, T. V., and Gomes, P. D. F., Lourenço, et al. (2018). Rapid isolation of culturable microalgae from a tropical shallow lake system. *J. Appl. Phycol.* 30 1807–1819. doi: 10.1007/s10811-018-1404-7

Gameiro, C., Zwolinski, J., and Brotas, V. (2011). Light control on phytoplankton production in a shallow and turbid estuarine system. *Hydrobiologia* 669:249. doi: 10.1007/s10750-011-0695-3

Grasshoff, K., Erhardt, M., and Kremling, K. (1983). *Methods of Seawater Analysis*. Weinheim: Verlag Chemie.

Grimm, A. M., and Tedeschi, R. G. (2009). ENSO and extreme rainfall events in South America. *J. Climate* 22, 1589–1609. doi: 10.1175/2008JCLI2429.1

Guillard, R. R. L. (1973). "Division rates," in *Handbook of Phycological Methods: Culture Methods and Growth Measurements*, ed J. R. Stein (Cambridge: Cambridge University Press), 289–312.

Guillard, R. R. L. (1975). "Culture of phytoplankton for feeding marine invertebrates," in *Culture of Marine Invertebrate Animals*, eds W. L. Smith and M. H. Chanley (New York, NY: Plenum Press), 26–60.

Hallegraeff, G. M., Anderson, D. M., Cembella, A. D., and Enevoldsen, H. O. (2004). *Manual on Harmful Marine Microalgae*. Paris: UNESCO.

Hallegraeff, G. M., and Hara, Y. (2003). "Taxonomy of harmful marine raphidophytes," in *Manual on Harmful Marine Microalgae*, eds G. M. Hallegraeff, D. M. Anderson, A. D. Cembella, and H. O. Enevoldsen, (Paris: International Oceanographic Commission), 511–522.

Hallegraeff, G. M., Munday, B. L., Banden, D. G., and Whitney, P. L. (1998). "*Chattonella marina* raphidophyte bloom associated with mortality of cultured bluefin tuna (*Thunnus maccoyii*) in South Australia," in *Harmful Microalgae, VIII Conference on Harmful Algae,* Vigo (International Oceanographic Commission of *UNESCO)*, 1–5.

Hamilton, M. A., Russo, R. C., and Thurston, R. V. (1977). Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. *Environ. Sci. Technol.* 11, 714–719. doi: 10.1021/es60130a004

Handy, S. M., Coyne, K. J., Portune, K. J., Demir, E., Doblin, M. E., Hare, C. E., et al. (2005). Evaluating vertical migration behavior of harmful raphidophytes in the

Delaware Inland Bays utilizing quantitative real-time PCR. *Aquat. Microb. Ecol.* 40, 121–132. doi: 10.3354/ame040121

Heisler, J., Glibert, P. M., Burkholder, J. M., Anderson, D. M., Cochlan, W., Dennison, W. C., et al. (2008). Eutrophication and harmful algal blooms: a scientific consensus. *Harmful Algae* 8, 3–13. doi: 10.1016/j.hal.2008.08.006

Higashi, A., Nagai, S., Salomon, P. S., and Ueki, S. (2017). A unique, highly variable mitochondrial gene with coding capacity of *Heterosigma akashiwo*, class *Raphidophyceae*. *J. Appl. Phycol.* 29, 2961–2969. doi: 10.1007/s10811-017-1142-2

Higgins, D. G., and Sharp, P. M. (1988). CLUSTAL: a package for performing multiple sequence alignment on a microcomputer. *Gene* 73, 237–244. doi: 10.1016/0378-1119(88)90330-7

Hiroishi, S., Okada, H., Imai, I., and Yoshida, T. (2005). High toxicity of the novel bloom-forming species *Chattonella ovata* (*Raphidophyceae*) to cultured fish. *Harmful Algae* 4, 783–787. doi: 10.1016/j.hal.2004.12.008

Hosoi-Tanabe, S., Otake, I., and Sako, Y. (2006). Phylogenetic analysis of noxious red tide flagellates *Chattonella antiqua, C. marina, C. ovata, and C. verruculosa (Raphidophyceae)* based on the rRNA gene family. *Fish. Sci.* 72, 1200–1208. doi: 10.1111/j.1444-2906.2006.01277.x

Imai, I. (1989). Cyst formation of the noxious red tide flagellate *Chattonella marina* (*Raphidophyceae*) in culture. *Mar. Biol.* 103, 235–239. doi: 10.1007/BF00543353

Imai, I., and Yamaguchi, M. (2012). Life cycle, physiology, ecology and red tide occurrences of the fish-killing raphidophyte *Chattonella*. *Harmful Algae* 14, 46–70. doi: 10.1016/j.hal.2011.10.014

Imai, I., Yamaguchi, M., and Hori, Y. (2006). Eutrophication and occurrences of harmful algal blooms in the Seto Inland Sea, Japan. *Plankton Benthos Res.* 1, 71–84. doi: 10.3800/pbr.1.71

Jeong, H. J., Seong, K. A., Kang, N. S., Yoo, Y. D., Nam, S. W., Park, J. Y., et al. (2010). Feeding by raphidophytes on the cyanobacterium *Synechococcus* sp. *Aquat. Microb. Ecol.* 58, 181–195. doi: 10.3354/ame01354

Joint, I. R., and Pomroy, A. J. (1981). Primary production in a turbid estuary. *Estuar. Coastal Shelf S.* 13, 303–316. doi: 10.1016/S0302-3524(81)80028-X

Jones, R. I. (1994). Mixotrophy in planktonic protists as a spectrum of nutritional strategies. *Marine Microbial Food Webs* 8, 87–96.

Jones, R. I. (2000). Mixotrophy in planktonic protists: an overview. *Freshwater Biol.* 45, 219–226. doi: 10.1046/j.1365-2427.2000.00672.x

Katano, T., Yoshida, M., Lee, J., Han, M. S., and Hayami, Y. (2009). Fixation of Chattonella antiqua and C. *marina (Raphidophyceae) using* Hepes-buffered paraformaldehyde and glutaraldehyde for flow cytometry and light microscopy. *Phycologia* 48, 473–479. doi: 10.2216/08-102.1

Khan, S., Arakawa, O., and Onoue, Y. (1995). Effects of physiological factors on morphology and motility of Chattonella antiqua (*Raphidophyceae*). *Bot. Mar.* 38, 347–354. doi: 10.1515/botm.1995.38.1-6.347

Kjerfve, B., Ribeiro, C. H. A., Dias, G. T. M., Filippo, A. M., and Quaresma, V. S. Q. (1997). Oceanographic characteristics of an impacted coastal bay: Baía de Guanabara, Rio de Janeiro, Brazil. *Cont. Shelf Res.* 17, 1609–1643. doi: 10.1016/S0278-4343(97)00028-9

Klöpper, S., John, U., Zingone, A., Mangoni, O., Kooistra, W. H. C. F., and Cambella, A. (2013). Phylogeny and morphology of a *Chattonella* (*Raphidophyceae*) species from the Mediterranean Sea: what is *C. subsalsa? Eur. J. Phycol.* 48, 79–92. doi: 10.1080/09670262.2013.771412

Kumar, S., Stecher, G., and Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33, 1870–1874. doi: 10.1093/molbev/msw054

Lewitus, A. J., Brock, L. M., Burke, M. K., DeMattio, K. A., and Wilde, S. B. (2008). Lagoonal stormwater detention ponds as promoters of harmful algal blooms and eutrophication along the South Carolina coast. *Harmful Algae* 8, 60–65. doi: 10.1016/j.hal.2008.08.012

Lewitus, A. J., Schmidt, L. B., Mason, L. J., Kempton, J. W., Wilde, S. B., Wolny, J. L., et al. (2003). Harmful algal blooms in South Carolina residential and golf course ponds. *Popul. Environ.* 24, 387–414. doi: 10.1023/A:1023642908116

Lincoln, R. A., Strupinski, K., and Walker, J. M. (2008). The use of *Artemia* nauplii (brine shrimp larvae) to detect toxic compounds from microalgal cultures. *Pharm. Biol.* 34, 384–389. doi: 10.1076/phbi.34.5.384.13255.

Lindholm, T., Öhman, P., Kurki-Helasmo, K., Kincaid, B., and Meriluoto, J. (1999). Toxic algae and fish mortality in a brackish-water lake in Åland, SW Finland. *Hydrobiologia* 397, 109–120.

Marshall, J. A., and Hallegraeff, G. M. (1999). Comparative ecophysiology of the harmful alga *Chattonella marina* (*Raphidophyceae*) from South Australian and Japanese waters. *J. Plankton Res.* 21, 1809–1822. doi: 10.1093/plankt/21.10.1809

Marshall, J. A., Nichols, P. D., Hamilton, B., Lewis, R. J., and Hallegraeff, G. M. (2003). Ichthyotoxicity of *Chattonella marina* (*Raphidophyceae*) to damselfish (*Acanthochromis polycanthus*): the synergistic role of reactive oxygen species and free fatty acids. *Harmful Algae* 2, 273–281. doi: 10.1016/S1568-9883(03)00046-5

Mayr, L. M., Tenenbaum, D. R., Villac, M. C., Paranhos, R., Nogueira, C. R., Bonecker, S. L., et al. (1989). "Hydrobiological characterization of Guanabara Bay," in *Coastlines of Brazil*, eds O. Maggon and C. Neves (NewYork, NY: American Society of Civil Engineers), 124–138.

Nakamura, Y., Takashima, J., and Watanabe, M. (1989). Chemical environment for red tides due to *Chattonella antiqua*. *J. Oceanogr. Soc. Japan* 45, 116–128. doi: 10.1007/BF02302618

Nakamura, Y., Umemori, T., Watanabe, M., Kulis, D. M., and Anderson, D. M. (1990). Encystment of *Chattonella antiqua* in laboratory cultures. *J. Oceanogr. Soc. Japan*, 46, 35–43. doi: 10.1007/BF02124813

Nakamura, Y., and Watanabe, M. M. (1983). Growth characteristics of *Chattonella antiqua*. Part 1. Effects of temperature, salinity, light intensity and pH on growth. *J. Oceanogr. Soc. Japan* 39, 110–114. doi: 10.1007/BF02070796

Nygaard, K., and Tobiesen, A. (1993). Bacterivory in algae: a survival strategy during nutrient limitation. *Limnol. Oceanogr.* 38, 273–279. doi: 10.4319/lo.1993.38.2.0273

Odebrecht, C., Villac, M. C., Abreu, P. C., Haraguchi, L., Gomes, P. D. F., and Tenenbaum, D. R. (2018). "Flagellates versus diatoms: phytoplankton trends in tropical and subtropical estuarine-coastal ecosystems" in *Plankton Ecology of the Southwestern Atlantic,* eds M. Hoffmeyer, M. Sabatini, F. Brandini, D. Calliari, and N. Santinelli (Cham: Springer), 249–267. doi: 10.1007/978-3-319-77869-3_12

Oliveira, E. N., Fernandes, A. M., Kampel, M., Cordeiro, R. C., Brandini, N., Vizon, S. B., et al. (2016). Assessment of remotely sensed chlorophyll-a concentration in Guanabara Bay, Brazil. *J. Appl. Remote Sens.* 10, 026003–026001. doi: 10.1117/1.JRS.10.026003

Onitsuka, G., Aokim, K., and Shimizu, M. (2015). Meteorological conditions preceding *Chattonella* bloom events in the Yatsushiro Sea, Japan, and possible links with the East Asian monsoon. *Fish. Sci.* 81, 123–130. doi: 10.1007/s12562-014-0812-0

Paranhos, R., Andrade, L., Mendonça-Hagler, L. C., and Pfeiffer, W. C. (2001). "Coupling bacterial density with activity in a tropical polluted coastal bay," in *Aquatic Microbial Ecology in Brazil*, Vol. 9, eds B. M. Faria, V. Farjalla, and F. A. *Esteves (Rio de Janeiro: Serie Oecologia Brasiliensis)*, 119–132.

Paranhos, R., and Mayr, L. M. (1993). Seasonal patterns of temperature and salinity in Guanabara Bay, Brazil. *Fresenius Environ. Bull.* 2, 647–652.

Paranhos, R., Pereira, A. P., and Mayr, L. M. (1998). Diel variability of water quality in a tropical polluted bay. *Environ. Monit. Assess.* 50, 131–141. doi: 10.1023/A:1005855914215

Parsons, T. R., Maita, Y., and Lalli, C. (1984). *A Manual of Chemical and Biological Methods for Seawater Analysis*. New York, NY: Pergamon Press.

Pennock, J. R., and Sharp, J. H. (1986). Phytoplankton production in the Delaware Estuary: temporal and spatial variability. *Mar. Ecol. Prog. Ser.* 34, 143–155. doi: 10.1016/0272-7714(87)90156-9

Portune, K. J., Coyne, K. J., Hutchins, D. A., Handy, S. M., and Cary, S. C. (2009). Germination of *Heterosigma akashiwo* and *Chattonella subsalsa* (*Raphidophyceae*) cysts in Delaware's Inland Bays, DE. *Aquat. Microb. Ecol.* 55, 229–239. doi: 10.3354/ame01292

Rogers, S. O., and Bendich, A. J. (1994). "Extraction of total cellular DNA from plants, algae and fungi," in *Plant Molecular Biology Mannual*, eds S. B. Gelvin and R. A Schilperoort (Netherlands: Springer), 183–190. doi: 10.1007/978-94-011-0511-8_12

Ryding, S.-O., and Rast, W. (1989). *The Control of Eutrophication of Lakes and Reservoirs*. Paris: UNESCO and The Parthenon Publishing Group.

Santos, V. S., Villac, M. C., Tenenbaum, D. R., and Paranhos, R. (2007). Auto- and heterotrophic nanoplankton and filamentous bacteria of Guanabara Bay (RJ, Brazil): estimates of cell/filament numbers versus carbon content. *Braz. J. Oceanogr.* 55, 133–143. doi: 10.1590/S1679-87592007000200006

Scholin, C. A., Herzog, M., Sogin, M., and Anderson, D. M. (1994). Identification of group- and strain-specific genetic markers for globally distributed

Alexandrium (*Dinophyceae*) II. Sequence analysis of a fragment of the LSU rRNA gene. *J. Phycol.* 30: 999–101. doi: 10.1111/j.0022-3646.1994.00999.x

Sevrin-Reyssac, J., Machado, M. C., Schutze, M. L. M., Ribas, S. G., Costa de Lima, I., Lima, C. A., et al. (1979). Biomasse et production du phytoplancton de la baie de Guanabara (État de Rio de Janeiro, Brésil) et du secteur óceanique adjacent. Variations de mai à juillet 1978. *B. Mus. Natl. Hist. Nat.* 4, 329–354.

Strom, S. L., Harvey, E. L., Frederickson, K. A., and Menden-Deuer, S. (2013). Broad salinity tolerance as a refuge from predation in the harmful raphidophyte alga *Heterosigma akashiwo* (*Raphidophyceae*). *J. Phycol.* 49, 20–31. doi: 10.1111/jpy.12013

Throndsen, J. (1997). "The planktonic marine flagellates," in *Identifying Marine Phytoplankton*, ed C. R. Thomas, (San Diego, CA: Academic Press), 591–729.

Tomas, C. R., Bridgers, A. M., and Smith, M. D. (2002). *Chattonella subsalsa Biecheler. North Carolina Sea Grant.* Marine Phytoplankton Identification Series. UNC-SG-02-04.

Valentin, J. L., Tenenbaum, D. R., Bonecker, A. C., Bonecker, S. L. C., Nogueira, C. R., and Villac, M. C. (1999). O Sistema Planctônico da Baía de Guanabara: Síntese do Conhecimento. Ecologia de Ambientes Costeiros do Estado do Rio de Janeiro. Series Oecologia Brasiliensis. *Rio de Janeiro*, 7, 35–59.

Villac, M. C., and Tenenbaum, D. R. (2010). The phytoplankton of Guanabara Bay, Brazil: I. historical account of its biodiversity. *Biota Neotrop.* 10, 271–293. doi: 10.1590/S1676-06032010000200030

Wang, G. (2017). Continued increase of extreme El Niño frequency long after 1.5°C warming stabilization. *Nat. Clim. Change* 7, 568–572. doi: 10.1038/nclimate3351

Wolter, K., and Timlin, M. S. (1993). "Monitoring ENSO in COADS with a seasonally adjusted principal component index," in *Proceedings of the 17th Climate Diagnostics Workshop, Norman, OK, NOAA/NMC/CAC, NSSL, Oklahoma Climate Survey, CIMMS and the School of Meteorology, University of Oklahoma*: Norman, OK, 52–57.

Wolter, K., and Timlin, M. S. (1998). Measuring the strength of ENSO events – how does 1997/98 rank? *Weather* 53, 315–324. doi: 10.1002/j.1477-8696.1998.tb06408.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Viana, Fistarol, Amario, Menezes, Carneiro, Chaves, Hargreaves, Silva-Lima, Valentin, Tenenbaum, Arruda, Paranhos and Salomon. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Marine Science

ORIGINAL RESEARCH
published: 27 March 2019
doi: 10.3389/fmars.2019.00145

Spatio-Temporal Pattern of Dinoflagellates Along the Tropical Eastern Pacific Coast (Ecuador)

Gladys Torres[1], Olga Carnicer[2], Antonio Canepa[3], Patricia De La Fuente[4], Sonia Recalde[1], Richard Narea[1], Edwin Pinto[1] and Mercy J. Borbor-Córdova[5]*

[1] Instituto Oceanográfico de la Armada del Ecuador, Guayaquil, Ecuador, [2] Escuela de Gestión Ambiental, Pontificia Universidad Católica del Ecuador Sede Esmeraldas (PUCESE), Esmeraldas, Ecuador, [3] Escuela Politécnica Superior, Universidad de Burgos, Burgos, Spain, [4] Institut de Ciències del Mar, CSIC, Barcelona, Spain, [5] Escuela Superior Politécnica del Litoral (ESPOL), Facultad de Ingeniería Marítima y Ciencias del Mar, Guayaquil, Ecuador

Among marine phytoplankton, dinoflagellates are a key component in marine ecosystems as primary producers. Some species synthesize toxins, associated with human seafood poisoning, and mortality in marine organisms. Thus, there is a large necessity to understand the role of environmental variables in dinoflagellates spatial-temporal patterns in response to future climate scenarios. In that sense, a monthly four-year (2013–2017) monitoring was taken to evaluate dinoflagellates abundances and physical-chemical parameters in the water column at different depths. Sampling sites were established at 10 miles in four locations within the Ecuadorian coast. A total of 102 taxa were identified, corresponding to 8 orders, 22 families, and 31 genera. Eight potentially harmful genera were registered but no massive blooms were detected. The most frequent dinoflagellates were *Gymnodinium sp*. and *Gyrodinium sp*. Environmental variables showed different mixing layer thickness and a conspicuous and deepening thermocline/oxycline/halocline and nutricline depending on annual and seasonal oceanographic fluctuations. This study confirms that seasonal and spatial distribution of the environmental variables are linked to the main current systems on the Eastern Tropical Pacific, thus the warm Panama current lead to a less dinoflagellates abundance in the north of Ecuador (Esmeraldas), while the Equatorial Upwelling and the cold nutrient-rich Humboldt Current influence dinoflagellates abundance at the central (Manta, La Libertad) and South of Ecuador (Puerto Bolivar), respectively. Inter-annual variability of dinoflagellates abundance is associated with ENSO and upwelling conditions. Climate change scenarios predict an increase in water surface temperature and extreme events frequency in tropical areas, so it is crucial to involve policy-makers and stakeholders in the implementation of future laws involving long-term monitoring and sanitary programs, not covered at present.

Keywords: dinoflagellates, HABs, ENSO, tropical Eastern Pacific, nutrients, upwelling, humboldt current

OPEN ACCESS

Edited by:
Jorge I. Mardones, Instituto de Fomento Pesquero (IFOP), Chile

Reviewed by:
Sai Elangovan S, National Institute of Oceanography (CSIR), India
Punyasloke Bhadury, Indian Institute of Science Education and Research Kolkata, India

***Correspondence:**
Olga Carnicer
olgacarnicer@gmail.com

Specialty section:
This article was submitted to Marine Ecosystem Ecology, a section of the journal Frontiers in Marine Science

Received: *10 September 2018*
Accepted: *07 March 2019*
Published: *27 March 2019*

Citation:
Torres G, Carnicer O, Canepa A, De La Fuente P, Recalde S, Narea R, Pinto E and Borbor-Córdova MJ (2019) Spatio-Temporal Pattern of Dinoflagellates Along the Tropical Eastern Pacific Coast (Ecuador). Front. Mar. Sci. 6:145. doi: 10.3389/fmars.2019.00145

INTRODUCTION

The Equatorial Pacific region is characterized by a unique complex oceanographic variability with a well-defined Equatorial Front where El Niño Southern Oscillation (ENSO) events eventually occur (Santos, 2006; Gierach et al., 2012). The Ecuadorian coast is divided in two biogeographical regions, a northern "Bay of Panama" ecoregion from Azuero Peninsula to Bahía de Caráquez and a

southern "Guayaquil" ecoregion from Bahía de Caráquez to the Illescas peninsula in Perú (Sullivan and Bustamante, 1999). The northern coast of Ecuador is influenced by the warm current of Panama and the current of El Niño, while the Humboldt current brings cold water to the southern Ecuadorian coast which contributes to outcrops formation in the area (Cucalon, 1989). The southern sector is the most productive area regarding fisheries and shrimp production (Pennington et al., 2006). Due to the variability found in oceanographic conditions over the Ecuadorian coast, there is a noticeable heterogeneity concerning physical, chemical, and biological parameters which leads to a high diversity in phytoplankton communities (Smayda, 2008) and the potential formation of harmful algae blooms (HABs).

The oceanographic conditions identified as drivers of HABs, are linked to those conditions associated to upwelling systems (cold nutrient-rich waters), high column water stratification (mostly associated to ENSO conditions), and long-term sea surface temperature (SST) increase due to climate change (Hallegraeff, 2010; McCabe et al., 2016; Kudela et al., 2017). Those studies that have related the particular oceanographic conditions with the occurrence of HABs in the Pacific Ocean (Brown et al., 2003; Franco-Gordo et al., 2004; Moore et al., 2010), have shown that those factors module the phytoplankton community and dynamics. In general, the phytoplankton community on the Eastern Tropical Pacific (ETP) region is characterized by relatively low levels of biomass, with the dominance of small species and rare large bloom-forming diatoms (Brown et al., 2003). Diatoms and dinoflagellates are two major groups of phytoplankton that flourish in the oceans (Abate et al., 2017), mostly registered in the south-eastern Equatorial Front (Jimenez-Bonilla, 1980; Torres and Tapia, 2002), and upwelling systems (Shulman et al., 2012). Particularly, dinoflagellates exhibit environmentally induced adaptation and survival to changing environmental conditions (Kudela et al., 2010).

Among the dinoflagellate community, there are some toxin producer dinoflagellates which may impact negatively marine ecosystems causing massive marine organism mortality, cause economic damage in coastal locations and affect human health by seafood consumption (Li et al., 2014). In addition, ballast waters contribute to the transfer of phytoplankton in commercial and oil tankers resulting in the dispersion of non-native phytoplankton species, increasing the risk of phytoplankton proliferation (Hallegraeff, 2003). Moreover, physiological changes may affect phytoplankton communities in response to climate change (Poloczanska et al., 2016) with consequences in their distribution and abundances worldwide. In that sense, it is important to perform studies related to phytoplankton biodiversity characterization and its spatio-temporal variability to guarantee an optimal integrated coastal management, contributing to future decisions from policy-makers to optimize waters vigilance.

Some countries of Latin America, such as Chile or Mexico, have implemented HABs monitoring regarding seafood production safety (Daguer et al., 2018; León-Muñoz et al., 2018). However, in Ecuador there is a lack of a national regulation plan, related to phytoplankton observations. Nevertheless, the Oceanographic Institute of Ecuador (INOCAR) has performed periodic monitoring since 1989 along the Ecuadorian coast. Torres (2015) observed the presence of algal proliferations along the Ecuadorian coast, which were more frequent in the Gulf of Guayaquil, where most aquaculture activities are located. Therefore, through the analysis of the INOCAR data bases, this study aims to provide information related to dinoflagellates abundances dynamic across a broad latitudinal area from 2013 to 2017 that constitute a base-line for future investigations.

MATERIALS AND METHODS

Study Area and Samples Collection

The study area was located between 0°89 North and 03°036 South, in the ETP Ocean. Along the study area, four geographic points were settled as sampling stations: three of them located 10 miles from the coast: (1) Esmeraldas (Galera San Francisco), (2) Manta, (3) La Libertad (Santa Elena Peninsula), and (4) Puerto Bolívar (**Figure 1**). All stations have an approximate depth of 100 m. except for Puerto Bolívar station, which is located approximately 27 miles, inside the submarine platform in a pit that is 9 m deep, northeast of Santa Clara Island in the Gulf of Guayaquil.

At each sampling station, 200 mL water samples were collected in plastic bottles from surface, 10, 20, 30, 40, 50, and 75 m depth using a 3 Liter volume Vand Dorn bottle, then preserved in neutral Lugol's solution (final concentration 0.4%). Samplings were performed monthly from February 2013 to December 2017, between 09h00 to 12h00 am.

Phytoplankton Identification and Abundance Estimation

Water samples were settled in 25 mL volume Utermöhl chambers (Utermöhl, 1958), and observed in a Leica (ML) inverted microscope after 24 h. Cell abundance estimation was performed in horizontal transects of the chamber at 400× magnification. Results are reported in cell. L^{-1}. The validity of names of the different taxa was checked on the World Register of Marine Species (Horton et al., 2018). Dinoflagellate species identification was based on Steidinger and Jangen (1997); Hoppenrath et al. (2009)Hoppenrath et al. (2014); Omura et al. (2012), and Lassus et al. (2016). The list of toxic species was checked on the Taxonomic Reference List of Harmful Micro Algae (Moestrup et al., 2009).

In Esmeraldas and Puerto Bolívar no sampling was performed during February to December 2016 due to economic issues.

Environmental Variables

Temperature and salinity vertical profiles were obtained using a SBE 19plus V2 SeaCAT Profiler CTD, at each sampling site. Data was processed using the SEABIRD software. Water samples for dissolved oxygen and nutrient estimation were collected with Van Dorn bottles at same depths as for phytoplankton analysis. Determination of dissolved oxygen for each sample was carried out *in situ* following the protocol for Dissolved Oxygen Test PEE/LAB-DOQ/01-INOCAR based on Apha (2005). Water samples for nutrient analysis were filtered with 0.45 μm millipore

filters and kept at −20°C. Nitrate, silicate and total phosphate were analyzed by techniques described in Test PEE/LAB-DOQ/01-INOCAR based on Strickland and Parsons (1972). For phosphates, as values in Equatorial Pacific waters are below 3 μmol. L^{-1}, the analysis followed is detailed in Part II of the protocol cited above. In Esmeraldas and Puerto Bolívar there is no data for dissolved oxygen and nutrients from February to December 2016 due to economic issues. In this study N/P is the ratio between Nitrate and Phosphate.

Data Analysis

Before any formal statistical analysis, an exploratory data analysis (EDA) was conducted to both, environmental and biological databases in order to avoid further statistical problems (i.e., proper family distribution of the variable, homoscedasticity, independence of the values), as suggested by Zuur et al. (2010). Beside this, the environmental variables were inspected in order to avoid collinearity (i.e., correlation among explanatory variables), thus all variables who showed a Pearson correlation coefficient (rho) higher than 0.7 were dropped from the analysis (**Supplementary Figure S1**). Community analysis considered the calculation of the common Shannon-Weaver diversity index and its spatio-temporal variability. From an ecological perspective those species with a percentage of occurrence higher than 2% were related with the environmental variables using a canonical correspondence analysis (CCA) for each year (ter Braak and Verdonschot, 1995) through the "cca" function of the *vegan* package (Oksanen et al., 2018). The significance of the models were achieved through the function "anova.cca" (from the vegan package, op.cit) which performs an ANOVA like permutation test using 999 permutations (Borcard et al., 2018). All the statistical analysis were conducted under the R software (R Core Team, 2018; version 3.4.4) and supervised by OneMind-DataScience[1].

RESULTS

Dinoflagellate Community

Dinoflagellate Occurrence

During the studied period, a total of 102 dinoflagellate taxa were identified, corresponding to 8 orders, 22 families and 31 genera (**Table 1**). The genera *Tripos* and *Protoperidinium* were the most diverse, comprising 22 and 16 species, respectively (**Table 1**).

The most dominating taxa were *Gymnodinium sp* (70% occurrence), *Gyrodinium sp* (53%), and *Gyrodinium acutum* (21%). Other representative genera were *Protoperidinium* spp (16%), *Oxytoxum* spp (14%), *Prorocentrum micans* (13%), *Scripsiella* spp (12%), *Gonyaulax* spp (10%), and *Oxytoxum scolopax* (9.5%). The rest of the taxa presented lower percentage of occurrence (**Table 1**).

With respect to the temporal variability of the species mentioned above (most representative in terms of occurrence), *Gymnodinium sp, Gyrodinium sp, Oxytoxum* spp, and *O. scolopax* were present during the entire study period (**Table 1**). *Gonyaulax* spp was present also for both seasons (wet and dry seasons) and all years except 2016, which was not observed (**Table 1**). *Gyrodinium acutum* and *Scripsiella* spp was found in both wet and dry seasons, but only for the 2015 to 2017 period (**Table 1**).

Protoperidinium spp was found also in both wet and dry seasons but for the 2013–2015 period (**Table 1**). Instead, for the years 2016 and 2017, *Protoperidinium* spp was only observed during the dry season. *P. micans* was only found in dry season for 2013 and for wet season in 2016; instead during the period 2015–2017, *P. micans* was present in both seasons (**Table 1**).

Spatio-Temporal Variability

The two most dominant species, *Gymnodinium sp* and *Gyrodinium sp,* were more abundant in Manta, with bloom formation up to 1.2×10^6 cell. L^{-1} for *Gymnodinium sp* in April 2017 (**Figure 2A**) and 5.4×10^4 cell. L^{-1} for *Gyrodinium sp* in February 2016 (**Figure 2B**).

Regarding cell abundance estimation, 2013 registered for the entire water column studied (0–75 m depth) the lowest abundances comparing with the rest of the years (**Figure 3**). For 2013, the maximum values were found at surface in La Libertad (mostly corresponding to March (2.8×10^5 cell. L^{-1}) and at 10 m depth in Puerto Bolívar (mostly in February, 2.0×10^5 cell. L^{-1}). In 2014, abundances were higher in the first 10 m of the water column except for Esmeraldas where abundances were similar in the first 30 m, being lower comparing with the other sampling sites for the same year (**Figure 3**). The maximum abundances in 2014, were observed at 10 m in Manta and at surface in Puerto Bolivar (with the maximum value corresponding to May, with 2.9×10^5 cell. L^{-1} and 3.8×10^5 cell. L^{-1}, respectively). In 2015, Esmeraldas registered again the lowest concentrations in the first layers, comparing with the other sampling sites for the same year. The same vertical pattern was observed, as in 2014, with similar abundances from surface until 30 m (**Figure 3**). Maximum of the year occurred in La Libertad at surface, in March (1.1×10^6 cell. L^{-1}) and at 20 m depth in Puerto Bolívar in July (maximum value of 4.5×10^5 cell. L^{-1}). No peaks were registered during the year 2016, but data is not available for Esmeraldas and Puerto Bolivar. Thus, the maximum concentrations registered were observed in the first 20 m for Manta and La Libertad sites (**Figure 3**). The maximum abundances were found at surface and at 75 m depth for Manta (in February with 1.7×10^5 cell. L^{-1} and 1.4×10^5 cell. L^{-1}, respectively) and at surface for La Libertad site (with maximum value for March with 1.0×10^5 cell. L^{-1}). The highest concentrations of the sampling period were reported in 2017 (**Figure 3**), mostly between January and June. The maximum abundance was found in Manta, in April at surface, 1.5×10^6 cell. L^{-1} and at 10 m depth in Manta with an abundance of 4.0×10^5 cell. L^{-1}. In February, at the first 10 m layer, Manta and Esmeraldas sites registered also high abundance values ($\sim 3.5 \times 10^5$ cell. L^{-1} and $\sim 2.5 \times 10^5$ cell. L^{-1}, respectively). The pattern of decreasing dinoflagellate concentration with depth, (very significant from 20 m onward) observed in the vertical abundances' distribution for the rest of the study period, in 2017 is not so marked (**Figure 3**). In 2017, high abundances are observed in more deep layers (30–40 m) respecting to the

[1]https://onemind-datascience.com/

TABLE 1 | Occurrence of dinoflagellates taxa (D, dry season; R, rainy season; + presence).

Order	Family	Species	Ocurrence (%)	2013		2014		2015		2016		2017	
				R	D	R	D	R	D	R	D	R	D
Dinophysiales	Amphisoleniaceae	*Dinophysis bidentata* Schröder, 1900	0,21	+	+				+				
	Dinophysiaceae	*Dinophysis acuminata* Claparéde and Lachmann, 1859	0,62						+	+		+	+
		Dinophysis caudata Saville-Kent, 1881	1,46	+	+		+	+	+			+	+
		Dinophysis cf. *rotondata* Claparéde and Lachmann, 1859	1,45						+			+	+
		Dinophysis ovum Schutt, 1895	1,45	+	+	+	+		+			+	+
		Dinophysis spp. Ehrenberg, 1839	2,56			+	+	+	+			+	+
		Ornithocercus quadratus Schutt, 1900	0,14										+
		Ornithocercus steinii Schutt, 1900	0,42		+		+		+				+
		Ornithocercus thumii (Schmidt) Kofoid and Skogsberg, 1928	0,14		+								
	Oxyphysaceae	*Phalacroma doryphorum* Stein, 1883	0,07										+
		Phalacroma rapa Jorgensen, 1923	0,07								+		
Gonyaulacales	Ceratiaceae	*Tripos boehmii* (H.W.Graham and Bronikovsky) F.Gómez, 2013	0,07			+							
		Tripos breve (Ostenfeld and Schmidt) F. Gómez, 2013	0,28					+	+	+			
		Tripos candelabrus (Ehrenberg) F. Gómez, 2013	0,35	+	+		+						+
		Tripos declinatus (G.Karsten) F.Gómez, 2013	0,83	+	+		+	+					+
		Tripos deflexus (Kofoid) F.Gómez, 2013	0,48		+		+			+	+	+	+
		Tripos dens (Ostenfeld and Johannes Schmidt) F.Gómez, 2013	0,97		+	+	+	+	+	+			+
		Tripos extensus (Gourret) F.Gómez, 2013	0,07										+
		Tripos falcatus (Kofoid) F.Gómez, 2013	0,21		+		+		+				
		Tripos falcatiformis (Jörgenen) F.Gómez, 2013	0,07			+							
		Tripos furca (Ehrenberg) F. Gómez, 2013	4,85	+	+	+	+	+	+	+		+	+
		Tripos fusus (Ehrenberg) F.Gómez, 2013	3,88	+	+	+	+	+	+	+	+	+	+
		Tripos hexacanthus (Gourret) F.Gómez, 2013	0,07						+				
		Tripos lunula (Schimper ex Karsten) F.Gómez, 2013	0,07										+
		Tripos massiliensis (Gourret) F.Gómez, 2013	0,35	+				+	+				+
		Tripos muelleri Bory de Saint-Vincent, 1824 , F. Gómez, 2013	1,11	+		+	+	+	+	+	+	+	+
		Tripos pentagonus (Gourret) F.Gómez, 2013 var. tenerum Jorgensen, 1920	3,11		+	+	+				+		
		Tripos pulchellus (Schröder) F.Gómez, 2013	0,21				+						
		Tripos spp. Tripos Bory de Saint-Vincent, 1823	0,28									+	
		Tripos strictus (Okamura and Nishikawa) F.Gómez, 2013	0,07							+			
		Tripos subrobustus (Jørgesen) F.Gómez, 2013	1,45	+		+	+	+	+	+	+	+	+
		Tripos teres (Kofoid) F.Gómez, 2013	0,28				+	+		+			
		Tripos trichoceros (Ehrenberg) Gómez, 2013	1,39		+	+	+	+	+	+		+	+

(Continued)

TABLE 1 | Continued

Order	Family	Species	Ocurrence (%)	2013		2014		2015		2016		2017	
				R	D	R	D	R	D	R	D	R	D
	Ceratocoryaceae	*Ceratocorys horrida* (Schütt) Kofoid, 1910	0,14				+						+
	Goniodomataceae	*Triadinium polyedricum* (Pouchet) Dodge, 1981	0,07										+
	Gonyaulacaceae	*Gonyaulax polygramma* Stein, 1883	3,05	+				+	+	+		+	+
		Gonyaulax spinifera (Claparède and Lachmann) Diesing, 1866	1,11						+	+		+	
		Gonyaulax spp. Diesing, 1866	9,83	+	+	+	+	+	+			+	+
		Gonyaulax turbynei Murray and Whitting, 1899	0,28	+				+	+				
		Lingulodinium polyedra (F.Stein) J.D.Dodge, 1989	0,07										+
		Lingulodinium spp. D.Wall, 1967	1,52	+			+		+	+	+	+	+
	Heterodiniaceae	*Heterodinium* spp. Kofoid, 1906	0,42					+					
	Ostreopsidaceae	*Alexandrium* spp. Halim, 1960	0,55						+	+	+	+	+
	Pyrophacaceae	*Pyrophacus steinii* (Schiller) Wall and Dale, 1971	0,62				+	+	+			+	+
Gymnodiniales	Gymnodiniaceae	*Amphidinium carterae* Hulburt, 1957	0,07									+	
		Amphidinium spp. Claparéde and Lachmann, 1859	0,48					+	+	+	+		
		Cochlodinium spp. Schütt, 1896	1,04				+	+	+			+	+
		Gymnodinium catenatum H.W.Graham, 1943	3,12	+		+	+	+	+		+	+	+
		Gymnodinium spp. Stein, 1878	69,94	+	+	+	+	+	+	+	+	+	+
		Gyrodinium spirale (Bergh) Kofoid and Swezy, 1921	3,39						+	+	+	+	+
		Gyrodinium spp. Kofoid and Swezy, 1921	52,7	+	+	+	+	+	+	+	+	+	+
		Gyrodinium acutum (Schütt) Kofoid and Swezy, 1921	24,72					+	+	+	+	+	+
		Margalefidinium catenatum (Okamura, 1916) F.Gómez, Richlen and D.M.Anderson, 2017	6,23				+	+	+	+	+	+	+
	Kareniaceae	*Karenia* spp. G.Hansen and Moestrup, 2000	0,07									+	
		Karenia mikimotoi (Miyake and Kominami ex Oda) Gert Hansen and Ø.Moestrup, 2000	0,28								+		+
	Polykrikaceae	*Polykrikos kofoidii* Chatton, 1914	0,07										
Noctilucales	Noctilucidae	*Noctiluca scintillans* (Macartney) Kofoid and Swezy, 1921	1,32				+	+	+	+	+		+
	Protodiniferaceae	*Pronoctiluca rostrata* F.J.R.Taylor, 1976	0,48									+	+
		Pronoctiluca spp Fabre-Domergue, 1889	0,07										+
Peridiniales	Oxytoxaceae	*Corythodinium tesselatum* (Stein) Loeblich Jr. and Loeblich, III, 1966	0,35								+		+
		Corythodinium sp 1 Loeblich Jr. and Loeblich III, 1966	0,07										+
		Corythodinium sp 2 Loeblich Jr. and Loeblich III, 1966	0,07										+
		Oxytoxum mediterraneum Schiller	0,14										+
		Oxytoxum scolopax Stein, 1883	9,56	+	+	+	+	+	+	+	+	+	+
		Oxytoxum sceptrum (F.Stein) Schröder, 1906	0,14	+									
		Oxytoxum turbo Kofoid, 1907	2,63	+		+	+					+	
		Oxytoxum spp. Stein, 1883	14,4	+	+	+	+	+	+	+	+	+	+

(Continued)

TABLE 1 | Continued

Order	Family	Species	Ocurrence (%)	2013		2014		2015		2016		2017	
				R	D	R	D	R	D	R	D	R	D
	Peridiniaceae	*Scrippsiella* Balech spp. ex A.R.Loeblich III, 1965	12,12					+	+	+	+	+	+
	Podolampadaceae	*Blepharocysta splendor-maris* (Ehrenberg) Ehrenberg, 1873	0,21						+				+
		Podolampas bipes Stein, 1883	0,21	+					+				+
		Podolampas palmipes Stein, 1883	1,66	+	+	+	+			+		+	+
		Podolampas spinifera Okamura, 1912	0,42		+	+	+						
	Protoperidiniaceae	*Preperidinium meunieri* (Pavillard) Elbrächter, 1993	0,21						+	+			+
		Protoperidinium bispinum (Schiller, 1937) Balech, 1974	0,14										+
		Protoperidinium brochii (Kofoid and Swezy, 1921) Balech, 1974	1,94	+	+	+	+		+			+	+
		Protoperidinium cassum (Balech, 1971) Balech, 1974	0,9				+	+	+	+			
		Protoperidinium compressum (Abé, 1927) Balech, 1974	0,9						+		+	+	+
		Protoperidinium depressum (Bailey, 1854) Balech, 1974	0,35	+					+	+		+	
		Protoperidinium elegans (Cleve, 1900) Balech, 1974	0,62		+		+		+	+			
		Protoperidinium latispinum (Mangin, 1926) Balech, 1974	0,48									+	+
		Protoperidinium longipes Balech, 1974	0,14									+	
		Protoperidinium oceanicum (VanHöffen, 1897) Balech, 1974	0,42			+	+		+				+
		Protoperidinium pentagonum (Gran, 1902) Balech, 1974	0,42	+	+					+		+	+
		Protoperidinium pedunculatum (Schütt, 1895) Balech, 1974	2,42	+	+	+		+	+	+		+	+
		Protoperidinium quarnerense (B.Schröder, 1900) Balech, 1974	2,22		+		+		+	+	+	+	+
		Protoperidinium simulum (Paulsen, 1931) Balech, 1974	5,12	+	+	+	+	+	+	+	+	+	+
		Protoperidinium steinii (Jørgensen, 1899) Balech, 1974	0,28						+				+
		Protoperidinium spp. Bergh, 1882	15,93	+	+	+	+	+	+	+		+	
Prorocentrales	Prorocentraceae	*Prorocentrum compressum* (J.W.Bailey) Abé J.D.Dodge, 1975	2,98	+				+	+	+	+	+	+
		Prorocentrum dentatum Stein, 1883	3,74						+	+	+	+	+
		Prorocentrum gracile Schütt, 1895	2,49			+	+	+	+	+	+	+	+
		Prorocentrum lima (Ehrenberg) F.Stein, 1878	1,73	+			+	+	+		+	+	+
		Prorocentrum mexicanum Osorio-Tafall, 1942	0,55						+			+	
		Prorocentrum micans Ehrenberg, 1834	12,88	+		+	+	+	+		+	+	+
		Prorocentrum scutellum Schröder, 1900	0,55					+	+				
		Prorocentrum spp. Ehrenberg, 1834	1,8	+		+		+	+			+	+
Pyrocystales	Pyrocystaceae	*Pyrocystis fusiformis* C.W.Thomson, 1876	2,08	+	+		+	+	+		+	+	+
		Pyrocystis hamulus var. semicircularis Schröder, 1900	0,14		+			+					
		Pyrocystis lunula (Schütt) Schütt, 1896	0,21		+								
		Pyrocystis pseudonoctiluca Wyville-Thompson, 1876	0,35								+	+	+
		Pyrocystis spp. Murray ex Haeckel, 1890	0,07						+				
Thoracosphaerales	Thoracosphaeraceae	*Goniodoma sp.* Stein, 1883	1,52	+					+	+	+		
		TOTAL		**36**	**31**	**28**	**43**	**41**	**63**	**40**	**33**	**53**	**70**

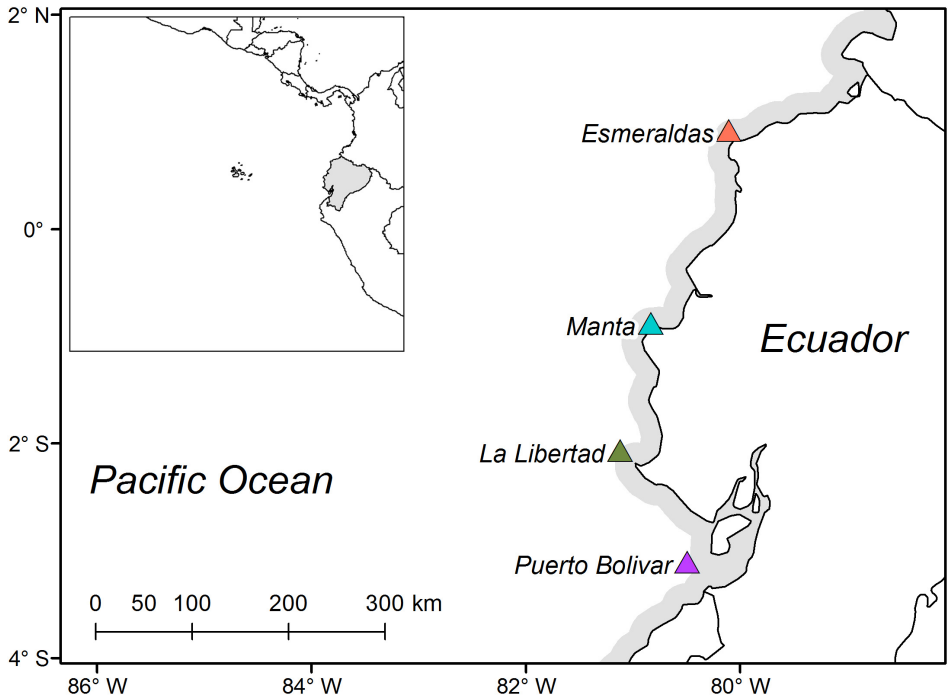


FIGURE 1 | Sampling sites; Esmeraldas (00°55′16.0″N; 80°06′24.0″O); Manta (00°52′45.0″S; 80°50′05.2″O); La Libertad (02°03′55.0″ S; 81°07′15.0″O); Puerto Bolivar (03°06′10.0″S; 80°29′47.0″O).

other years (**Figure 3**) (i.e., at 30 m in May in Puerto Bolivar with 3.8 × 10^5 cell. L^{-1} and at 50 m in April in Manta with 3.9 × 10^5 cell. L^{-1}).

Species Richness

Species richness values ranged from zero to a maximum of 21 species recorded in Puerto Bolivar during 2015 (August). Higher values were found in 0 m with a negative trend with depth (**Figure 4**). At surface waters (0 m) Manta and Puerto Bolívar showed the highest average values (median value of 5 for both sites), followed by Esmeraldas and La Libertad (median value of 4 for both sites). At 10 m depth the pattern is the same, except Puerto Bolívar that showed lower values (median of 4 species). Respect to the temporal variability, there is a general increase pattern along the study period, visualized as a predominance of observations above the average value across the time span of the study. In addition, along time series there are several periods were the species richness values showed the highest values. Those periods were between July to September 2013; the period between April to June 2014; February and March of 2015 in La Libertad; April and May of 2015 in Puerto Bolivar and Esmeraldas; the period of August and October in Puerto Bolivar where its maximum value was recorded; beginning of 2016, particularly for Puerto Bolivar and Manta and finally, most notably a general increase in richness values during 2017 starting in February and ending during May for the northern sites (Esmeraldas and Manta) and ending in September for southern sites (La Libertad and Puerto Bolívar). A new increase at the end of the study period (November – December) was evident for all sites. maximum richness was found in August 2015 in Puerto Bolivar at 10 and 75 m depth (20 and 21 taxa, respectively). Richness above 10 taxa were also observed the same year at same depths in La Libertad (February and March) and in 2017 at surface and 10 m in Manta (April), Puerto Bolivar (April, May, July and August) and Esmeraldas (May) (**Figure 4**).

Dinoflagellate Diversity

Diversity (Shannon–Wiener index) values ranged from 0 to 2.35 with southern stations (Puerto Bolívar and La Libertad) showing the highest values (2.21 and 2.35, respectively). In addition, higher richness values were found in surface waters across sampling stations (probably influenced by the richness gradient, see previous section) and along the sampling period. However, in some occasions high richness values were found in deeper waters during February to March 2013 (mostly for Manta and Puerto Bolivar), during the period between February to May of 2014 (except for Manta), between February to May 2015, between August to October 2015 and since the year 2017 where a general increase in diversity index was found. This general increase for 2017 showed a maximum for all sites during April – May (**Supplementary Figure S2**).

Harmful Algae

A total of 8 potential harmful dinoflagellate species were observed in low abundances belonging to 4 orders and

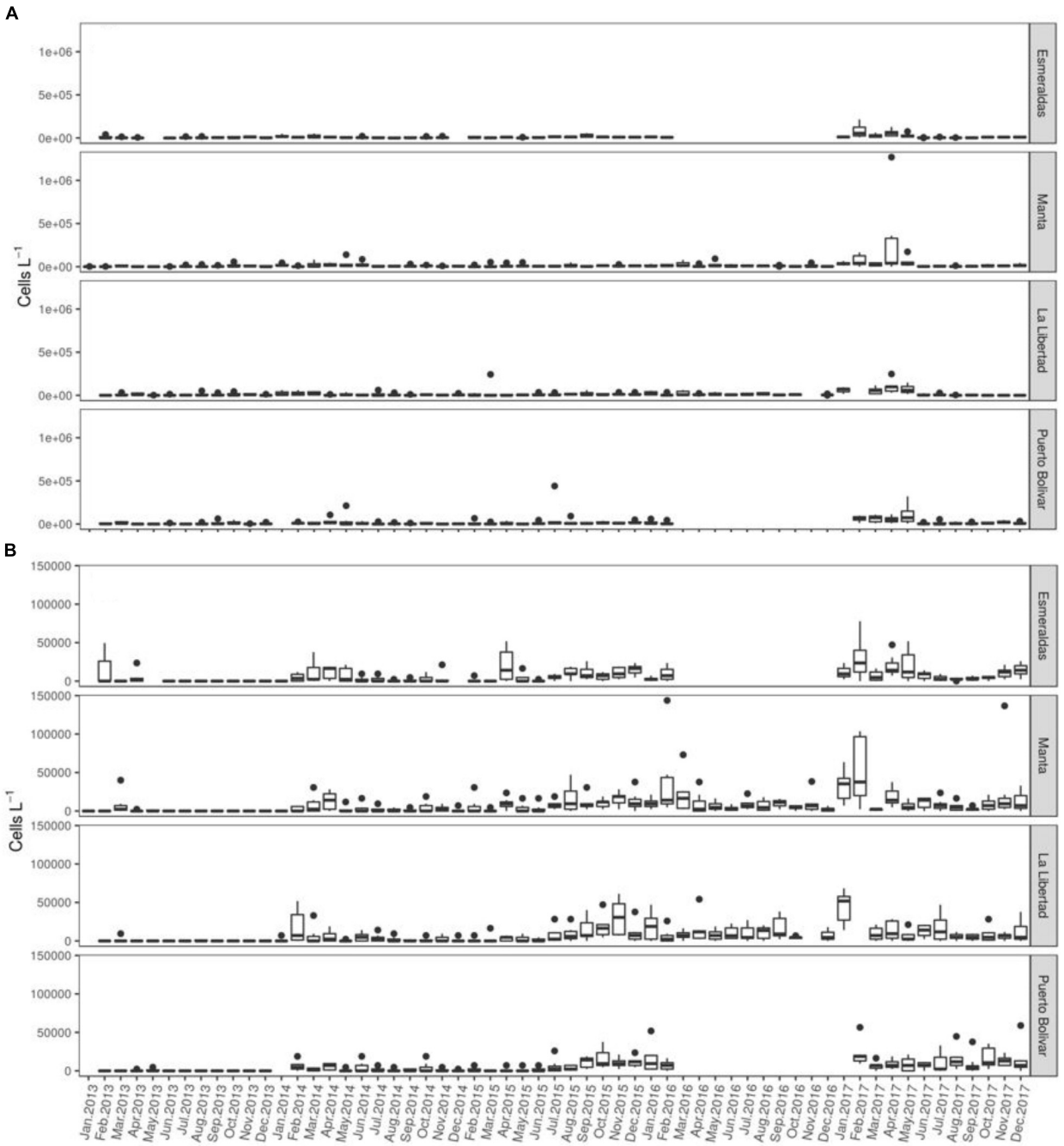


FIGURE 2 | Dinoflagellate cell abundance estimation (cell. L^{-1}). Spatio-temporal variability of **(A)** *Gymnodinium* spp. **(B)** *Gyrodinium* spp. Abundance are expressed as cells L^{-1}.

6 families, *Alexandrium* sp, *Cochlodinium catenatum*, *Dinophysis acuminata, Dinophisis ovum, Gymnodinium* cf. *catenatum, Karenia* sp., *Prorocentrum lima* and *Prorocentrum mexicanum*. The taxa that showed the highest occurrence were *C. catenatum* and *G.* cf. *catenatum* (6 and 3%, respectively).

The highest cell abundance was recorded by *C. catenatum* in May and June 2014 (7.5×10^4 and 9.6×10^4 cell. L^{-1}) in Puerto Bolivar. In general, the species was present in southern stations. Contrary, *G.* cf. *catenatum* was registered more times in northern stations, mainly in Esmeraldas, maximum abundances were observed in February 2013 (4×10^4 cell. L^{-1}). *P. lima* had an occurrence of 1.5%, the highest abundance was recorded in February – March 2015 at Puerto Bolívar at surface (9.1×10^4 cell. L^{-1}). *Alexandrium* spp, *Karenia* spp, *P. mexicanum, D.*

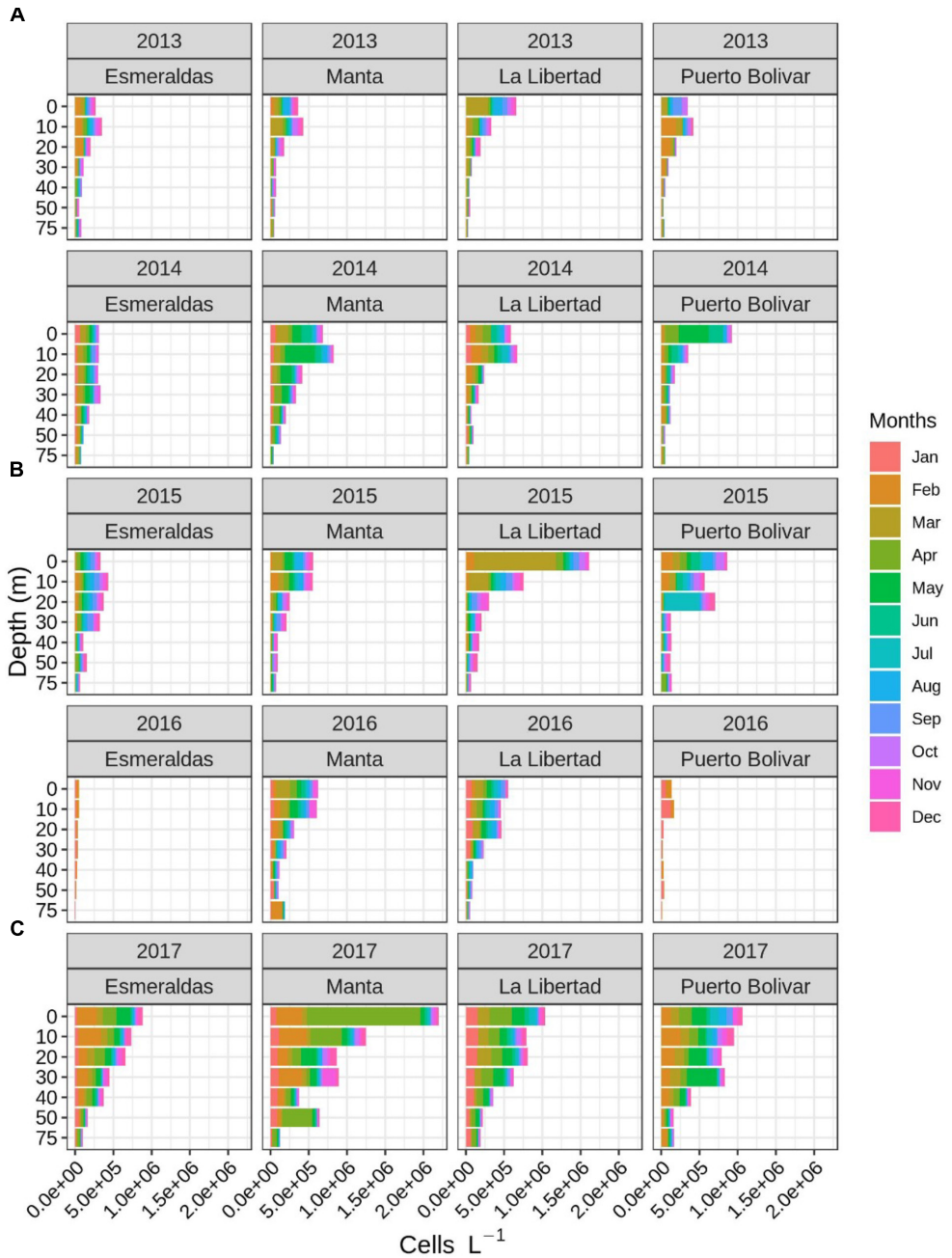


FIGURE 3 | Spatial (horizontal and vertical) and temporal distribution of the dinoflagellate's abundance (cell. L^{-1}) across the years (rows), sampling sites (columns), and seasonality represented by the sampling month (color bar).

acuminata, *D. ovum*, recorded the lowest occurrences (<1%) (**Table 1**).

Environmental Variables

Temperature and Salinity

In general, water temperature was higher in Esmeraldas compared to the rest of the stations during the studied period with a maximum in surface of 28.86°C in May 2017 (**Figure 5A**).

Concerning temporal variability, lowest temperatures (<22°C) registered in the first 10 m of the water column, occurred in 2013 from June to August in Puerto Bolivar, August and November in La Libertad, March and September in Manta and March in Esmeraldas (**Figure 5A**).

The next 2 years, 2014 and 2015, lowest temperatures were observed in February and March in Puerto Bolivar and La Libertad in surface and 10 m while in Esmeraldas and Manta there were also present at 20 m. In Puerto Bolivar and La Libertad

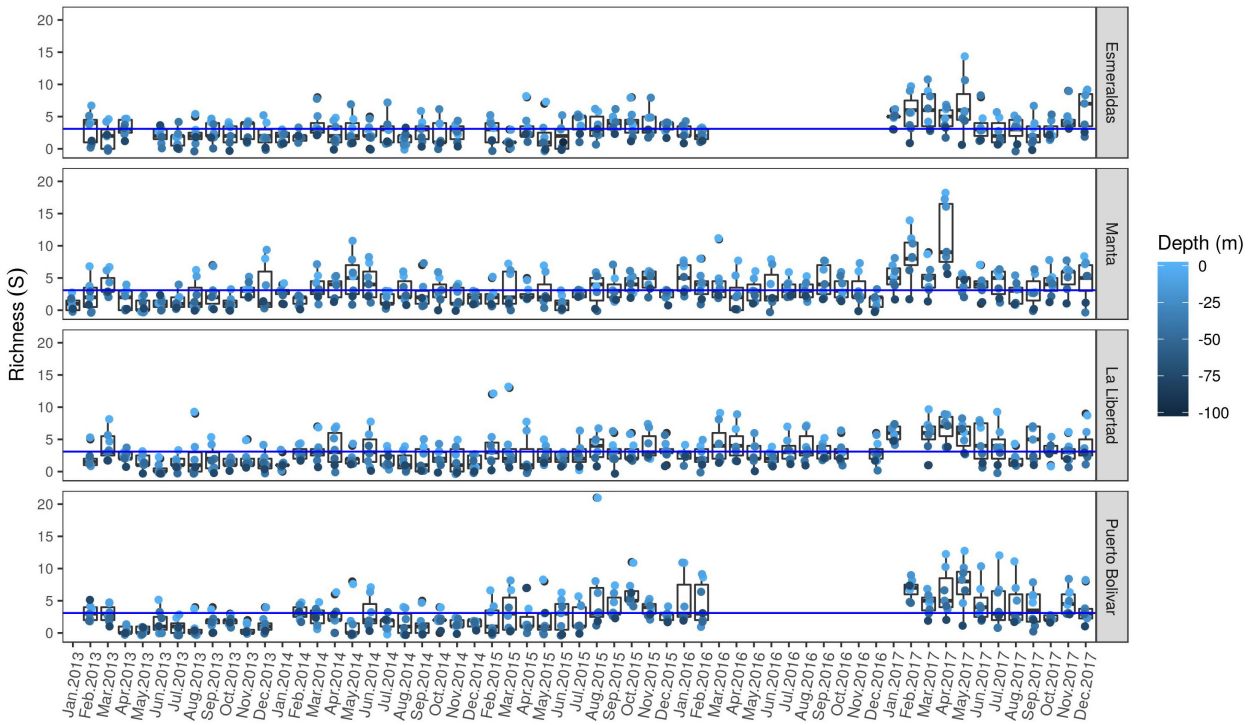


FIGURE 4 | Spatio-temporal variability of dinoflagellates community richness. Color gradient scale represents the sampling depth. Horizontal (blue) line represents the average value for the whole study coverage.

the lowest values occurred in September in all depths of the water column (**Figure 5A**).

In 2016, lowest temperatures were reported in March in Manta for the first 20 m and in April in Puerto Bolívar from 10 m (**Figure 5A**).

During 2017, lowest temperatures were registered in February and March for Esmeraldas, Manta and La Libertad, while in Puerto Bolivar there were also registered in April (**Figure 5A**).

Contrary, higher temperatures (>25°C) registered in 2013 occurred from April to December in the first 40 m in Esmeraldas and Manta, however, in La Libertad and Puerto Bolívar, warmer waters were limited to the first layers, 20 and 10 m, respectively (**Figure 5A**).

In 2014 and 2015, Esmeraldas and Manta registered warmer waters in the 50 m of the water column from April to December, evident in La Libertad and Puerto Bolivar (**Figure 5A**).

The thermocline was deeper in northern stations, Esmeraldas and Manta (between 30 and 50 m), while in La Libertad and Puerto Bolivar it was between 10 and 30 m (**Figure 5A**). Annual vertical profiles of monthly mean temperature are available in **Supplementary Figure S3**.

Lower salinity values (<32) were observed mainly in Esmeraldas in 2013, 2014, and 2017. In the other stations, values were between 33 and 34 during the studied period in the first 30–40 m of the water column. Saltier water layers (>35.00) were present in deeper layers (between 40 and 100 m) with the exception in 2015 and 2016 where in February, April, August and October, higher salinities were observed up to 20 m (**Figure 5B**). Annual vertical profiles of monthly mean salinity are available in **Supplementary Figure S4**.

Nutrients and Dissolved Oxygen

Regarding nutrients, highest nitrate concentrations (5 a 27.6 μg-at·L^{-1}) were registered from 20 to 50 m, mainly during the warm season (February and March) and dry season (From July to October) in all sampling sites (**Figure 6A**). Lowest nitrate concentrations (<1 μg-at·L^{-1}) were observed in surface, up to 30 m and there was not a seasonal trend among sampling sites. In general, Esmeraldas registered lowest values compared with the rest of sampling sites. Annual vertical profiles of monthly mean nitrate are available in **Supplementary Figure S5**.

Concerning phosphates, highest concentrations (>1 μg-at·L^{-1}) were registered in 2013 in Manta and Esmeraldas in the first 50 m of the water column in February and March and between 30 and 50 m from May to September. For the rest of the years, higher values decreased between 0.3 and 1.9 ug.at/l and no seasonal pattern was observed among stations (**Figure 6B**).

Lowest phosphate concentrations (<0.2 μg-at·L^{-1}) were mainly observed in Esmeraldas without a particular pattern. Manta also registered low values but less frequency. Annual vertical profiles of monthly mean phosphate are available in **Supplementary Figure S6**.

Moreover, high silicate concentrations (>5 μg-at·L^{-1}) were registered in 2013, 2014, and 2017, with a slightly variability among sampling sites (**Figure 6C**). Annual

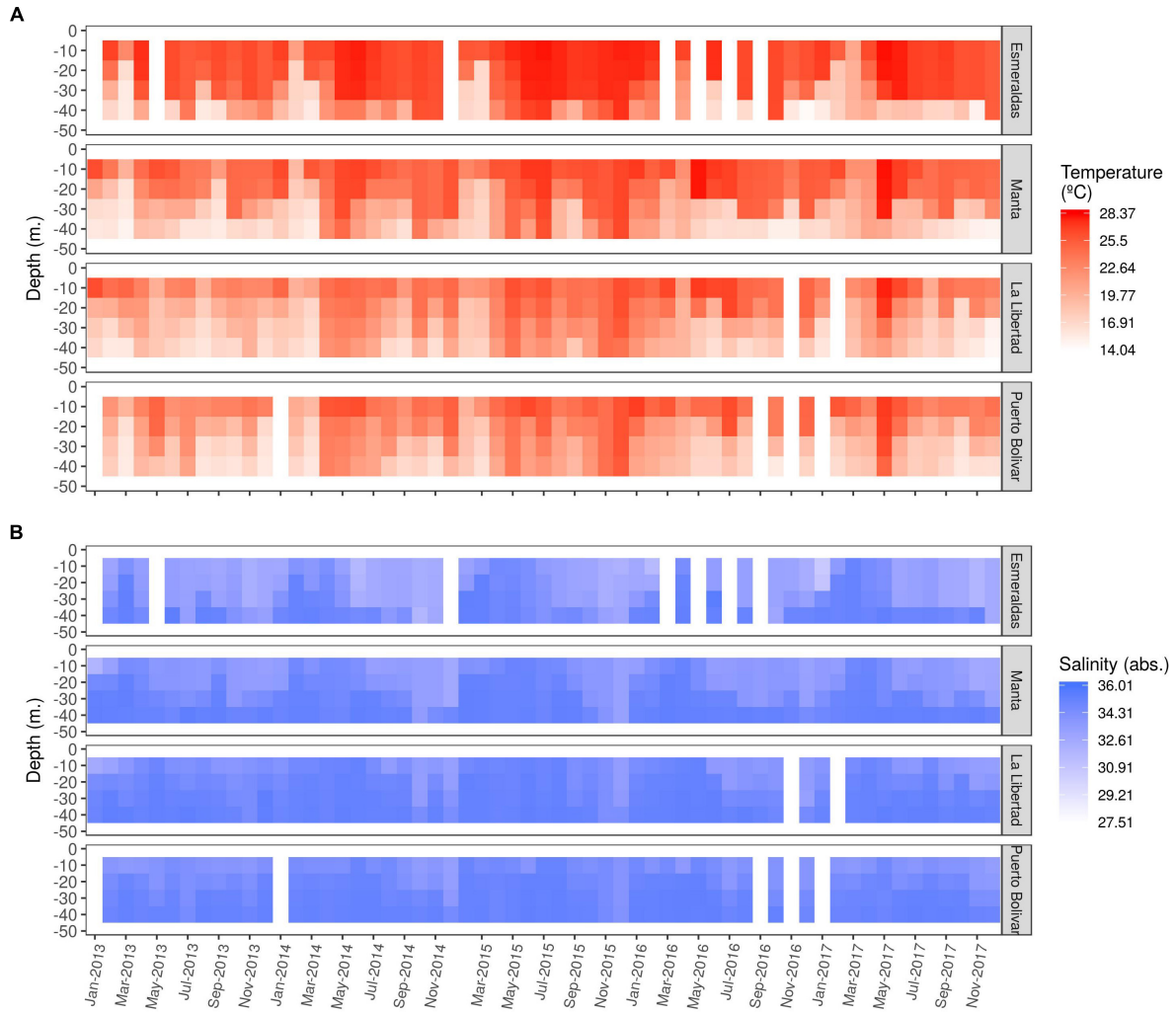


FIGURE 5 | Spatio-temporal variability of **(A)** Temperature and **(B)** Salinity.

vertical profiles of monthly mean silicates are available in **Supplementary Figure S7**.

Concerning dissolved oxygen, values in all sampling sites were between 4 and 5 mL. L^{-1} in the first 30–40 m approximately in Esmeraldas and Manta, while in the southern sites, this layer was found in higher depth (between 20 and 30 m). More oxygenic waters (>5 mL. L^{-1}) were found occasionally in surface and 10 m layer in February and March 2013 and 2017, between April and June 2016. Lower values of dissolved oxygen (<2.5 mL·L^{-1}) were observed between 40 and 50 m (**Figure 6D**). Annual vertical profiles of monthly mean silicates are available in **Supplementary Figure S8**.

Relationship With Environmental Variables

For those species with a percentage of occurrence higher than 2.8% the association between the environmental variables and the structure of dinoflagellates was inspected through CCA per year. Results showed for year 2013 a significant proportion of total constrained inertia (TCI) explained by the environmental variables (TCI = 8%, p-value < 0.05) with the first two axis explaining more than 80% of this constrained inertia (**Table 2**). Permutation showed that salinity, phosphate and temperature significantly affected the community structure for this year (**Table 3**). The community composition (CCA analysis) showed that the species, *Protoperidinium simulum* and *Prorocentrum compressum*, were positively correlated with salinity and with N/P (this last being non-significant). The taxa *Oxytoxum sp* and *Protoperidinium sp*. were positively associated with high values of nitrite. The group formed by *Gymnodinium* cf. *catenatum*, *Oxytoxum turbo* and *P. micans* were positively associated with high values of temperature and negatively with salinity and N/P values. N/P is the ratio between nitrate and phosphate in this study, low N/P ratio have been associated to HAB occurrence. In relation with salinity the species *O. scolopax*, *Gyrodinium sp* and *Gonyaulax polygramma* showed a negative association with this variable. Finally, the

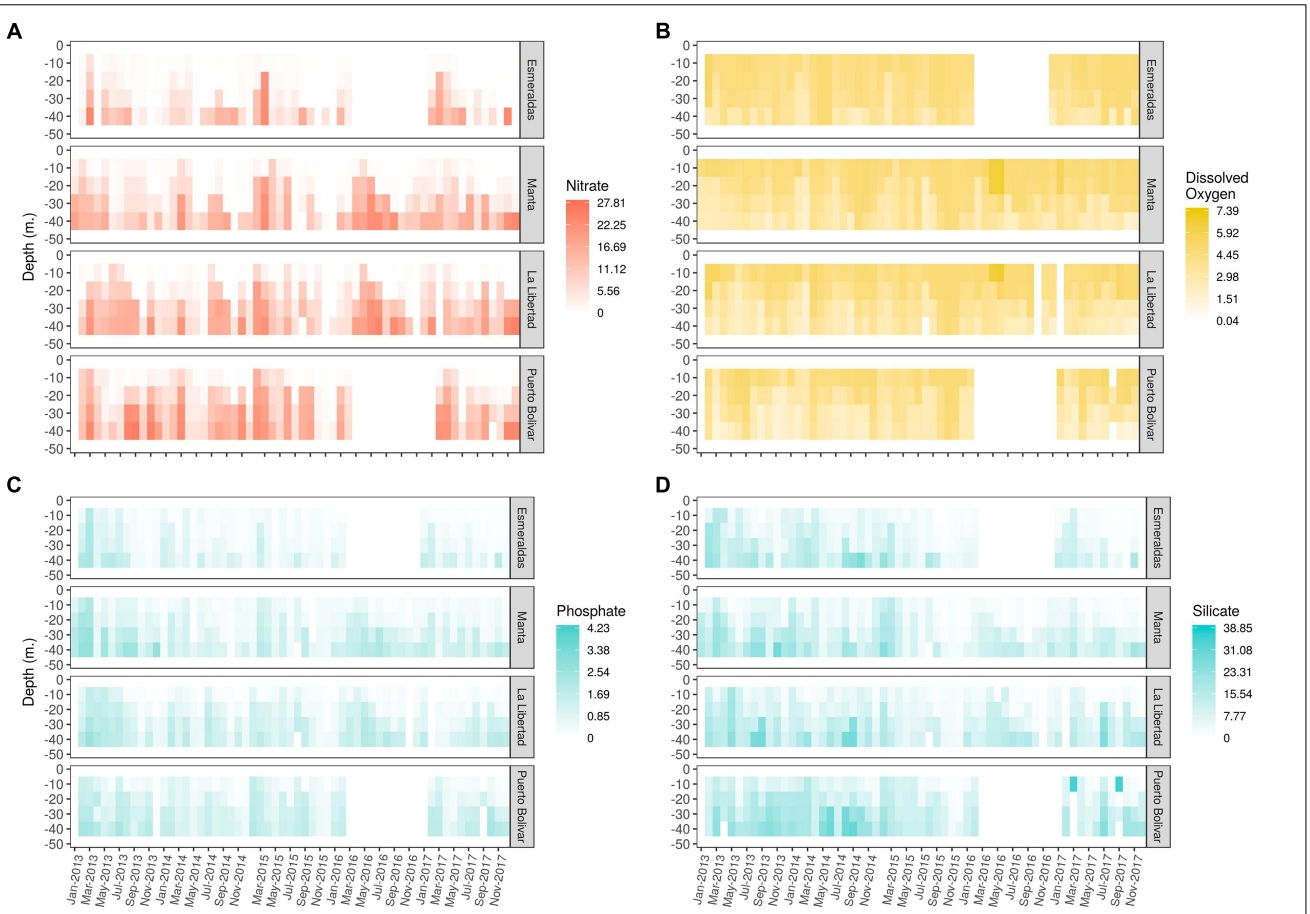


FIGURE 6 | Spatio-temporal variability of **(A)** Nitrate, **(B)** Phosphate, **(C)** Dissolved oxygen, and **(D)** Silicate.

group formed by the species *Gonyaulax sp*, *Gymnodinium sp*, *Tripos fusus* and *Tripos furca* were negatively associated with nitrite and phosphate, but showed an unimodal response with the rest of the variables (a central position in the CCA-Triplot, **Figure 7A**).

For year 2014 a lower than previous year, but still significant proportion of TCI explained by the environmental variables (TCI = 5.8%, p-value < 0.05) with the first two axis explaining 78.8% of this constrained inertia (**Table 2**). For this year (same as 2013) salinity, temperature and phosphate significantly affected the community structure (**Table 3**). Moreover, a clear positive correlation between phosphate with nitrite and N/P (both being non-significant) and those three variables showed a negatively association with temperature (**Figure 7B**). The association of species with environmental variables showed that *Dinophysis sp* was positively associated with N/P and nitrite – phosphate variables. With respect to salinity *Oxytoxum turbo* was positively associated whereas *T. fusus*, *O. scolopax* and *P. simulum* were negatively associated. *T. furca* and *C. catenatum* (in a lesser extent) showed a positive association with temperature. The rest of the species showed a central position in the CCA-Triplot representing unimodal response to the environmental variables (**Figure 7B**).

For year 2015 the proportion of TCI explained by the environmental variables was also significant (TCI = 5.8%, p-value < 0.05) with the first two axis explaining the highest amount of this constrained inertia along the study period (89.4%, **Table 2**). During 2015 the temperature, the N/P ratio and salinity were those variables which significantly explained the dinoflagellate's community structure (**Table 3**). A positive relationship was observed among salinity and nitrite and phosphate and a negative association between temperature and N/P (**Figure 7C**). From the species-specific relationship, the CCA analysis revealed a positive association between *Dinophysis sp* and *P. simulum* with N/P ratio and negatively with temperature. The species *Gymnodinium* cf. *catenatum* was associated with positive values of nitrite and phosphate and in a lesser extent, showing a non-linear response, with salinity. Finally, the species *P. micans*, *P. compressum* and *T. furca* were associated to low values of salinity (negative relationship) (**Figure 7C**). Interestingly, this year none species was associated with high, but low temperature values.

For year 2016 and 2017 a decrease in the TCI from 7.8% to 5.5 was evident. However, for both years the model was able to significantly explain this constrained inertia with the first two axes explaining 82.6% and 65.1% of this constrained

TABLE 2 | Summary table of canonical correspondence analysis (CCA) statistics per year.

Year	*n*	TMI	TCI (%)	F-statistic (*p*-value)	CI-1 (%)	CI-2 (%)	CI (%)
2013	223	4.9	8.0	3.82 (<0.05)	52.5	27.9	80.4
2014	274	4.1	5.8	3.32 (<0.01)	58.2	20.6	78.8
2015	271	4.2	6.9	3.96 (<0.01)	64.5	24.9	89.4
2016	179	1.95	7.8	2.93 (<0.01)	52.6	30.0	82.6
2017	309	1.82	5.5	3.55 (<0.01)	45.1	20.0	65.1

The total number of observations (n), the total model inertia (TMI), the proportion of total constrained inertia explained by the environmental variables [TCI (%)], the model significance at $\alpha = 0.05$ (p-value), the proportion of constrained inertia explained by axis 1 [CI-1 (%)] and by axis 2 [CI-2 (%)], and the total proportion of constrained inertia explained by the first two axis CI (%) is showed for each year.

inertia, respectively (**Table 2**). Particularly during 2016 the environmental variables who were significantly associated with the structure of the dinoflagellate community were temperature, nitrite and phosphate (**Table 3**). Salinity and phosphate were positively correlated and temperature and nitrite were negatively correlated. The CCA analysis for this year showed that *Gymnodinium* cf. *catenatum* was correlated positively with salinity and negatively with nitrite. The species *Gyrodinium acutum* was associated with high values of phosphate with a non-lineal response (center position of the species related with the environmental vector). The species *C. catenatum*, *O. scolopax*, *Scripsiella sp*, *G. polygramma*, *T. furca*, *Protoperidinium sp* and *Gymnodinium sp.* showed a negative association with phosphate and *Oxytoxum sp*, *Gyrodinium spirale*, *G. polygramma, Scripsiella sp*, *O. scolopax*, and *Gymnodinium sp* a negatively association with nitrite (**Figure 7D**). During 2017 all the environmental variables were significant when related to the observed dinoflagellate community structure (**Table 3**). Among the environmental variables, a negative association was evident for salinity and phosphate (**Figure 7E**). From the community composition perspective, the species *P. simulum*, *T. fusus*, *Gymnodinium* cf. *catenatum,* and *Prorocentrum dentatum* showed a clear positive relationship with nitrite and negatively with salinity and with the N/P ratio. The species *T. fusus*, *P. compressum*, *O. scolopax,* and *Gyrodinium sp* were positively associated with phosphate and negatively with salinity. For this year, non-species were related positively with temperature, but some species showed a negative association with this environmental variable (e.g., *Oxytoxum turbo*, *Gyrodinium spirale*, *Oxytoxum sp,* and *Gyrodinium sp).*

Interestingly, the total model inertia (TMI), which represents the total variability n-dimensional inside the model, remained more or less constant for the first 3 years (2013–2015) ranging from 4.1 to 4.9. However, during years 2016 and 2017 this TMI was significant lower with values for 2016 of 1.95 and for 2017 a TMI of 1.82 (**Table 2**).

DISCUSSION

Dinoflagellate Community

In this study, there were reported a total of 97 taxa, corresponding to 8 orders, 22 families, and 31 genera during the sampling period from 2013 to 2017 in 4 stations in the coast of Ecuador at different depths. Phytoplankton dynamics across the ETP coast is more characterized in other countries and there is a lack of information in the central area corresponding to the Ecuadorian coast. Interestingly, recent studies regarding phytoplankton distribution in response to ENSO events have been published (Conde and Prado, 2018; Conde et al., 2018), but, unfortunately, species identification is not indicated as a result. Another recent study explored the oceanography of red tides using remote sensing data from 1997 to 2017, confirmed that potential HABs have been dominated by dinoflagellates during wet season mostly at the Gulf of Guayaquil (Borbor-Cordova et al., 2019). Furthermore, dinoflagellates abundance reported in the present study was not high and the number of proliferations was low, only *Gymnodinium sp* exceeded 10^6 cell. L^{-1} in April 2017. In the coast of Ecuador, INOCAR and other researchers have reported blooms of dinoflagellates, for example *Gymnodinium sp* was reported in 2003, 2004, 2005 reaching high concentrations (1×10^5 cell. L^{-1} – 1.1×10^7 cell. L^{-1}) in the Gulf of Guayaquil both in wet and dry season (Torres and Tapia, 2002; Torres et al., 2017).

It is important to mention that species identification resulted difficult to perform without specific equipment and adequate formation. In that sense, some of the taxa found in the area were not identified at species level. Dinoflagellates identification under inverted microscope based on morphological features constitutes a challenge since some species from several genera share the same plate pattern and overlap in size (e.g., the genus *Ostreopsis* – Carnicer et al., 2016). In the last decades, molecular techniques have allowed to perform accurate identifications in dinoflagellates using rDNA sequences (Litaker et al., 2007). Unfortunately, there is no molecular characterization of marine dinoflagellates in the Ecuadorian coasts, with the exception of *Ostreopsis* cf. *ovata* (Carnicer et al., 2016). It is important for Ecuadorian institutions to invest in the implementation of molecular techniques for future investigations, especially important to identify potentially toxic species.

Higher species richness in the water column was observed in surface layers, with a maximum of 21 taxa. These results are in agreement with a study performed in 2015 at the southern coast of the country, in El Oro province, where they reported species richness average between 28 and 23 dinoflagellates in surface samples (Prado et al., 2015; Conde et al., 2018). The time series length of the present study avoids us to conduct a formal time series

TABLE 3 | Summary table of the permutation tests over the CCA results.

Variable	2013		2014		2015		2016		2017	
	F-statistic	***p*-value**	**F-statistic**	***p*-value**	**F-statistic**	***p*-value**	**F-statistic**	***p*-value**	**F-statistic**	***p*-value**
Temperature (Anomaly)	**5.78**	**<0.01**	4.22	<0.01	**6.76**	**<0.01**	**2.00**	**<0.05**	**3.57**	**<0.01**
Salinity (Anomaly)	**3.80**	**<0.05**	5.47	<0.01	**5.66**	**<0.05**	1.25	n.s.	**4.19**	**<0.01**
Phosphate	**5.04**	**<0.01**	3.78	<0.01	1.74	n.s.	**5.13**	**<0.01**	**4.6**	**<0.01**
Nitrite	0.91	n.s.	0.82	n.s.	0.31	n.s.	**2.40**	**<0.05**	**4.61**	**<0.01**
N/P	1.44	n.s.		n.s.	**3.60**	**<0.05**	1.99	n.s.	**3.67**	**<0.05**

Significant environmental variables (terms) are showed in bold and the value of the F statistic (an p-value) are also given. n.s., means non-significant term.

analysis to statistically prove this increase pattern observed. In addition, some oscillatory behavior is evident and probably associated with the interannual variability pattern (Conde et al., 2018).

In 2015, associated with El Niño ENSO event, higher dinoflagellate species richness values were registered during the wet season in La Libertad, Puerto Bolivar and Esmeraldas, reaching a maximum of 28 species in dry season in Puerto Bolivar, that could be linked with upwelling and higher nutrients concentrations (Borbor-Cordova et al., 2019). In 2017, higher values are also observed in each station with the highest found in Puerto Bolivar in agreement with Prado et al. (2015), associating the event with the presence of the Humboldt current. The higher amount of species during El Niño ENSO events can be linked with the transport of species from the advected waters due to the current, as well as upwellings occurring in the equatorial region (Pennington et al., 2006; Gierach et al., 2012).

Regarding potential harmful dinoflagellates, a total of 8 taxa were observed, *Alexandrium* sp, *Cochlodinium* sp., *D. acuminata*, *D. ovum*, *Gymnodinium* cf. *catenatum*, *Karenia sp., P. lima* and *P. mexicanum* but not HABs were observed. In Torres (2015), a total of 131 HABs were registered from 1968 to 2009 in the same sampling sites as the present study, where toxic dinoflagellate community was similar to the species listed above. Unfortunately, no statistics analysis was performed in Torres (2015), however, they concluded that during wet-warm season the highest number of HABs events occurred, being more abundant in the Gulf of Guayaquil, southern station. Due to the low abundances registered in the present study, CCA analysis was not possible to perform with toxic species, which do not allow to confirm this hypothesis. Furthermore, an historical reconstruction of blooms from 1997 to 2017 in the Ecuadorian coast, highlighted the presence of toxic species such as *Gymnodinium* cf. *catenatum, P. micans, C. catenatum* and *Dinophysis caudata* among others. As for the present study, lower abundances were registered for toxic species, slightly higher in the Gulf of Guayaquil than in La Libertad and Manta (Borbor-Cordova et al., 2019). Unfortunately, Esmeraldas was not considered in the study and data corresponded only to wet season.

During these studies performed by the INOCAR, no toxic analysis has been performed in water samples due to a lack of specialized laboratory. This fact highlights the necessity of specific equipment acquisition by the government in order to control the presence of toxins in the productive areas, not only limited to monitoring phytoplankton abundances.

Environmental Variables and Seasonality

Overall, in this study, SST and salinity anomalies, nutrients, as well as the N/P ratio were strongly associated to the spatio-temporal distribution of dinoflagellate community (**Figure 7**). Studies elsewhere have found that HAB have been related during positive anomalies of SST, stratification with a deeper thermocline, pulses of upwelling during summer or dry season and the biological interaction among phytoplankton community (Hallegraeff, 2010; Díaz et al., 2016), some of these conditions were found in this analysis.

The environmental variables presented in this study confirm the main patterns of current systems on the ETP, which are driven by cold Humboldt Current in the central and south coast of Ecuador (La Libertad and Puerto Bolivar), associated with the equatorial upwelling (EU) system and the warm Panama current toward the north of Ecuador (Esmeraldas), these oceanographic settings drives the biogeochemical conditions that may promote extensive phytoplankton growth and potential HABs (Cucalon, 1989; Pennington et al., 2006). The north of the Ecuadorian coast is under the influence of the inter-tropical convergence zone (ITCZ), receiving higher levels of precipitation as well as runoff from Panama Gulf generating a low salinity and warmer temperatures which lead to stratified conditions, shallow halocline-nutricline (30–40 m), and low nutrients concentration (Pennington et al., 2006). Those conditions are reflected in the northern station of Esmeraldas, where minimum dinoflagellate abundances were reported (**Figure 2** and **Supplementary Figure S2**) compared to other stations, highlighting the influence of the oceanographic characteristics and strong seasonality of the ETP (Chavez et al., 2002).

Oceanographic studies have established the seasonal pattern of the coastal EU at the center of Ecuador, and the upwelling from the Humboldt Current on the south of the Ecuadorian Coast (Pennington et al., 2006). Seasonality of those upwelling system are reflected in all stations (based on higher nutrient concentrations and lower temperatures) except at Esmeraldas, being deeper and high nutrient concentration during wet season (December to May) but also between August to September. It is expected that Manta is mostly influenced by the EU while at La Libertad and Puerto Bolivar it is the upwelling of Humboldt Current, considered one of the most productive of

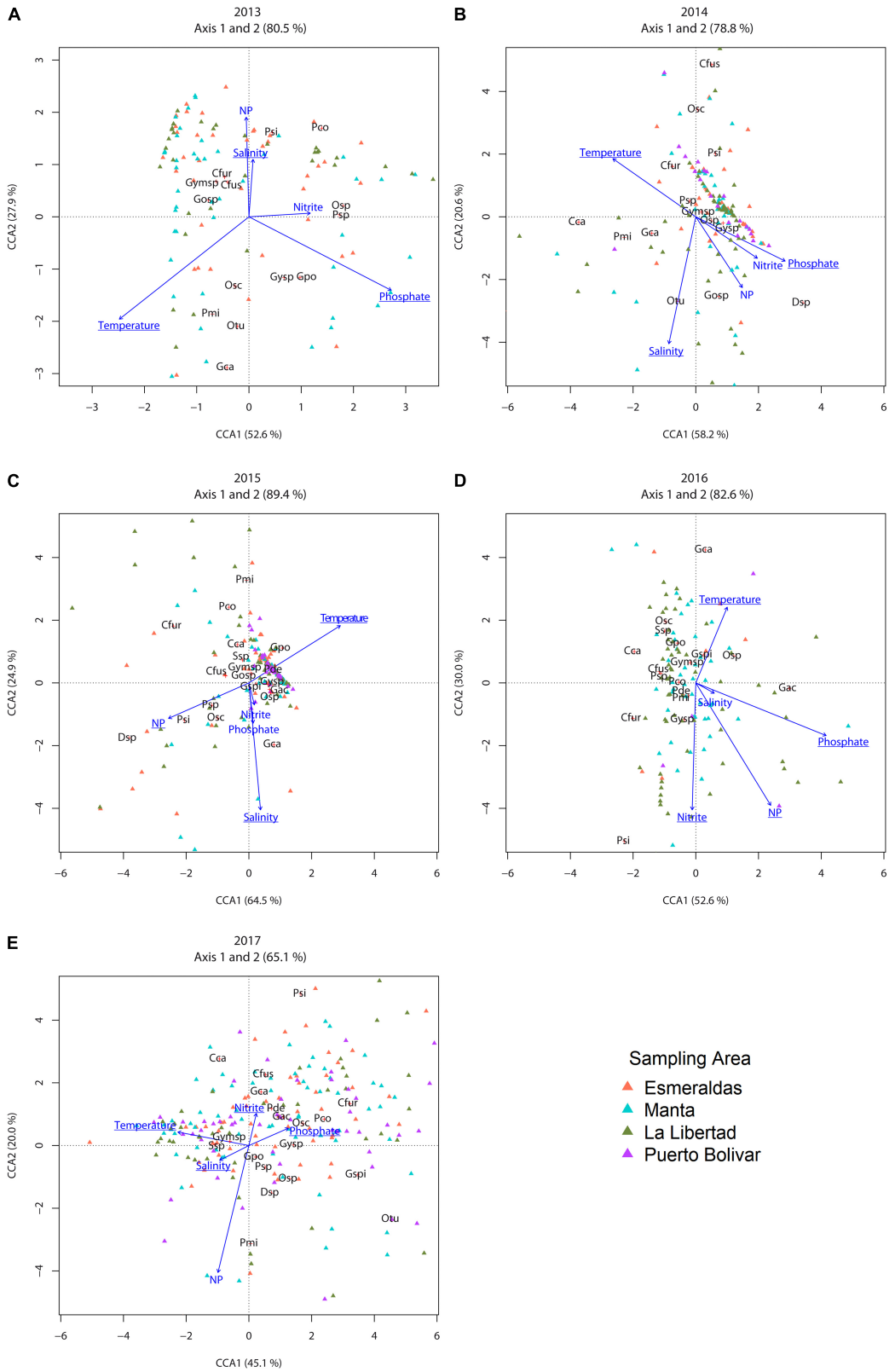


FIGURE 7 | Canonical correspondence analysis triplot for **(A)** 2013, **(B)** 2014, **(C)** 2015, **(D)** 2016, and **(E)** 2017. Sampling stations are shown as triangles with color-code according to the sampling area (figure legend). The species (with occurrence >2%) present in the community CCA analysis follows: **Otu**,

(Continued)

FIGURE 7 | Continued
Oxytoxum turbo; **Pco**, *Prorocentrum compressum*; **Gpo**, *Gonyaulax polygramma*; **Gca**, *Gymnodinium* cf. *catenatum*; **Gspi**, *Gyrodinium spirale*; **Pde**, *Prorocentrum dentatum*; **Cfus**, *Tripos fusus*; **Cfur**, *Tripos furca;* **Psi**, *Protoperidinium simulum;* **Cca**, *Cochlodinium catenatum;* **Osc**, *Oxytoxum scolopax;* **Gosp**, *Gonyaulax sp;* **Ssp**, *Scripsiella sp;* **Pmi**, *Prorocentrum micans;* **Osp**, *Oxytoxum sp;* **Psp**, *Protoperidinium sp;* **Gac**, *Gyrodinium acutum*; **Gysp**, *Gyrodinium sp;* **Gymsp**, *Gymnodinium sp.* Environmental (explanatory) variables are indicated in blue ["Temperature anomaly" (abbreviated in the figure as "Temperature," °C), "Absolute salinity anomaly" (abbreviated in the figure as "Salinity," "Nitrite," "Phosphate," and the ratio nitrate – phosphate (abbreviated in the figure as "N/P"); bold underline correspond to significant explanatory variables according to permutation test (see methods)]. The percentage of variability (inertia) explained is indicated on each axis.

the world (Chavez et al., 2002; Pennington et al., 2006; Oyarzún and Brierley, 2018). In this study, highest concentrations of dinoflagellates were found in Manta (April 2017), La Libertad (March 2015), and Puerto Bolivar (May 2014 and May 2017), during wet season. During this period, there is a large amount of nutrients coming with the intense runoff from the agricultural Guayas Basin (Borbor-Cordova et al., 2006) and interestingly, in the present study it is reported higher levels of nutrients, mainly nitrate and phosphate (**Supplementary Figures S5**, **S6**), coming from deeper water, suggesting advection from the coastal Humboldt Upwelling in dry season with peaks on August. Thus, data on this study suggests that a combination of extreme hydrodynamic conditions, climate variability associated to the warm phase of the Pacific Decadal Oscillation (PDO) and ENSO in the coast of Ecuador (2015–2016), influencing salinity and SST anomalies and nutrient limitation conditions, led to shifts in the phytoplankton community and maybe to HABs.

In this region phytoplankton productivity and growth are influenced by the upwelling dynamics which can be associated to the pycnoline-nutricline-oxycline depth, and their variability along the coast is driven by local specific conditions of coastal waves, seasonality and inter-annual variability of ENSO cycle (Montecino and Lange, 2009). The thermo-nutricline determines the supply of limiting nutrients (related to N/P ratio) to the euphotic zone and influencing the phytoplankton community assemblage during each ENSO event. This is evidenced in the present study with the inter-annual variability of dinoflagellates associated to ENSO events which is characterized by high positive anomaly of SST, changes on the timing of seasonality and strengthening (cold phase) or weakening (warm phase) the coastal upwelling (Pizarro and Montecinos, 2004; Pennington et al., 2006; Montecino and Lange, 2009). Similar dynamics have been reported in North and ETP during ENSO events (Chavez et al., 2002; Jacox et al., 2016). Previous ENSO studies pointed out that southwest winds weakness lead to a coastal upwelling decline which diminish phytoplankton blooms occurrence (Tam et al., 2008; Wang et al., 2008; Montecino and Lange, 2009). However, during warm phase ENSO event in 2015 occurred anomalous conditions related with sustained wind stress, intermittent coastal upwelling, that could be the reason why dinoflagellates were able to persist even in a nutrient limited environment, and warm stratified conditions (Smayda and Reynolds, 2001; Du et al., 2015; McCabe et al., 2016; McKibben et al., 2017; Conde and Prado, 2018).

During the period of this study (2013–2017), 2013 is considered a normal year with the lower abundances of dinoflagellates of the study, a weak ENSO warm phase in 2014, followed by a strong warm phase ENSO in 2015–2016 (with a different seasonality of the one in 1997–1998) beginning a transition to cold phase ENSO during the dry season 2016 is extended to 2017 characterized by abundance of dinoflagellates (see **Figure 3**). Each year of the study develop specific environmental conditions; however, SST anomalies, salinity and nutrients persistently drove the dinoflagellates community for all the study period (see **Figure7**).

In 2013 a normal year with a slightly cold SST anomaly (see **Supplementary Figure S9**), lower salinities, and nutrients were the drivers for the dinoflagellates. *Gymnodinium* cf *catenatum, Oxytoxum turbo* and *P. micans* were associated to less saline, low nutrients and warm waters typical conditions during wet season in Esmeraldas influenced by the Panama Current. While *P. simulum, P. compressum, Oxytoxum sp*, and *Protoperidinium sp* are positively related to nutrient and salinity which are characteristics of upwelled waters, suggesting a preference of nutrient-rich water along the coast of Ecuador (**Figure 7A**).

In 2014, considered a weak ENSO year with ONI strong anomalies (>1.5) during wet season decreasing rapidly toward December 2014 (see **Supplementary Figure S9**). *T. fusus, O. scolopax and P. simulum* were associated to low salinity conditions mostly on Esmeraldas, Manta and La Libertad suggesting iN/Put of freshwater runoff and precipitation. While *Dinophysis sp* was associated to nutrients from upwelled waters from EU system at the Esmeraldas station, the upwelling process is related with the nitrate distribution at 30–50 m (**Supplementary Figure S5**). *T. furca* and *C. catenatum* were associated to positive anomalies of temperature, suggesting that have adapted to the warm conditions on the Esmeraldas site.

Although the SST anomalies of ENSO events in 2015 were of similar magnitude to those in 1997–1998, the responses were diverse in relation to the oceanographic characteristic of the equatorial front (Conde et al., 2018). By April 2015, the thermocline deepened to 40–75 m until December 2015 generating stable thermal stratification, but nutrients were sustained by a weak upwelling and a pycnocline-nutricline between 30–50 m. Even though the nutrients were reduced at the surface layer (see **Figures 6**, **7**), surprisingly the abundance levels of dinoflagellates in La Libertad and Puerto Bolivar where high between 10–30 m (see **Figure 3**). By August 2015, nutrients (nitrate, and phosphate) were upwelled between 30 and 50 m at La Libertad, and Puerto Bolivar. These conditions of thermal stratification, deepened thermocline, slightly upwelling, light for growth and ciliate prey (*Mesodinium rubrum*) appear to establish the optimal combination for the development of potential HABs such as *Dinophysis sp and P. simulum* (Reguera et al., 2012; Díaz et al., 2013). *G. catenatum* have affinity for the nitrate and phosphate and less with salinity, bloom of this specie has been

related to advected vegetative populations to the coasts during the relaxation of coastal upwelling (Bravo and Figueroa, 2014). *P. micans, P. compressum,* and *T. furca* were associated to less saline water characteristic of the Panama current or influenced by precipitation at the northern stations of Esmeraldas and Manta. Considered an extreme ENSO year and warm PDO, expecting a limited algal growth, surprisingly there was a relative high abundance of dinoflagellate and higher richness reflexed in the diversity of species (21). If this increase in richness is a pattern of the ENSO need to be verified in other years.

Year 2016 shows the transition between a warm ENSO to cold and normal conditions, with ONI decreasing from 2.5 in January to – 0.7 in September in the dry season (see **Supplementary Figure S9**). In this year, *Gymnodinium* cf. *catenatum* appeared during dry season associated with salinity and inversely with nitrite at the Manta and La Libertad, unfortunately no data in Esmeraldas and Puerto Bolivar are available. *Gymnodinium* cf. *catenatum* has been recognized to cause paralytic shellfish poisoning across regions and as a survivor by using strategies based on its motile forms and cysts in plankton assemblages and in surface sediments (Yamamoto et al., 2004; Bravo and Figueroa, 2014). *Gymnodinium* cf. *catenatum* is also identified as one of ten most damaging potential domestic target species, considering the environmental and economic impact in aquaculture and also in human health (Hallegraeff, 2003). *C. catenatum* is another specie that seems to be associated to the upwelling at La Libertad in dry season.

During 2017, persistent negative SST anomalies associated to upwelling and water nutrients richness lead to an increase in the productivity of the region in all the ETP (Brown et al., 2015). Concentrations of dinoflagellates increased and reached their maximum in all the stations due to conditions of a persistent upwelling specially in Puerto Bolivar station. Some of the species that were associated with upwelled nutrients were *P. simulum, T. furca, Gymnodinium* cf. *catenatum* and *P. dentatum.*

In the present study, the most abundant species were *Gymnodinium sp* and *Gyrodinium sp* which were present every year in both seasons of the studied period and registered up to 40 m depth. Those species correspond to free living large cell dinoflagellates, ubiquitous in the Pacific Ocean, previously registered in México, California and Ecuador (Gomez, 2005; Meave-del Castillo et al., 2012; Torres, 2015). Both species appeared in all coastal stations during the seasonal transition in the weak ENSO in 2014 and strong ENSO in 2015 (**Figure 2** and **Table 1**) indicating their capacity to adjust to warm and deeper thermo-nutricline.

Even though some microalgae exhibit a rapid adaptability to high temperatures in short-term experiments, the physiological plasticity and genetic response of many microalgae under future environmental conditions is unknown (Hallegraeff, 2010). Organism size and elemental composition of the phytoplankton community will influence processes at the level of individuals, populations, communities and ecosystems (Finkel et al., 2010). Some researchers have found that dinoflagellates are species with a smaller surface and have the ability to grow and sustain during ENSO, ocean heatwaves, stratified conditions, and nutrient poorer waters (Smayda and Reynolds, 2003; Glibert et al., 2005). However, there is also evidence that nutrient enriched waters can stimulate dinoflagellates blooms (Glibert et al., 2005), therefore, dinoflagellates are considered strategist and survivors either in extreme conditions.

The contrasting behavior of *Tripos* species in divergent lake types (with different climatic, morphometric, geological, hydrological, and trophic features) explains the existence of ecotypes of these species adapted to diverse environmental conditions and exhibiting high intra- and inter-population and morphological variability (Cavalcante et al., 2016). *T. furca* is also known to perform active vertical migration, depending on nutrients and water temperature in both natural and laboratory conditions, which means that different species of dinoflagellates have different ecological abilities. These abilities are possibly linked with their ecological responses for surviving under local environmental conditions. *T. furca* has a competitive advantage because of physiological adaptations to low nutrient concentration waters (Baek et al., 2011). Therefore, dinoflagellates can alter their vertical position in the water column by swimming, which allows them to maintain an optimal depth in terms of light or nutrients. Consequently, the actual duration of high irradiance in the coastal bays is considered to regulate the maintenance of the bloom (Baek et al., 2011).

In general, under N or P limitation or some specific N/P ratio, some toxic dinoflagellates are probably outcompeted, and toxin production may be an adaption strategy to offset the ecological disadvantages of dinoflagellates with low nutrient affinity (Smayda, 1997). How these biochemical shifts shape phytoplankton community structure and the presence of potentially toxic species need to be explored in Ecuador.

Montecino and Lange (2009) proposed that during warm ENSO as 2015, there is microbial trophic web path mostly dominated by small-sized phytoplankton, in this study taxa such as *Gonyaulax polygrama, P. dentatum* and *Gynmodinum spp.* were characterized to be trophic web dominated. Still it is not very clear which are the main ecological drivers to explain interannual variability of phytoplankton community structure, including toxin producer species. In addition, mixotrophy relationships need to be explored because many of the motile bloom formers are mixotrophic protists (Stoecker et al., 2017). Some argued that PDO is a good indicator to explain those shifts on the community structure and potential toxins generation (Du et al., 2015). Unfortunately, in this study only dinoflagellates community was considered, so it is not possible to confirm this ascertain. An effort in future works need to be done in order to encompass more taxa to better understand phytoplankton distribution in the area.

CONCLUSION

Dinoflagellates abundances reported in the present study were not elevated, and no HABs were observed in the studied period. The taxa presented in this study represents a baseline in which future work may referred in order to understand certain dinoflagellate dynamics. In synthesis, the environmental variables and the oceanographic ENSO index served to understand how the current system of the ETP, their thermocline

dynamics, and the coastal upwelling affect euphotic zone nutrient supply and hence, dinoflagellates abundance and richness along the coast of Ecuador.

Moreover, toxic species were detected and, considering that in future decades it is predicted an increase in seawater temperature and extreme events frequency (IPCC, 2014), phytoplankton dynamics may be influenced. In order to be able to predict future scenarios of HABs, it is important to study their distribution in relation to environmental variables in areas where extreme events may affect their proliferations. Thus, it is crucial to involve policy-makers and stakeholders in the implementation of environmental policies and climate change adaptation strategies involving long-term monitoring and sanitary programs, not covered at present.

AUTHOR CONTRIBUTIONS

GT, SR, RN, and EP made substantial contributions to conception and design of the work and conducted the sampling, nutrient, and dinoflagellates analysis. OC, GT, MB-C, PDLF, and AC made substantial contributions in data analysis and interpretation and participated in drafting the article. MB-C gave the final approval of the version to be submitted.

FUNDING

This research has been developed as an international and inter-institutional collaboration among different research institutes and universities. We would like to acknowledge the following projects and institutional support: Project "Oceanic Early Warning System," funded by National Secretary of Science and Technology (SENESCYT) and INOCAR, Project T2-DI-2014 "Climate Variability and Harmful Algae Bloom Interaction and their impact on human health on the coast of Ecuador," funded by the Escuela Superior Politecnica del Litoral (ESPOL).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2019.00145/full#supplementary-material

FIGURE S1 | Collinearity analysis of the environmental variables measured in the study. The Pearson's correlation coefficients for each pair of variables are shown in the upper-right panel with the size of the text proportional to its value. Significance code represents: ***p-value < 0.001. Additionally, the density histogram of each variable is represented in the diagonal and a scatter plot with a smoothing spline (in red) is shown in the left-lower panel.

FIGURE S2 | Spatio-temporal variability of dinoflagellates community Shannon-Wiener (H) index. Color gradient scale represents the sampling depth. Horizontal (blue) line represents the average value for the whole study coverage.

FIGURE S3 | Annual vertical profiles of monthly mean temperature (°C) for **(A)** Esmeraldas, **(B)** Manta, **(C)** Libertad, and **(D)** Puerto Bolívar.

FIGURE S4 | Annual vertical profiles of monthly mean salinity for **(A)** Esmeraldas, **(B)** Manta, **(C)** Libertad, and **(D)** Puerto Bolívar.

FIGURE S5 | Annual vertical profiles of monthly mean nitrate for **(A)** Esmeraldas, **(B)** Manta, **(C)** Libertad, and **(D)** Puerto Bolívar.

FIGURE S6 | Annual vertical profiles of monthly mean phosphate for **(A)** Esmeraldas, **(B)** Manta, **(C)** Libertad, and **(D)** Puerto Bolívar.

FIGURE S7 | Annual vertical profiles of monthly mean silicate for **(A)** Esmeraldas, **(B)** Manta, **(C)** Libertad, and **(D)** Puerto Bolívar.

FIGURE S8 | Annual vertical profiles of monthly mean dissolved oxygen for **(A)** Esmeraldas, **(B)** Manta, **(C)** Libertad, and **(D)** Puerto Bolívar.

FIGURE S9 | Temporal series of ENSO index **(A)** ONI (1+2), **(B)** ONI 3.4, and **(C)** MEI index.

REFERENCES

Abate, R., Gao, Y., Chen, C., Liang, J., Mu, W., Kifile, D., et al. (2017). Decadal variations in diatoms and dinoflagellates on the inner shelf of the east china sea. *Chin. J. Ocean. Limnol.* 35, 1374–1386. doi: 10.1007/s00343-017-6029-1

Apha. (2005). *Standard Methods for the examination of Water and Wasterwater*, 21 Edn. Washington, D.C: American Public Health Association.

Baek, S. H., Shin, H. H., Choi, H.-W., Shimode, S., Hwang, O. M., Shin, K., et al. (2011). Ecological behavior of the dinoflagellate *Ceratium furca* in jangmok harbor of jinhae Bay. *Korea. J. Plankton Res.* 33, 1842–1846. doi: 10.1093/plankt/fbr075

Borbor-Cordova, M., Boyer, E. W., McDowell, W. H., and Hall, A. S. (2006). Nitrogen and phosphorus budgets for a tropical agricultural watershed: guayas. ecuador. *Biogeochemistry* 79, 135–161. doi: 10.1007/s10533-006-9009-7

Borbor-Cordova, M., Torres, G., Mantilla-Saltos, G., Casierra-Tomala, A., Bermudez, R., Renteria, W., et al. (2019). Oceanography of harmful algal blooms on the ecuadorian coast (1997-2017): integrating remote sensing and biological data. *Front. Mar. Sci.* 6:13. doi: 10.3389/fmars.2019.00013

Borcard, D., Gillet, F., and Legendre, P. (2018). *Numerical Ecology with R*, 2nd Edn. New York, NY: Springer International Publishing. doi: 10.1007/978-3-319-71404-2

Bravo, I., and Figueroa, R. I. (2014). Towards an ecological understanding of dinoflagellate cyst functions. *Microorganisms* 2, 11–32. doi: 10.3390/microorganisms2010011

Brown, J. N., Langlais, C., and Gupta, A. S. (2015). Projected sea surface temperature changes in the equatorial Pacific relative to the warm Pool edge. *Deep Sea Res. Part 2 Top. Stud. Oceanogr.* 113, 47–58. doi: 10.1016/j.dsr2.2014.10.022

Brown, S. L., Landry, M. R., and Neveux, J. (2003). Microbial community abundance and biomass along a 180 transect in the equatorial pacific during an el niño-southern oscillation cold phase. *J. Geophys. Res. Oceans* 108, 1–15. doi: 10.1029/2001JC000817

Carnicer, O., García-Altares, M., Andree, K. B., Diogène, J., and Fernández-Tejedor, M. (2016). First evidence of Ostreopsis cf. ovata in the eastern tropical Pacific Ocean. Ecuadorian coast. *Bot. Mar.* 59, 267–274. doi: 10.1515/bot-2016-0022

Cavalcante, K. P., Cardoso, L. S., Sussella, R., and Becker, V. (2016). Towards a comprehension of *Ceratium* (*Dinophyceae*) invasion in brazilian freshwaters: autecology of C. furcoides in subtropical reservoirs. *Hydrobiologia* 771, 265–280. doi: 10.1007/s10750-015-2638-x

Chavez, F. P., Pennington, J. T., Castro, C. G., Ryan, J. P., Michisaki, R. P., Schlining, B., et al. (2002). Biological and chemical consequences of the 1997–1998 El Nino in central california waters. *Prog. Oceanogr.* 54, 205–232. doi: 10.1016/S0079-6611(02)00050-2

Conde, A., Hurtado, M., and Prado, M. (2018). Phytoplankton response to a weak El Niño event. *Ecol. Indic.* 95, 394–404. doi: 10.1016/j.ecolind.2018.07.064

Conde, A., and Prado, M. (2018). Changes in phytoplankton vertical distribution during an El Niño event. *Ecol. Indic.* 90, 201–205. doi: 10.1016/j.ecolind.2018.03.015

Cucalon, E. (1989). "Oceanographic Characteristics of the Coast of Ecuador," in *A Sustainable Shrimp Mariculture Industry for Ecuador*, eds S. Olsen and

L. Arriaga (Narragansett, RI: Coastal Resources Center, University of Rhode Island).

Daguer, H., Hoff, R. B., Molognoni, L., Kleemann, C. R., and Felizardo, L. V. (2018). Outbreaks, toxicology, and analytical methods of marine toxins in seafood. *Curr. Opin. Food Sci.* 24, 43–55. doi: 10.1016/j.cofs.2018.10.006

Díaz, P., Ruiz-Villarreal, M., Pazos, Y., Moita, T., and Reguera, B. (2016). Climate variability and Dinophysis acuta blooms in an upwelling system. *Harmful Algae* 53, 145–153. doi: 10.1016/j.hal.2015.11.007

Díaz, P. A., Reguera, B., Ruiz-Villarreal, M., Pazos, Y., Velo-Suárez, L., Berger, H., et al. (2013). Climate variability and oceanographic settings associated with interannual variability in the initiation of *Dinophysis acuminata* blooms. *Mar. Drugs* 11, 2964–2981. doi: 10.3390/md11082964

Du, X., Peterson, W., and O'Higgins, L. (2015). Interannual variations in phytoplankton community structure in the northern california current during the upwelling seasons of 2001-2010. *Mar. Ecol. Prog. Ser.* 519, 75–87. doi: 10.3354/meps11097

Finkel, Z., Beardall, J., Flynn, K., Quigg, A., Ress, T., and Raven, J. (2010). Phytoplankton in a changing world: cell size and elemental stoichiometry. *J. Plankton Res.* 32, 119–137. doi: 10.1093/plankt/fbp098

Franco-Gordo, C., Godímez-Domínguez, E., Filonov, A., Tereshchenko, I., and Freire, J. (2004). Plankton biomass and larval fish abundace prior to and during the El Niño period of 1997-1998 along the central pacific coast of méxico. *Prog. Oceanogr.* 63, 99–123. doi: 10.1016/j.pocean.2004.10.001

Gierach, M. M., Lee, T., Turk, D., and McPhaden, M. J. (2012). Biological response to the 1997–98 and 2009–10 El Niño events in the equatorial Pacific Ocean. *Geophys. Res. Lett.* 39:L10602. doi: 10.1029/2012GL051103

Glibert, P. M., Anderson, D. M., Gentin, P., Granéli, E., and Sellner, K. G. (2005). The global complex phenomena of harmful algal blooms. *Oceanography* 18, 137–147. doi: 10.5670/oceanog.2005.49

Gomez, F. (2005). A list of free-living dinoflagellate species in the world's oceans. *Acta Bot. Croat.* 64, 129–212.

Hallegraeff, G. M. (2003). "Harmful Algal Blooms: A Global Overview," in *Manual on Harmful Marine Microalgae. Monogr. Oceanogr. Methodol*, 2nd Edn, eds M. Hallegraeff, D. M. Anderson, and A. D. Cembella (Paris: IOC-UNE-SCO).

Hallegraeff, G. M. (2010). Ocean climate change, phytoplankton community responses, and harmful algal blooms: a formidable predictive challenge. *J. Phycol.* 46, 220–235. doi: 10.1111/j.1529-8817.2010.00815.x

Hoppenrath, M., Elbrächter, M., and Debres, G. (2009). *Marine Phytoplankton*. Stuttgart: Schweizerbart Science Publishers.

Hoppenrath, M., Murray, S. A., Chomérat, N., and Horiguchi, T. (2014). *Marine Benthic Dinoflagellates - Unveiling Their Worldwide Biodiversity*. Stuttgart: Schweizerbart Science Publishers.

Horton, T., Kroh, A., Ahyong, S., Bailly, N., Boury-Esnault, N., Brandão, S. N., et al. (2018). *World Register of Marine Species*. Available at: http://www.marinespecies.org

Ipcc. (2014). in *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, eds R. K. Pachauri and L. A. Meyer (Geneva: IPCC).

Jacox, M. G., Hazen, E. L., Zaba, K. D., Rudnick, D. L., Edwards, C. A., Moore, A. M., et al. (2016). Impacts of the 2015–2016 el niño on the california current system: early assessment and comparison to past events. *Geophys. Res. Lett.* 43, 7072–7080. doi: 10.1002/2016GL069716

Jimenez-Bonilla. (1980). Composicion distribucion de biomasa plancton frente costa ecuatorial. *Acta Oceanográ. Del Pac.* 1, 19–64.

Kudela, R., Berdalet, E., Enevoldsen, H., Pitcher, G., Raine, R., and Urban, E. (2017). GEOHAB-the global ecology and oceanography of harmful algae "bloom" program: motivation, goals and legacy. *Oceanography* 30, 12–21. doi: 10.5670/oceanog.2017.106

Kudela, R., Seeyave, M. S., and Cochlan, W. P. (2010). The role of nutrients in regulation and promotion of harmful algal blooms in upwelling systems. *Prog. Oceanogr.* 85, 122–135. doi: 10.1016/j.pocean.2010.02.008

Lassus, P., Chomérat, N., Hess, P., and Nézan, E. (2016). "Toxic and harmful microalgae of the World Ocean," in *Micro-algues Toxiques Et Nuisibles De l'Océan Mondial. IOC Manuals and guides, 68 (Bilingual English/French)*, (Copenhagen: International Society for the Study of Harmful Algae).

León-Muñoz, J., Urbina, M. A., Garreaud, R., and Iriarte, J. L. (2018). Hydroclimatic conditions trigger record harmful algal bloom in western patagonia (summer 2016). *Sci. Rep.* 8:1330. doi: 10.1038/s41598-018-19461-4

Li, H., Tang, H., Shi, X., Zhang, C., and Wang, X. (2014). Increased nutrient loads from the changiiang (yangtze) river have led to increased harmful algal blooms. *Harm. Algae* 39, 92–101. doi: 10.1016/j.hal.2014.07.002

Litaker, W. R., Vandersea, M. W., Kibler, S. R., Reece, K. S., Stokes, N. A., Lutzoni, F. M., et al. (2007). Recognizing dinoflagellate species using ITS rDNA sequences. *J. Phycol.* 43, 344–355. doi: 10.1111/j.1529-8817.2007.00320.x

McCabe, R. M., Hickey, B. M., Kudela, R. M., Lefebvre, K. A., Adams, N. G., Bill, B. D., et al. (2016). An uN/Precedented coastwide toxic algal bloom linked to anomalous ocean conditions. *Geophys. Res. Lett.* 43, 366–310. doi: 10.1002/2016GL070023

McKibben, S. M., Peterson, W., Wood, A. M., Trainer, V. L., Hunter, M., and White, A. E. (2017). Climatic regulation of the neurotoxin domoic acid. *Proc. Natl. Acad. Sci. U.S.A.* 114, 239–244. doi: 10.1073/pnas.1606798114

Meave-del Castillo, M. E., Zamudio-Resendiz, M. E., and Castillo-Rivera, M. (2012). Riqueza fitoplanctonica de la bahia de acapulco y zona costera aledana, guerrero, mexico. *Acta Bot. Mex. Núm.* 100, 405–487. doi: 10.21829/abm100.2012.41

Moestrup, Ø, Akselmann, R., Fraga, S., Hoppenrath, M., Iwataki, M., Komárek, J., et al. (2009). *IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae*. Available at: http://www.marinespecies.org/hab

Montecino, V., and Lange, C. (2009). The humboldt current system: ecosystem components and processes, fisheries, and sediment studies. *Prog. Oceanogr.* 83, 65–79. doi: 10.1016/j.pocean.2009.07.041

Moore, S. K., Mantua, N. J., Hickey, B. M., and Trainer, V. L. (2010). The relative influences of el niño – southern oscillation and pacific decadal oscillation on paralytic shellfish toxin accumulation in pacific northwest shellfish. *Limnol. Oceanograp.* 55, 2262–2274. doi: 10.4319/lo.2010.55.6.2262

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2018). *Community Ecology PackageR package version 2.5-2*. Available at:https://CRAN.R-project.org/package=vegan.

Omura, T., Iwataki, M., Borja, V. M., Takayama, H., and Fukuyo, Y. (2012). *Marine Phytoplankton of the Western Pacific*. Tokyo: Koseishakoseikaku.

Oyarzún, D., and Brierley, C. M. (2018). The future of coastal upwelling in the humboldt current from model projections. *Clim. Dyn.* 52, 599–615. doi: 10.1007/s00382-018-4158-7

Pennington, J. T., Mahoney, K. L., Kuwahara, V. S., Kolber, D. D., Calienes, R., and Chavez, F. P. (2006). Primary production in the eastern tropical Pacific: a review'. *Prog. Oceanogr.* 69, 285–317. doi: 10.1016/j.pocean.2006.03.012

Pizarro, O., and Montecinos, A. (2004). Interdecadal variability of the thermocline along the west coast of south america. *Geophys. Res. Lett.* 31:L20307. doi: 10.1029/2004GL020998

Poloczanska, E. S., Burrows, M. T., Brown, C. J., García Molinos, J., Halpern, B. S., Hoegh-Guldberg, O., et al. (2016). Responses of marine organisms to climate change across oceans. *Front. Mar. Sci.* 3:62. doi: 10.3389/fmars.2016.00062

Prado, M., Trocholli, L., and Moncayo, E. (2015). Cambios estructurales del microfitoplancton en la zona costera de la provincia el oro-ecuador en temporada seca. *Bol. Inst. Oceanogr. Venezuela* 54, 139–152.

R Core Team (2018). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. Available at: https://www.R-project.org/

Reguera, B., Velo-Suárez, L., Raine, R., and Park, R. G. (2012). Harmful *Dinophysis* species: a review. *Harm. Algae* 14, 87–106. doi: 10.1016/j.hal.2011.10.016

Santos, J. L. (2006). The impact of el niño - southern oscillation events on south america. *Adv. Geosci.* 6, 221–225. doi: 10.5194/adgeo-6-221-2006

Shulman, I., Penta, B., Moline, M. A., Haddock, S. H. D., Anderson, S., Oliver, M. J., et al. (2012). Can vertical migrations of dinoflagellates explain observed bioluminescence patterns during an upwelling event in Monterey Bay, California? *J. Geophys. Res.* 117:C01016. doi: 10.1029/2011JC007480

Smayda, T. J. (1997). What is a bloom? A commentary. *Limnol. Oceanogr.* 42, 1132–1136. doi: 10.4319/lo.1997.42.5_part_2.1132

Smayda, T. J. (2008). Complexity in the eutrophication-harmful algal bloom relationship, with comment on the importance of grazing. *Harm. Algae* 8, 140–151. doi: 10.1016/j.hal.2008.08.018

Smayda, T. J., and Reynolds, C. S. (2003). Strategies of marine dinoflagellate survival and some rules of assembly. *J. Sea Res.* 49, 95–106. doi: 10.1016/S1385-1101(02)00219-8

Smayda, T. J., and Reynolds, C. S. (2001). Community assembly in marine phytoplankton: application of recent models to harmful dinoflagellate blooms. *J. Plankton Res.* 23, 447–461. doi: 10.1093/plankt/23.5.447

Steidinger, K. A., and Jangen, K. (1997). "Chapter 3 - Dinoflagellates," in *Identifying Marine Phytoplankton*, ed. C. R. Tomas (San Diego: Academic Press) doi: 10.1016/B978-012693018-4/50005-7

Stoecker, D., Hansen, P., Caron, D., and Mitra, A. (2017). Mixotrophy in the marine plankton. *Ann. Rev. Mar. Sci.* 9, 311–335. doi: 10.1146/annurev-marine-010816-060617

Strickland, J. D. H., and Parsons, T. R. (1972). *A Practical Hand Book of Seawater Analysis*, 2nd Edn. Ottawa: Fisheries Research Board of Canada.

Sullivan, S. K., and Bustamante, G. (1999). *Setting Geographic Priorities for Marine Conservation in Latin America and the Caribbean*. Arlington: The Nature Conservancy.

Tam, J. J., Taylor, M. H., Blaskovic, V., Espinoza, P., Ballón, R. M., Díaz, E., et al. (2008). Trophic modeling of the northern humboldt current ecosystem, part i: comparing trophic linkages under la niña and el niño conditions. *Prog. Oceanogr.* 79, 352–365. doi: 10.1016/j.pocean.2008.10.007

ter Braak, C. J. F., and Verdonschot, P. F. M. (1995). Canonical correspondence analysis and related multivariate methods in aquatic ecology. *Aqu. Sci.* 57:255. doi: 10.1007/BF00877430

Torres, G. (2015). Evaluación de mareas rojas durante 1968-2009 en ecuador. *Acta Oceanograì. Del Paciìfico* 20, 89–98.

Torres, G., Recalde, S., Narea, R., Renteria, W., Troccoli, L., and Tinoco, O. (2017). Variabilidad espacio-temporal del fitoplancton y variables oceanográficas en el golfo de guayaquil durante 2013-15. *Rev. Del Inst. De Invest.* 20, 70–79.

Torres, G., and Tapia, M. (2002). Distribución del fitoplancton en la región costera del mar ecuatoriano, durante diciembre 2000. *Acta Oceanográ. Del Pacífico* 11, 63–72.

Utermöhl, H. (1958). Zur vervollkomnung der quantitativen phytoplankton-meth-odik mitteilungen. *Int. Ver. Theor. Ange-w. Limnol.* 9, 1–38.

Wang, S., Tang, D., He, F., Fukuyo, Y., and Azanza, R. V. (2008). Occurrences of harmful algal blooms (HABs) associated with ocean environments in the South China Sea. *Hydrobiologia* 596, 79–93. doi: 10.1007/s10750-007-9059-4

Yamamoto, T., Oh, S. J., and Kataoka, Y. (2004). Growth and uptake kinetics for nitrate, ammonium and phosphate by the toxic dinoflagellate *Gymnodinium catenatum* isolated from Hiroshima Bay, Japan. *Fish. Sci.* 70, 108–115. doi: 10.1111/j.1444-2906.2003.00778.x

Zuur, A. F., Ieno, E. N., and Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods Ecol. Evol.* 1, 3–14. doi: 10.1111/j.2041-210X.2009.00001.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Torres, Carnicer, Canepa, De La Fuente, Recalde, Narea, Pinto and Borbor-Córdova. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Marine Science

REVIEW
published: 05 April 2019
doi: 10.3389/fmars.2019.00148

A Review on the Biodiversity and Biogeography of Toxigenic Benthic Marine Dinoflagellates of the Coasts of Latin America

Lorena María Durán-Riveroll[1,2], Allan D. Cembella[2] and Yuri B. Okolodkov[3]*

[1] CONACyT-Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Mexico City, Mexico, [2] Alfred-Wegener-Institut, Helmholtz-Zentrum für Polar-und Meeresforschung, Bremerhaven, Germany, [3] Instituto de Ciencias Marinas y Pesquerías, Universidad Veracruzana, Veracruz, Mexico

Many benthic dinoflagellates are known or suspected producers of lipophilic polyether phycotoxins, particularly in tropical and subtropical coastal zones. These toxins are responsible for diverse intoxication events of marine fauna and human consumers of seafood, but most notably in humans, they cause toxin syndromes known as diarrhetic shellfish poisoning (DSP) and ciguatera fish poisoning (CFP). This has led to enhanced, but still insufficient, efforts to describe benthic dinoflagellate taxa using morphological and molecular approaches. For example, recently published information on epibenthic dinoflagellates from Mexican coastal waters includes about 45 species from 15 genera, but many have only been tentatively identified to the species level, with fewer still confirmed by molecular criteria. This review on the biodiversity and biogeography of known or putatively toxigenic benthic species in Latin America, restricts the geographical scope to the neritic zones of the North and South American continents, including adjacent islands and coral reefs. The focus is on species from subtropical and tropical waters, primarily within the genera *Prorocentrum*, *Gambierdiscus*/*Fukuyoa*, *Coolia*, *Ostreopsis* and *Amphidinium*. The state of knowledge on reported taxa in these waters is inadequate and time-series data are generally lacking for the prediction of regime shift and global change effects. Details of their respective toxigenicity and toxin composition have only recently been explored in a few locations. Nevertheless, by describing the specific ecosystem habitats for toxigenic benthic dinoflagellates, and by comparing those among the three key regions - the Gulf of Mexico, Caribbean Sea and the subtropical and tropical Pacific coast, insights for further risk assessment of the global spreading of toxic benthic species is generated for the management of their effects in Latin America.

Keywords: benthic dinoflagellates, polyether toxins, dinoflagellate biodiversity, phycotoxins, chemical ecology

OPEN ACCESS

Edited by:
Juan Jose Dorantes-Aranda, University of Tasmania, Australia

Reviewed by:
Gustaaf Marinus Hallegraeff, University of Tasmania, Australia
Patricia A. Tester, Ocean Tester, LLC., United States

****Correspondence:***
Lorena María Durán-Riveroll
lorena.duran@awi.de;
lduran@conacyt.mx

Specialty section:
This article was submitted to Marine Ecosystem Ecology, a section of the journal Frontiers in Marine Science

Received: *30 November 2018*
Accepted: *08 March 2019*
Published: *05 April 2019*

Citation:
Durán-Riveroll LM, Cembella AD and Okolodkov YB (2019) A Review on the Biodiversity and Biogeography of Toxigenic Benthic Marine Dinoflagellates of the Coasts of Latin America. Front. Mar. Sci. 6:148. doi: 10.3389/fmars.2019.00148

INTRODUCTION TO BENTHIC DINOFLAGELLATES

Benthic dinoflagellates are important components of marine ecosystems, implicated in benthic food webs and species interactions, including bacterial associations, and also contribute to community diversity. Such species are common in shallow waters, and are frequently found attached to diverse substrates through the production of polysaccharide filaments and mucous

layers (Honsell et al., 2013). In natural habitats, they tend to thrive in undisturbed locations and accumulate under calm water conditions. Benthic dinoflagellates do not create "blooms" in the conventional sense, as planktonic species do in the water column, but often yield dense aggregations or colonies of attached cells. In cases where dense cell aggregations are associated with harmful events, they are frequently referred to as "benthic harmful algal blooms" (or bHABs) but this terminology is not precisely defined. Whereas benthic dinoflagellates are better known and more completely described from tropical and subtropical waters, species are found globally even from temperate, sub-arctic and polar environments. Although optimal growth conditions are still unknown for many benthic species, their high relative cell abundance and diversity in shallow subtropical and tropical waters indicates that higher cell growth is favored at elevated water temperatures. In the Caribbean, there is an apparent correlation between high water temperature and *Gambierdiscus* Adachi & Fukuyo cell abundance (Tester et al., 2010), but this relationship is not clear for other benthic dinoflagellates.

Many benthic species are considered to be low-light ("shade") adapted, and can therefore flourish in deeper waters within tropical environments with higher water transparency, while some species can even tolerate high irradiance (Richlen and Lobel, 2011). Tropical waters with abundant benthic dinoflagellate communities are usually characterized by high salinity, but there is little direct evidence from culture experiments or observations of natural populations that such high salinities are necessary for growth.

Benthic dinoflagellates comprise both phototrophic and heterotrophic species, and mixotrophic capacity is also common. In fact, it is likely that all benthic species are capable of facultative heterotrophy and may be less dependent on inorganic nutrient concentration than phototrophic pelagic species, since they can acquire organic nutrients not only from the surrounding waters but also from the sediments or other surfaces upon which they live (Litaker et al., 2010).

Although benthic and, in particular, epiphytic dinoflagellates are designated as such because of their typical preference for substrate attachment, the vegetative cells of free-living species are flagellated and are fully capable of detachment and motility. They can occupy a wide variety of micro-niches provided by these alternative substrate habitats, compared to plankton species in the water column. For example, the genus *Ostreopsis* Schmidt is widely distributed in both tropical and temperate waters, often in shallow sheltered embayments or reefs, adhering to different substrates such as macroalgae, seagrasses, pebbles, rocks, coral rubble, mussel shells, soft sediments and invertebrates, but can also be found in the water column (Tindall and Morton, 1998; Totti et al., 2010; Accoroni et al., 2015). Many benthic dinoflagellates are photosynthetic endosymbionts ("zooxanthellae"), e.g., *Symbiodinium* spp. or the species from other recently described closely related genera (LeJeunesse et al., 2018), among corals, sea anemones, nudibranchs and other sessile and vagile macrobenthic invertebrates, but none of those endosymbionts are known to be toxigenic.

A comprehensive understanding of the diverse life history strategies of benthic dinoflagellates is still lacking and is even more limited than that available for free-living planktonic species. A few life history studies have been performed on *Gambierdiscus* and *Ostreopsis* (Bravo et al., 2014), for which both meiosis and gametogenesis have been described, but much remains unresolved with reference to sexual and asexual cycles. High apparent morphological variability, e.g., within *Ostreopsis*, could correspond to alternative life history stages, but this has not been confirmed.

OCEAN ENVIRONMENT, HUMAN HEALTH AND SOCIO-ECONOMIC CONSEQUENCES

Among the plethora of benthic dinoflagellates, several species collected primarily from seagrasses and macroalgal substrates are known to produce potent biotoxins (Boisnoir et al., 2018). In fact, as a proportion of the described taxa of free-living marine dinoflagellates, epiphytic and sand-dwelling species are heavily represented among known toxigenic genotypes. Such toxins from benthic dinoflagellates are often found within complex toxic species assemblages and are therefore of great concern worldwide (Ben-Gharbia et al., 2016), but associations of individual dinoflagellate species with particular toxin chemotypes is often unclear. There is a critical lack of research on bHABs, based on the objective knowledge of the real threat that these organisms represent. This is particularly true in Latin America, although there have been significant improvements in addressing these phenomena by regional research institutions (Barón-Campis et al., 2014) and the associated toxin syndromes by health authorities. Major global efforts have now been initiated to describe and understand the dynamics and biodiversity of bHAB populations (Berdalet et al., 2016) and to develop effective strategies to monitor and mitigate the consequences.

Benthic HABs are of particular significance in tropical and subtropical regions, for coastal and island communities where the ocean is crucial for seafood production for local inhabitants, and also for global trade. These regions are frequently threatened by human illnesses and marine faunal toxicity associated with toxic benthic and epiphytic dinoflagellates (Berdalet et al., 2017). The toxic effects on humans and other organisms often occurs when bHABs are present in persistently high cell abundances, but with certain species, these effects can be expressed via biomagnification even when highly toxigenic populations are present at low cell densities (Berdalet et al., 2016).

In Latin America, several human intoxication syndromes are caused by benthic dinoflagellates due to the bioaccumulation of their toxins within the food web and transfer into seafood species. For example, on the northwestern Pacific coast of Mexico, several cases of human poisoning were a direct result of the consumption of fish species of the families Serranidae and Lutjanidae (Sierra-Beltrán et al., 1998). In general, in Latin America there is a high local consumption of fish and shellfish from commercial and artisanal fisheries, including non-common species such as octopus and sea cucumber with poorly described accumulation kinetics, and from emerging aquaculture activities (Barón-Campis et al., 2014). The economic

consequences of bHABs on human health and losses to the fishing, aquaculture and tourism industries in Latin America have not been comprehensively investigated, but informal estimates suggest that these are substantial (Cuellar-Martinez et al., 2018).

Monitoring programs for bHAB species and their toxins in seafood are sporadic on a global scale. In Latin America only a few countries have implemented routine monitoring programs and these focus exclusively on commercial species (Cuellar-Martinez et al., 2018). With such inadequate monitoring protocols to protect human health, some fish species have been banned for human consumption, e.g., in Australia and French Polynesia, because of the risk of CFP (Lehane, 2000). This is also the case in Quintana Roo on the Caribbean coast of Mexico, where fishing, selling and consumption of the great barracuda (*Sphyraena barracuda* Walbaum), a known ciguateric fish, is forbidden (García-Mendoza et al., 2016; López, 2018). In spite of the health risks posed by bHAB toxins, many toxins are still not well characterized structurally or toxicologically, and reliable quantitative analysis and assay methods for such toxins are still emerging.

BIOGEOGRAPHY OF TOXIGENIC BENTHIC DINOFLAGELLATES

The distribution of benthic dinoflagellates within Latin America is expected to reflect adaptive capacity, tolerance and resilience of species within ecological niches defined by the ambient environmental conditions. For these reasons, the distribution patterns are considered herein from the perspective of linked or contiguous functional ecosystems, which can only be roughly defined, rather than by geographical or geopolitical boundaries (**Table 1** and **Figure 1**). The temperate and Arctic eastern coasts of North America (north of Mexico) are therefore not considered within the purview of this review on toxigenic benthic dinoflagellates. On a cautionary note, it should be recognized that reported species distribution may more accurately reflect the intensity of regional surveys rather than a true biogeographical pattern. Taxonomic revisions can also bias interpretation of biogeographical species patterns. For example, *Prorocentrum rhathymum* is considered here as distinct from *P. mexicanum* because these taxa are morphologically and ecologically separable (Cortés-Altamirano and Sierra-Beltrán, 2003; An et al., 2010), although it has been claimed that they are synonymous (Steidinger and Tangen, 1997; Faust and Gulledge, 2002; Lim et al., 2013; Gómez et al., 2017a; Steidinger, 2018). A final caveat regarding biogeographical description of benthic dinoflagellate species distribution is due to incomplete knowledge of the linkage between species descriptors, either morphological or molecular, and actual or potential toxigenicity at the local population level.

Eastern Pacific Coast of Latin America

With respect to biogeographical distribution of potentially toxigenic species of the eastern Pacific of Latin America, the region is defined to include the coast of Mexico, Guatemala, El Salvador, Honduras, Nicaragua, Costa Rica, Panama, Colombia, Ecuador, Peru and Chile.

TABLE 1 | Potentially toxic benthic dinoflagellate species reported in Latin America and adjacent subtropical waters.

Geographical Location	Species	Reference
Eastern Pacific coast of Latin America		
Mexico	***Amphidinium***	Sierra-Beltrán et al., 1998; Núñez-Vázquez et al., 2000; Cortés-Lara et al., 2005; Núñez-Vázquez, 2005; Okolodkov and Gárate-Lizárraga, 2006; Gárate-Lizárraga et al., 2007; Gárate-Lizárraga, 2012; Morquecho-Escamilla et al., 2016, 2017; Gallegos-Mendiola et al., 2017; Sepúlveda-Villarraga et al., 2017; Gárate-Lizárraga et al., 2018
	A. cf. *carterae*	
	A. operculatum	
	A. carterae	
	Gambierdiscus	
	G. cf. *carolinianus*	
	G. cf. *carpenteri*	
	G. toxicus (?)	
	Fukuyoa	
	F. yasumotoi	
	Ostreopsis	
	O. lenticularis	
	O. ovata	
	O. siamensis	
	Prorocentrum	
	P. concavum	
	P. lima	
	P. maculosum	
	P. mexicanum	
El Salvador	*Gambierdiscus* spp.	Quintanilla and Amaya, 2016
	Ostreopsis spp.	
	Prorocentrum	
	P. lima	
Costa Rica	***Amphidinium***	Vargas-Montero et al., 2012
	A. carterae	
	Coolia	
	C. tropicalis	
	Gambierdiscus spp.	
	Ostreopsis	
	O. siamensis	
	Prorocentrum	
	P. concavum	
Ecuador	***Ostreopsis***	Carnicer et al., 2016
	O. cf. *ovata*	
Subtropical and tropical Atlantic Ocean and the Caribbean Sea		
Cuba	***Gambierdiscus***	Delgado et al., 2002; Delgado-Miranda, 2005; Moreira-González et al., 2016
	G. caribaeus	
	G. toxicus (?)	
	Ostreopsis	
	O. lenticularis	
	Prorocentrum	
	P. concavum	
	P. mexicanum	
	P. hoffmannianum	
	P. lima	
Puerto Rico	***Gambierdiscus***	Tosteson et al., 1986
	G. toxicus (?)	
	Ostreopsis	
	O. lenticularis	
Bahamas	***Coolia***	Karafas and Tomas, 2015
	C. santacroce	
Dominican Republic	***Coolia***	Karafas et al., 2015; Karafas and Tomas, 2015
	C. palmyrensis	
	C. malayensis	
British and United States Virgin Islands	***Coolia***	Carlson and Tindall, 1985; Litaker et al., 2009; Karafas et al., 2015; Karafas and Tomas, 2015; Rains and Parsons, 2015
	C. santacroce	
	C. malayensis	

(Continued)

TABLE 1 | Continued

Geographical Location	Species	Reference
	Gambierdiscus	
	G. toxicus (?)	
	G. caribaeus	
	G. belizeanus	
	Ostreopsis	
	O. lenticularis	
	Prorocentrum	
	P. mexicanum	
	P. concavum	
	P. lima	
Venezuela	***Amphidinium***	Valerio-González and Díaz-Ramos, 2008; Navarro-Vargas et al., 2014; Marchan-Álvarez et al., 2017
	A. carterae	
	Ostreopsis	
	O. siamensis	
	O. ovata	
	O. lenticularis	
	Prorocentrum	
	P. lima	
	P. mexicanum	
	P. concavum	
	P. emarginatum	
Colombia	***Gambierdiscus***	Mancera-Pineda et al., 2014; Arbelaez et al., 2017; Quintana-M and Mercado-Gómez, 2017
	G. toxicus	
	(*G. belizeanum*?)	
	Ostreopsis	
	O. cf. *ovata*	
	O. lenticularis	
	Prorocentrum	
	P. hoffmannianum	
	P. lima	
	P. mexicanum	
	P. emarginatum	
Belize	***Coolia***	Faust, 1990a,b, 1993c,d, 1994, 1995, 1996, 1997, 1999, 2000, 2004; Faust and Gulledge, 1996; Morton and Faust, 1997; Faust and Steidinger, 1998; Faust et al., 2008; Litaker et al., 2009
	C. tropicalis	
	Gambierdiscus	
	G. belizeanus	
	G. caribaeus	
	G. carptenteri	
	Fukuyoa	
	F. ruetzleri	
	Prorocentrum	
	P. hoffmannianum	
	P. maculosum	
Mexico	***Amphidinium***	Almazán-Becerril, 2000; Hernández-Becerril and Almazán Becerril, 2004; Almazán-Becerril et al., 2015
	A. carterae	
	Coolia	
	C. cf. *tropicalis*	
	Gambierdiscus	
	G. belizeanus	
	G. toxicus (?)	
	Fukuyoa	
	F. yasumotoi	
	Ostreopsis	
	O. heptagona	
	O. siamensis	
	Prorocentrum	
	P. arenarium	
	P. belizeanum	
	P. concavum	
	P. emarginatum	
	P. hoffmannianum	
	P. lima	
	P. maculosum	
	P. rhathymum	
Gulf of Mexico		
Mexico	***Amphidinium***	Okolodkov et al., 2007, 2009; Barón-Campis et al., 2014; Okolodkov et al., 2014; Martínez-Cruz et al., 2016; Estrada-Vargas et al., 2017
	A. cf. *carterae*	
	A. cf. *operculatum*	
	Gambierdiscus	
	G. toxicus	
	(*G. caribaeus*?)	
	G. caribaeus	
	Gambierdiscus sp.	
	Ostreopsis	
	Ostreopsis sp.	
	Prorocentrum	
	P. cf. *lima*	
	P. lima	
	P. rhathymum	
	Prorocentrum sp.	
Florida Straits, Keys and southeastern U.S. Atlantic coasts		
Florida Keys	***Amphidinium***	Mitchell, 1985; Bomber et al., 1988, 1989; Litaker et al., 2010
	A. klebsii	
	Coolia	
	Coolia santacroce	
	Gambierdiscus	
	G. toxicus (?)	
	G. belizeanus	
	G. carolineanus	
	Fukuyoa	
	F. ruetzleri	
	Ostreopsis	
	O. heptagona	
	O. siamensis	
	Prorocentrum	
	P. lima	
	P. rhathymum	
	P. emarginatum	
	P. concavum	
Southwestern Atlantic		
Brazil	***Ostreopsis***	Gómez et al., 2017b; Nascimento et al., 2017
	O. cf. *ovata*	
	Prorocentrum	
	P. caipirignum	

Species recognized as toxigenic were compiled from the IOC-Harmful Microalgal Species list (Moestrup et al., 2009) plus new valid toxigenic taxa not yet included on this list.

The Pacific coastal waters of Baja California Sur, Mexico, have been relatively well explored for the distribution of benthic dinoflagellates. Four putatively toxic *Ostreopsis* species have been found: *O. lenticularis, O. marina, O. ovata* and *O. siamensis* (Sierra-Beltrán et al., 1998; Núñez-Vázquez et al., 2000; Cortés-Lara et al., 2005; Gárate-Lizárraga, 2005; Núñez-Vázquez, 2005; Okolodkov and Gárate-Lizárraga, 2006). The latter species was also reported from Isabel Island, Nayarit, 70 km from the mainland (Cortés-Lara et al., 2005; Cortés-Altamirano et al., 2011). *Gambierdiscus toxicus* has been found in several studies in the adjacent waters of Baja California Sur [see references in Okolodkov and Gárate-Lizárraga (2006)]. Some benthic dinoflagellate species isolated from Bahía de La Paz and Bahía

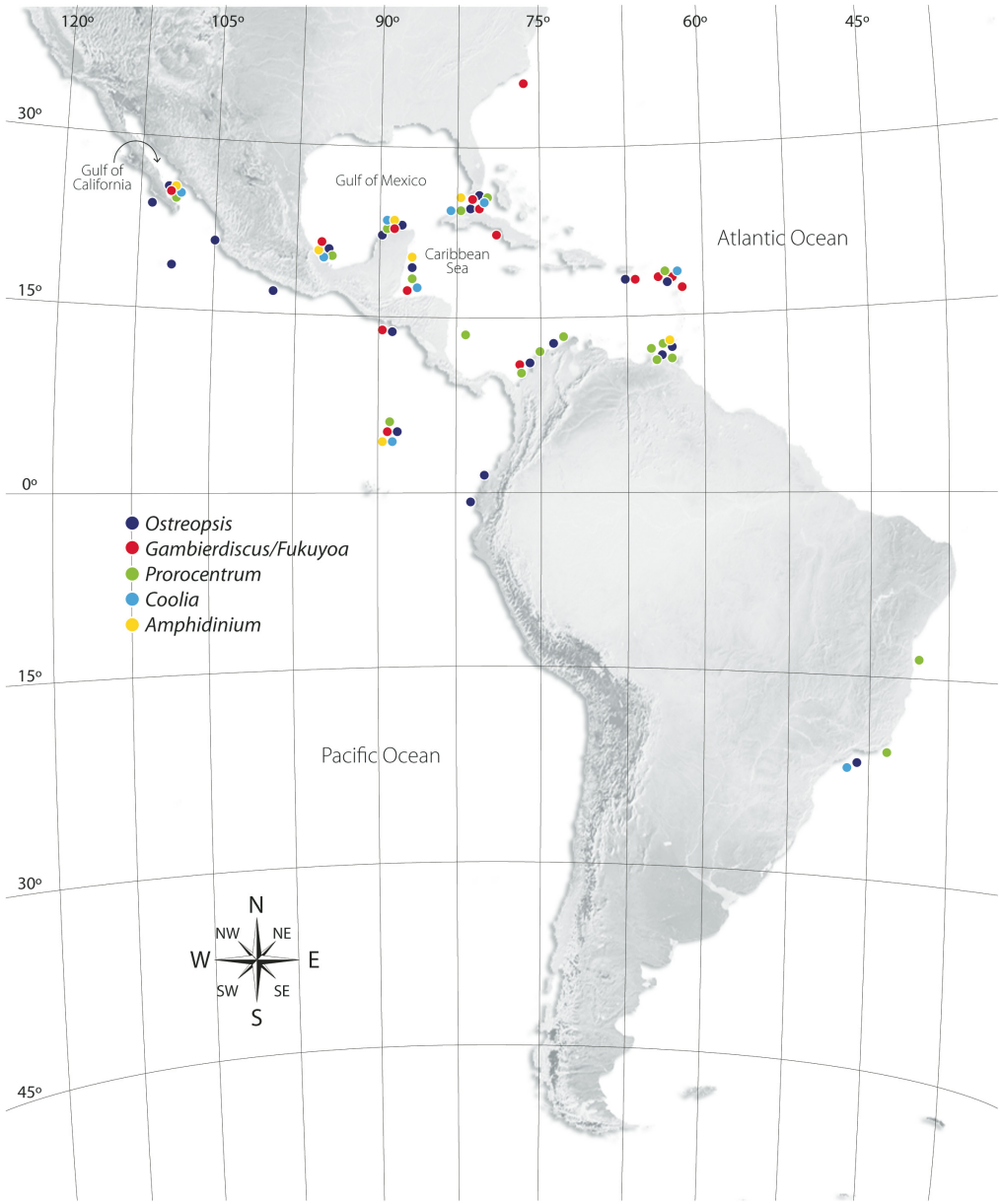


FIGURE 1 | Known biogeographical distribution of dinoflagellate genera with toxigenic benthic members from tropical and subtropical waters of Latin America, as well as the Caribbean Sea and adjacent coasts of the Americas. Genera including toxigenic benthic species were obtained from the IOC-Harmful Microalgal Species list (Moestrup et al., 2009), but the map may include taxa not yet confirmed as toxigenic at the specified locations.

Concepción in the southern Gulf of California are maintained in culture: *Prorocentrum belizeanum, P.* cf. *concavum, P. lima, P. maculosum, O.* cf. *ovata* and *P. rhathymum,* (Morquecho-Escamilla et al., 2016), and confirmation of toxin production is currently underway. Further macroalgal samples collected at Isla San José and Bahía de La Paz in 2011, 2014, and 2016, yielded cultures of *Amphidinium operculatum*, *Bysmatrum gregarium*, *Coolia canariensis*, *Fukuyoa yasumotoi*, *G.* cf. *carolinianus*, *G.* cf. *carpenteri*, *O. marina*, *O. ovata, P. concavum, P. fukuyoi, P. lima* and *P. rhathymum* (Morquecho-Escamilla et al., 2017). *Ostreopsis ovata* has also been observed along the Pacific coastal state of Guerrero (Gallegos-Mendiola et al., 2017). At San Juan de la Costa and Isla Gaviota in Bahía de La Paz, *A.* cf. *carterae, C. malayensis, O.* cf. *lenticularis* and *Prorocentrum* sp. were found associated with brown algae (Sepúlveda-Villarraga et al., 2017). In the waters of the Archipielago de Revillagigedo (Isla San Benedicto, Isla Socorro and Isla Roca Partida), state of Colima, about 720–970 km west of the continent, *O. lenticularis* was also reported in 2001–2017 (Gárate-Lizárraga et al., 2018).

The Pacific coast of Central America has only been sporadically explored for benthic dinoflagellates. Nevertheless, both toxigenic genera *Gambierdiscus* and *Ostreopsis*

(not identified to species) were found among macroalgal samples from the Los Cóbanos reef zone along the west coast of El Salvador, whereas *P. lima* was the most abundant species (Quintanilla and Amaya, 2016). Analysis of samples taken in Costa Rica (2006–2011) at Cocos Island, 532 km southwest of the continental mainland, revealed *A. carterae*, *C. tropicalis, C.* cf. *areolata,* unidentified benthic *Gambierdiscus* (presumably two species), *P. concavum* and *O. siamensis* (Vargas-Montero et al., 2012). The first evidence of *O.* cf. *ovata* in the eastern tropical Pacific Ocean was provided from the Ecuadorian coast at Estero del Plátano (Esmeraldas Province) and Playa Mateo (Manabí Province) and multiple isolates have been successfully cultured from the region (Carnicer et al., 2016). Recently, a study from the Galapagos Marine Reserve and the Marine Reserve of Galera de San Francisco, in Ecuador, reported the genus *Ostreopsis* as a common inhabitant in both places, showing a great abundance and diversity of species in the Galapagos. They also reported members of the genera *Gambierdiscus*, *Prorocentrum* Ehrenb., *Amphidinium* Clap. & J. Lachm., and *Coolia* Meunier (Yépez-Rendón et al., 2018).

The paucity of information on toxigenic benthic dinoflagellates from the Chilean coast and the absence from the distributional map (**Figure 1**) may be derived from multiple but not mutually exclusive factors. First, the cold-water regime defined by the Humboldt Current influence may be less favorable to the development of bHABs. In consequence, the lack of bHAB events has reduced the relative urgency for research and monitoring of these species and their toxins in fisheries and aquaculture operations. This should not be interpreted to conclude, however, that these toxigenic benthic species are necessarily absent from the region.

Subtropical and Tropical Atlantic Ocean and the Caribbean Sea

In the Northern Hemisphere, the subtropical North Atlantic Ocean and the Caribbean Sea are not discontinuous in terms of water mass movement motility of benthic and epiphytic species and therefore cannot be reliably separated in terms of ecological niches and ecotypes of benthic dinoflagellates. For this reason, subtropical and tropical regions belonging to this biogeographical cluster are considered to include the Eastern Florida coast and Keys, Bahamas, Turks and Caicos above the Caribbean proper, the large Caribbean islands of the Greater Antilles (Cuba, Haiti/Dominican Republic, Puerto Rico, Jamaica) and a myriad of other islands belonging to the Lesser Antilles, such as Anguilla, Antigua and Barbuda, Aruba, Barbados, Curaçao, Guadalupe, Dominica, Martinique, Montserrat, and St. Lucia, etc., and island chains continuing toward the South American coast. The mainland coasts of Belize, Venezuela and Colombia are also linked to this system.

In the northern Caribbean from Cuba, *Prorocentrum belizeanum, P. concavum* and *P. mexicanum* were associated with nine macroalgal species collected at Bahía de Cienfuegos and at Jaimanitas Inlet, northeast of Havana (Delgado et al., 2002). Within the annual cycle from 2011 to 2012, in a shallow-sheltered reef lagoon offshore Bahía de Cienfuegos, the reportedly toxic and potentially toxic benthic dinoflagellates included *Gambierdiscus caribaeus, Ostreopsis* cf. *lenticularis* (dominant), *P. hoffmannianum, P. lima* and *P. mexicanum* (Moreira-González et al., 2016). Along the southern Cuban coast, a high diversity of *Gambierdiscus* species has been revealed by a molecular method (Díaz-Asencio et al., 2016), but the qPCR technique has not been widely applied in Latin America for comparison. The epiphytic dinoflagellate taxocoenosis was studied during two annual cycles (1999 to 2000 and 2001 to 2002) at Jaimanitas (Delgado et al., 2002; Delgado-Miranda, 2005); *G. toxicus* and *P. lima* were reported as the dominant species, but the former is a misidentification, according to Litaker et al. (2009, 2010). *Coolia monotis*, *O. lenticularis* and *P. belizeanum* were also found from Jaimanitas and the latter species is maintained in culture (Morquecho-Escamilla et al., 2016). An early publication on epibenthic dinoflagellates from Puerto Rico (Tosteson et al., 1986) identified two species: *G. toxicus* and *O. lenticularis*, collected at La Parguera on the southwest coast, but, evidently, *G. toxicus* was misidentified.

The first studies on ciguatera from waters around the Caribbean were conducted in the British and United States Virgin Islands (Coral Bay) of the Lesser Antilles (Carlson and Tindall, 1985). Both epibenthic and planktonic dinoflagellates were collected biweekly throughout a year; five dominant epibenthic species that may contribute to ciguatera were identified: *Gambierdiscus* sp. (referred to as *G. toxicus*), *O. lenticularis*, *P. rhathymum, P. concavum,* and *P. lima. Coolia* sp. (referred to as *C. monotis*) was reported among the dominant putatively non-toxic benthic species. Much later, *G. caribaeus* and *G. belizeanus* were collected and brought into culture from St. Tomas, United States Virgin Islands and Saint-Barthélemy Island (Litaker et al., 2009; Rains and Parsons, 2015; Boisnoir et al., 2019). Recently, based on morphometric and phylogenic analyses, two new toxic species, *Coolia palmyrensis* and *C. santacroce* were described from the northern coast of the Dominican Republic, and the United States Virgin Islands (Saint Croix), respectively (Karafas et al., 2015; Karafas and Tomas, 2015). The latter species was also later found further north in the Bahamas.

From the northeastern coast of Venezuela, near Tortuga Island, Mochima Bay, and the Gulf of Cariaco, epiphytic dinoflagellates associated with the seagrass *Thalassia testudinum* Banks ex König from 0.5 to 30 m water depth were found sporadically over two years (Valerio-González and Díaz-Ramos, 2008). *Prorocentrum lima, P. rhathymum* and *P. compressum* were reported as the most abundant putatively toxic species. In the Gulf of Cariaco (Turpialito Bay), epibenthic dinoflagellates were also sampled biweekly for six months from different substrates (*T. testudinum*, two brown algae, three green seaweeds and plastic bottles). *Ostreopsis siamensis, P. rhathymum* and *P. lima* were present at the highest abundances among nine species observed (Navarro-Vargas et al., 2014). Typically, planktonic species, *P. minimum* and *P. micans*, were also found, but they are not considered part of the epibenthic taxocoenosis, and in any case are presumably non-toxigenic. Several potentially toxic benthic species were found in the sand of two beaches of Margarita Island: *Amphidinium carterae*,

O. ovata, O. siamensis, O. lenticularis and *P. lima,* among which *P. lima, O. ovata* and *O. siamensis* were the most abundant (Marchan-Álvarez et al., 2017).

Epibenthic dinoflagellate taxocoenosis studies from the seagrass *T. testudinum* along the Caribbean Sea coast of Colombia, from Chengue Bay and Chengue Lagoon, found *O.* cf. *ovata, P. hoffmannianum, P. lima* and *P. rhathymum* as the most common among 14 species in these two localities (Arbelaez et al., 2017). At San Andres Island, 12 species collected from drifting brown macroalgae and seagrass samples revealed *P. emarginatum* and *P. belizeanum* at the highest cell concentrations (Mancera-Pineda et al., 2014). In the Gulf of Morrosquillo, six dinoflagellate species were found attached to 26 macroalgal and seagrass species (Quintana-M and Mercado-Gómez, 2017). In general, with respect to the species composition and cell abundances of epibenthic dinoflagellates, the potentially toxic species, *O. lenticularis, O. ovata* and *P. lima* were the most abundant. The *Gambierdiscus* taxon found at San Andres Island was referred to as *G. toxicus*, as illustrated in two SEM micrographs in antapical view, but the taxonomic and biogeographic revision of the genus *Gambierdiscus* (Litaker et al., 2009, 2010) was not taken into account. The illustrated species may be *G. belizeanus*, judging from the shape of the 1p thecal plate and the cell width/depth ratio (Y.B. Okolodkov, pers. observation).

Intensive taxonomic observations of benthic dinoflagellate species from mangrove environments along the western Caribbean coast of Belize have led to the description of an array of new morphological species of *Coolia, Gambierdiscus, Ostreopsis*, and *Prorocentrum* (Faust, 1990b, 1993c,d, 1994, 1995, 1997, 1999; Faust and Morton, 1996; Faust et al., 2008; Litaker et al., 2009). Known species from Belize comprise: *C. tropicalis* (referred to as *C. monotis*); members of the *Gambierdiscus* group (*Fukuyoa ruetzleri* = *G. ruetzleri*, *G. caribaeus, G. carpenteri,* and including toxigenic *G. belizeanus*); *Ostreopsis* spp. of unknown toxicity or considered non-toxic in the region (*O. labens, O. marina, O. belizeana, O. caribbeana*); and *Prorocentrum* species (*P. caribbaeum, P. elegans, P. foraminosum, P. formosum, P. norriseanum, P. reticulatum, P. ruetzlerianum, P. sabulosum, P. sculptile, P. tropicalis*), with several known toxigenic species (*P. arenarium, P. belizeanum*, *P. hoffmannianum, P. levis, P. maculosum*). Cultures developed from the material collected at South Water Cay and Carrie Bow Cay were particularly useful in the description of the new species, *G. caribaeus, G. carpenteri* and *F. ruetzleri* (Litaker et al., 2009). Benthic dinoflagellate taxocoenosis in Belize has been studied from a variety of habitats including floating detritus, coral rubble, sand sediment, lagoon water, seaweeds and seagrasses from intertidal mangrove islands in the barrier reef ecosystem (Faust, 1993b, 1995, 2000, 2004; Faust and Morton, 1996; Morton and Faust, 1997; Faust and Gulledge, 2002; Faust et al., 2005). Ontogenetic, morphological and autoecological studies have focused mainly on toxic *P. lima* (Faust, 1990a, 1991, 1993a), *Coolia* sp. [referred to as *C. monotis* (Faust, 1992)], and the non-toxigenic species *Bysmatrum subsalsum* (Faust, 1996; Faust and Steidinger, 1998). Information on the problems in species identification, and details on the morphology, ecology and toxicity of benthic dinoflagellates from Belize is also included in the accompanying reviews (Faust et al., 1999; Faust and Gulledge, 2002).

The morphotaxonomy of epibenthic dinoflagellates from the Caribbean Sea coast of Mexico has been more thoroughly investigated than from other Caribbean areas, with the possible exception of Belizean reef ecosystems. Three key species linked to ciguatera fish poisoning (CFP), *Gambierdiscus* sp. (referred to as *G. toxicus*), *G. belizeanus,* and *Fukuyoa yasumotoi* have been identified from both epibenthos and net phytoplankton samples collected along the eastern Yucatán Peninsula coast, including the northern part of the Mesoamerican Reef System (Hernández-Becerril and Almazán Becerril, 2004). In addition to these potentially ciguateric species, descriptions and illustrations of four benthic *Prorocentrum* species have also been provided (Almazán-Becerril, 2000). Later, 24 epiphytic dinoflagellate species, including thirteen *Prorocentrum* species, were found from the same area (Almazán-Becerril et al., 2015; Irola-Sansores et al., 2018). A list of potentially toxic species from the northern portion of the Mesoamerican Reef System includes *A.* cf. *carterae, C.* cf. *tropicalis, O. heptagona, O. siamensis* and various *Prorocentrum* species, among which *P. arenarium, P. belizeanum, P. concavum, P. emarginatum, P. hoffmannianum, P. lima, P. maculosum* and *P. rhathymum* have been reported as toxigenic.

Gulf of Mexico

The Gulf of Mexico is linked hydrodynamically to the Caribbean Sea and adjacent waters and via the Florida Straits to the western Atlantic coasts of Florida and the Carolinas. The latter coasts are not technically part of Latin America but can be considered as a warm water sub-regional extension for investigating the biogeography and taxocoenosis of toxigenic benthic dinoflagellates.

The coastal waters of northern Yucatan, along with the Florida Keys, are the most studied in the Greater Caribbean Region with respect to benthic dinoflagellate biogeography. Most investigations focusing primarily on seasonal changes in benthic dinoflagellate taxocoenosis have been conducted along the southern Gulf of Mexico in the coastal waters of the Mexican states of Veracruz and Yucatan. In 2005, at two sampling sites in the Sistema Arrecifal Veracruzano National Park, 17 dinoflagellate species were found associated with 23 macroalgal and two seagrass species (Okolodkov et al., 2007). More recently, *Amphidinium* cf. *carterae, Amphidinium* cf. *operculatum, Bysmatrum* cf. *caponii, Cabra* cf. *matta, Coolia* cf. *monotis, Ostreopsis* sp., *Prorocentrum* cf. *lima,* and *P. rhathymum* were found in a reef zone at Chachalacas, Veracruz (Estrada-Vargas et al., 2017). In 2008–2009, seasonal changes were followed at ten sampling sites in the Chelem lagoon system and in the area exposed to the open sea at Dzilam de Bravo in the coast of Yucatan (Okolodkov et al., 2014). *Prorocentrum lima* and *P. rhathymum* were the most abundant species among 20 dinoflagellate taxa identified and associated with four seagrass and 33 macroalgal species. *Gambierdiscus toxicus* reported in Yucatan waters (Okolodkov et al., 2009) should be reconsidered as *G. caribaeus*, based on the description of the latter new species (Litaker et al., 2009), and known low toxicity (Litaker et al., 2017). A survey in 2011 of benthic dinoflagellates along

the northern coast of the Yucatan Peninsula, within a wide range of habitats (exposed coast, semi-closed brackish water and hypersaline lagoons, fringe mangroves) from Celestún to El Cuyo found 26 dinoflagellate taxa associated with macroalgae, seagrasses, sponges and surface sediments (Aguilar-Trujillo et al., 2014). Changes in the benthic dinoflagellate taxocoenosis (composed of 25 species) were followed in August–December 2011, in the coastal waters of the northern Yucatan Peninsula (Dzilam de Bravo – San Crisanto area), before and after a pelagic bloom that lasted about 150 days (Aguilar-Trujillo, 2017; Aguilar-Trujillo et al., 2017). *Prorocentrum rhathymum* and *P.* cf. *sipadanensis* were co-dominant before the bloom, but only the latter remained dominant after the bloom. Along the northern Yucatan coast, unidentified species of *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* have also been found (Barón-Campis et al., 2014). Monitoring of the seagrass *Thalassia testudinum* over an annual cycle (2012 to 2013) revealed 16 epiphytic dinoflagellate species, with *P. lima* and *P.* cf. *sipadanensis* as the most abundant (Martínez-Cruz et al., 2016).

The benthic habitat in the northwestern Gulf of Mexico is not considered to be suitable for optimal growth of benthic dinoflagellates–muddy bottoms characterize the region, but thousands of oil platforms and hundreds of artificial reefs along this basin provide substrates for these organisms to attach to and to reproduce. In 2007, members of the genus *Gambierdiscus* were reported as epiphytes on six oil platforms (Villareal et al., 2007). This study was of particular interest regarding how human activities can allow for the expansion of toxic species into places where they were not found previously, or where the natural substrate was not suitable for survival and growth. In the northern Gulf of Mexico, United States, a great diversity of species of the genus *Gambierdiscus* has been reported, including *G. belizeanus, G. caribaeus, G. carolinianus, G. carpenteri* and *Gambierdiscus* ribotype 2 (Tester et al., 2013). This study revealed that some *Gambierdiscus* species are able to grow throughout the year at the Flower Garden Banks National Marine Sanctuary located 185 km offshore of Galveston, Texas in the northwestern Gulf of Mexico.

Florida Straits, Keys and Southeastern Atlantic Coast of the United States

The annual cycle (1983 to 1984) of the benthic dinoflagellate taxocoenosis (<38 μm size-fraction) was studied at five locations in the Florida Keys: the Upper Matecumbe Key, Long Key, Knight Key, Bahia Honda Key, and Raccoon Key (Mitchell, 1985). *Amphidinium klebsii*, *Coolia monotis* and *Prorocentrum lima* were the dominant species found together with *P. rhathymum*, *P. emarginatum*, and *Bysmatrum subsalsum*; their autecology was studied in detail, with respect to seasonality, spatial heterogeneity and preference for macrophyte substrates among 15 macroalgal species and *Thalassia testudinum*. The seasonality of *Gambierdiscus* sp. (referred to as *G. toxicus*) was investigated from field sub-samples taken at Knight Key (Bomber et al., 1988); the influence of temperature, salinity and light on seasonality, growth rate and toxicity was determined from unialgal culture experiments under controlled laboratory conditions. The field samples collected at Knight Key in 1983 to 1984 (38–250 μm size-fraction) also served a study of substrate specificity and the nutrition of five benthic dinoflagellate species (*Gambierdiscus* sp. referred to as *G. toxicus, Ostreopsis heptagona, O. siamensis, P. lima, P. rhathymum* and *P. concavum*) from the seagrass *T. testudinum* and 14 macroalgal species (Bomber et al., 1989). Cultures developed from single-cell isolates from samples collected from Marathon Key were assigned to *Gambierdiscus belizeanus,* and isolates from Cape Fear, North Carolina were the basis for descriptions of the new species, *G. carolinianus* and *F. ruetzleri* (Litaker et al., 2009).

Southwestern Atlantic

Along the Brazilian coasts, *Ostreopsis* cf. *ovata* and *Coolia malayensis* were reported in the coastal waters of Ubatuba, east of São Paulo (Gómez et al., 2017b). A new toxic epiphytic species, *Prorocentrum caipirignum*, was described (Nascimento et al., 2017) from isolates collected from Rio de Janeiro (Arraial do Cabo) and Bahía (Garapuá, Ilha de Tinharé, Cairu). The lack of reports of toxigenic benthic dinoflagellates (and absence from **Figure 1**) from the Argentine coast further south, likely reflects the low relative cell abundance in colder waters influenced by the Malvinas Current. The limited or negligible impacts of toxigenic benthic dinoflagellates on aquaculture and fisheries activities should not be interpreted, however, to conclude that such species are absent.

TAXONOMY AND BIODIVERSITY

A comprehensive worldwide guide for identifying marine benthic dinoflagellates includes 190 described species from 45 genera (Hoppenrath et al., 2014), which is less than a tenth of the known extant dinoflagellate species diversity (Taylor et al., 2008; Hoppenrath et al., 2009). Confirmed toxigenic dinoflagellates with well-defined toxin composition are all marine free-living obligate or facultative photoautotrophs, although many have mixotrophic capabilities. In contrast, among freshwater dinoflagellates, only a single putatively toxic species (*Naiadinium polonicum* (Wołosz.) Carty) has been described, and it is planktonic (Moestrup and Calado, 2018). Toxigenic dinoflagellates comprise more than 90 species (Moestrup and Calado, 2018), including 22 athecate members from the Gymnodiniales and Amphidiniales. There are at least 34 toxigenic benthic species and therefore in comparison to planktonic forms, toxic benthic and epiphytic species are heavily represented among free-living marine dinoflagellates. The known toxigenic marine benthic species are found frequently in Latin America (**Table 1** and **Figure 1**), and often in high relative cell abundance, but toxigenicity and toxin composition are typically unconfirmed.

Morphological Taxonomy

The first described benthic dinoflagellate species (*Amphidinium operculatum, Coolia monotis, Ostreopsis siamensis,* and *Prorocentrum lima*) were characterized morphologically as early as the late 19th to beginning of the 20th century. A few

more species were described throughout the 1940s and 1950s (e.g., *Sinophysis microcephalus* Nie and Wang, 1944, *Roscoffia capitata* Balech, 1956, *Amphidinium carterae* Hulburt 1957), but most benthic dinoflagellates have been described based upon morphological characteristics since the late 1970s. These species include well-known and widely distributed taxa such as *Gambierdiscus toxicus, P. rhathymum, P. concavum, P. emarginatum, O. lenticularis*, and *O. heptagona* and comprise all the major known toxigenic phenotypes. Originally, new species descriptions were based exclusively on the vegetative cell morphology and thecal plate tabulations, and this has continued during the first decade of the 21th century.

The vegetative cells of most benthic dinoflagellates are relatively large (>10 μm) and often heavily armored (but not always, e.g., athecate *Amphidinium* spp.) and thus preserve well for optical- and electron-microscopic analysis. It is perhaps surprising, therefore, that an expert dinoflagellate taxonomist can usually distinguish planktonic taxa at species level easier than benthic ones. This may be due to the unusually high degree of intraspecific morphological plasticity among populations of benthic dinoflagellate species. Furthermore, most cells of benthic dinoflagellates are bilaterally symmetrical and strongly dorso-ventrally compressed - a probable adaptation to their specific habitats (surfaces of seagrasses, macroalgae or invertebrates, narrow spaces between sand particles, etc). These morphological constraints create difficulties for detailed microscopic analysis of the diagnostic apical platelets of genera such as *Prorocentrum* Ehrenb. (in the periflagellar area), *Amphidiniopsis* Wołosz., *Cabra* Murray & Patterson emend. Chomerat, Couté & Nézan, *Plagiodinium* Faust & Balech, *Planodinium* Saunders & Dodge, *Sinophysis* Nie & Wang, *Thecadinium* Kof. & Skogsb., i.e., genera with reduced epitheca.

Until the past decade, identification of benthic dinoflagellates at species level has been substantially, if not exclusively, based on the theca morphology, in particular, on the plate arrangement (typically Kofoidean tabulation notation). According to Hoppenrath et al. (2013), asymmetry or symmetry of the theca, in combination with the shape of the periflagellar area, is constant within a species; however, the stability of pore patterns is not known to date. The high degree of morphological variability and intraspecific plasticity in details of the thecal structure and ornamentation highlight the difficulties in relying exclusively on these phenotypic characteristics for distinguishing benthic dinoflagellate species. Lately, high cryptic diversity has been revealed for some previously considered "good" species, such as *P. lima*, *O. ovata* or *C. monotis*, presently referred to as species complexes (Leaw et al., 2010; Zhang et al., 2015; Accoroni and Totti, 2016). Problems in the strict application of the morphospecies concept, particularly for desmokont dinoflagellates, are well illustrated by the uncertainty in species boundaries of potentially toxic species such as *P. hoffmannianum, P. belizeanum, P. maculosum* and *P. faustiae* (Hoppenrath et al., 2013). Patterns of the periflagellar platelets seem to be species-specific for most benthic *Prorocentrum* species, as shown in Hoppenrath et al. (2013). Nevertheless, confirmed identification of *P. lima,* one of the most common toxigenic epibenthic species of the genus and widely distributed in both temperate and tropical regions, is particularly problematic. The morphospecies *P. lima* comprises high cryptic diversity; thus, referring to it as the "*Prorocentrum lima* complex" is recommended (Aligizaki, 2009). Our morphological observations of recent isolates from the Caribbean and Gulf of Mexico coast of 23 monoclonal cultures of the *Prorocentrum lima* complex under an epifluorescence microscope showed much greater variability in cell shape than expected; some cells could be easily (if not incorrectly) identified as another *Prorocentrum* species but the thecal pore pattern remained stable. The fact that most species of benthic dinoflagellates belong to *Prorocentrum* also contributes to the above-mentioned methodological difficulties in morphologically discriminating between benthic and planktonic thecate species.

The problem of distinguishing between *P. rhathymum* and *P. mexicanum* (both reported as toxic) still remains. Although they are usually regarded as distinct morphospecies, *P. rhathymum* is sometimes considered a junior synonym to *P. mexicanum*. In addition to subtle morphological differences, the former species is epibenthic whereas the latter is typically planktonic (Cortés-Altamirano and Sierra-Beltrán, 2003). If this habitat distinction is valid, most, if not all, records of *P. mexicanum* in benthic habitats are likely misidentifications. Species boundaries between *P. emarginatum* and *P. sculptile* and between *P. faustiae* and *P. concavum* (all reported as toxigenic except for *P. sculptile*) are not clear, and more investigations are required to compare specimens from natural populations and cultured isolates of these taxa (Hoppenrath et al., 2014; Lassus et al., 2016).

Ostreopsis species are in general difficult to distinguish because they have the same thecal plate tabulation (Kofoidean formula) and that is similar to the genus *Coolia*; within *Ostreopsis* there may be only slight differences in cell shape and sometimes in the presence of only one thecal pore type (as in most species) or two types (as in *O. lenticularis*). Identification at species level is particularly problematic due to imprecision in the original descriptions and confusion in subsequent interpretations (Hoppenrath et al., 2014). The first apical (1') plate is the most critical for identification. Probably, only *O. heptagona* has the plate features that would allow a specialist to identify it unequivocally - a long 2' plate that has a wide contact with the 3' plate on the epitheca, and a very characteristic 1p plate that narrows from the sulcus toward the center of the hypotheca.

After re-investigation of the original description of *Coolia* species (Hoppenrath et al., 2014), the species are mainly distinguished on the basis of small differences in the shape and size of the 1' and 7' plates on the epitheca, in combination with the presence or absence of a suture between the 2''' and 2'''' plates on the hypotheca.

Gambierdiscus toxicus, the type species of the genus, has been re-described (Litaker et al., 2009), and subsequently, the following morphological features have been used to distinguish between species: shape (narrow or broad) and size of the 1p plate on the hypotheca; a smooth or areolated thecal surface; and the shape of 2' plate, which can be quantified by the ratio of the 2'/2" suture length to the 2'/4" suture length (Hoppenrath et al., 2014). Within the morphologically related genus *Fukuyoa* F. Gómez, D. Qiu, R.M. Lopes & S. Lin, the species are mainly distinguished

by the cell size and shape (globular or laterally flattened, unlike anterior-posteriorly compressed *Gambierdiscus sensu stricto*), the proportions between the cell length, width and depth, the shape of the 2"" plate (Litaker et al., 2009), and the shape of the 1' plate (Gómez et al., 2015).

Amphidinium is an athecate genus, with poorly defined morphological characteristics. The genus is found in a wide variety of marine habitats including benthic sandy and epiphytic substrates, and within the water column. Vegetative cells are generally dorsoventrally compressed with a minute, triangular or crescent-shaped, usually left-deflected epicone. The type species of the genus, *A. operculatum*, was re-described, and the genus was redefined; based on morphological and molecular phylogenetic analyses, six distinct species were identified (Jørgensen et al., 2004; Murray et al., 2004). Furthermore, the genus *Prosoaulax* Calado & Moestrup was erected for some freshwater species previously ascribed to *Amphidinium* (Calado and Moestrup, 2005). Among toxigenic benthic *Amphidinium* species, the two most frequently reported, *A. carterae* and *A.* cf. *operculatum* are distinguished mainly by the cell size and shape of the epicone. Furthermore, some authors consider that *A. operculatum* and *A. klebsii* are synonyms (Dodge, 1982); Karafas et al. (2017); however, recommended elimination of *A. klebsii* as an accepted taxonomic entity. Examination of live *Amphidinium* cells is always preferable; otherwise, for morphological study, Lugol's iodine-fixed cells are better than formalin- or ethanol-fixed ones. The number and the shape of chloroplasts and the position of the ring-shaped starch-sheathed pyrenoid can be additional useful features to identify species and to distinguish among other *Amphidinium* species (Karafas et al., 2017).

Molecular Genetic Approaches to Taxonomy and Phylogenetic Reconstruction

The advent of molecular technologies, particularly metagenomics and targeted gene sequencing, has revolutionized our understanding of biodiversity of microeukaryotes in marine ecosystems, revealing cryptic species and permitting definition of biogeographical distributional patterns of species occurrence. Within the last decade, morphological descriptors are frequently supplemented by molecular methods applied to sequencing key genes (rDNA, ITS) for taxonomic and phylogenetic reconstruction for benthic dinoflagellates (e.g., Litaker et al., 2009). Among toxigenic HAB taxa, molecular approaches have been essential in establishing the structure and function of toxin biosynthetic genes and the mechanisms whereby gene expression is regulated. As of yet, there are no diagnostic gene probes for the polyketide synthase (PKS) genes presumably responsible for the biosynthesis of the array of polyether toxins produced among various benthic dinoflagellates.

Application of ecogenomics and phylogenetic reconstruction approaches to bHAB taxa (almost all dinoflagellates) has been rather sporadic and often geographically limited, with very little information available for toxigenic benthic dinoflagellates from Latin America. Nevertheless, a few key studies have addressed this issue from a global perspective, but which involve populations from the Americas. Molecular evidence supports the contention that both *P. belizeanum* and *P. hoffmannianum* belong to the *P. hoffmannianum* species complex in which clades are separated by geographical region. Thus, four ribotypes can be distinguished from diverse geographical locations: from Belize, Florida-Cuba, India and Australia (Herrera-Sepúlveda et al., 2015). Similarly, application of molecular tools to the genus *Gambierdiscus*, originally believed to be monotypic, has resolved several cryptic taxa within this genus into distinct species, and is adding to an ever increasing list of new descriptions (Larsson et al., 2018). Recently, combined morphological and molecular genetic analysis was also successful in separating a new genus, *Fukuyoa*, with putative toxigenicity, and similar to but distinct from *Gambierdiscus* (Gómez et al., 2015).

Establishing the link between toxigenicity and phylogenetic relatedness among benthic dinoflagellates continues to be hampered by the lack of targeted gene probes for toxin biosynthetic genes and limited analysis of toxin spectra from natural populations and cultured isolates. Nevertheless, there are ongoing attempts to define patterns of toxigenicity and taxonomic affiliations. Currently, three genetic clades of *Ostreopsis* cf. *ovata* have been shown to occur in overlapping geographical areas (Penna et al., 2005), and several different toxicity patterns have been described (Carnicer et al., 2016). In this investigation, thirteen isolates from the coast of Ecuador in the eastern Pacific Ocean, including a geographical area from which no information existed, were analyzed for *Ostreopsis* genotypes and toxicity of the *O.* cf. *ovata* complex. By analyzing the ITS and 5.8S rDNA, the isolates were found to be identical and to cluster within the Atlantic/Indian/Pacific clade.

Beyond the contribution to resolution of taxonomic issues and cryptic species, functional genomic approaches will play an increasing role in tracking shifts in gene frequency of HAB species over seasonal and multi-annual cycles. Although such work has already been initiated for planktonic HAB species at the population level, e.g., for diversity of *Alexandrium minutum* along the coast of France (Le Gac et al., 2016), to our knowledge there are no published corresponding studies for bHAB species within tropical or subtropical ecosystems in Latin America. Such studies are critical to understanding the population dynamics of bHABs and associated toxin distribution because the ecological niche definitions and contributions to community structure are expected to be rather different for benthic species compared with their planktonic counterparts.

DETERMINATION OF TOXIN COMPOSITION, OCCURRENCE AND ASSOCIATED SYNDROMES

Most Latin American countries have developed and implemented some capability for monitoring potentially toxic phytoplankton blooms and/or the presence of their toxins above regulatory limits in production and harvest zones for mollusks for human consumption (Secretaría de Salud, 2015). Phycotoxin monitoring programs in Latin America have tended to focus on quantitative assay or analysis of hydrophilic toxins in

shellfish, primarily the paralytic shellfish toxins (PSTs) (or saxitoxins) and domoic acid, associated with planktonic blooms of dinoflagellates (*Alexandrium* spp., *Gymnodinium catenatum*, *Pyrodinium bahamense*) or chain-forming diatoms (*Pseudo-nitzschia* spp.), respectively. In Latin America, somewhat less monitoring attention has been directed to the lipophilic phycotoxins from planktonic dinoflagellate blooms, such as the diarrhetic shellfish toxins (DSTs; okadaic acid/dinophysistoxins) from *Dinophysis* spp., and brevetoxins from *Karenia brevis*, mainly in the Gulf of Mexico. With respect to the toxins generated by bHABs and the associated benthic dinoflagellate species, there are no comprehensive monitoring programs implemented within Latin America.

Benthic dinoflagellates are capable of synthesizing a wide array of bioactive molecules, particularly cyclic polyether compounds, often considered as "toxins" because of their potent effects in mammalian systems and/or cytotoxicity. These toxins are, in general, lipophilic compounds, and thus highly soluble in organic solvents, but their polarities are extremely variable (Blanco et al., 2005), and among analogs they differ markedly in specific toxicity and species occurrence (**Figures 2–5** and **Table 2**).

Ciguatoxins (CTXs) (**Figures 2A–E**) have been directly implicated in CFP (Lehane, 2000), but early studies also presumed that maitotoxins (MTXs) (**Figure 2F**) were involved in the diverse CFP syndrome (Yasumoto and Murata, 1993). Ciguatoxins are known to act on site 5 of the voltage-gated sodium channels (Na_V) with great affinity, affecting normal cellular function through the elevation of cytoplasmic Na^+ and Ca^{2+} concentrations. Other biological activities have also been reported, such as the blockage of voltage-gated potassium channels (K_V), activation of L-type Ca^{2+} channels, and accumulation of nitric oxide in cells (Kumar-Roiné et al., 2008).

Maitotoxins (**Figure 2F**) are water-soluble polyethers, structurally similar to CTXs but more hydrophilic due to numerous hydroxyl and sulfate groups. When injected intraperitoneally into mice, MTX is among the most potent known natural toxins (Estacion, 2000), with toxicity even higher than for CTX, but less potent when administered orally (Wang, 2008; Kohli et al., 2014). The role of MTX analogs in CFP has not been clearly established, although accumulation in carnivorous fish tissues such as viscera, liver and muscle has been confirmed (Kohli et al., 2014). Recent toxicological studies have shown that MTXs are probably not a significant factor in CFP because of their low oral potency and higher water solubility (compared to CTXs).

Circumstantial association of CFP with toxigenic benthic dinoflagellates has led to the historical view that the toxin syndrome represents a synergistic effect of a complex suite of diverse metabolites produced by various benthic dinoflagellates (primarily *Gambierdiscus*, *Amphidinium*, *Coolia*, *Ostreopsis*, and *Prorocentrum* species). *Gambierdiscus* species are still considered the ultimate origin of most toxins involved in CFP, but structural elucidation of many CFP toxins has traditionally been conducted on tissue extracts of fish and other marine fauna, rather than directly from the putative toxigenic dinoflagellates. With the advent of advanced mass spectrometric and NMR approaches, it is now confirmed that members of the genus *Gambierdiscus* (and closely related *Fukuyoa*) produce multiple analogs of toxins such as CTXs and MTXs, known to be associated with CFP, whereas the other implicated genera do not. For some years, the genus *Gambierdiscus* was thought to be monotypic, but increased interest and the use of molecular tools for taxonomic identification have shown that this genus comprises multiple species, and an increasing list of new taxa (Larsson et al., 2018). A new related toxigenic genus, *Fukuyoa*, has also been described (Gómez et al., 2015) but few strains of this genus have been established in culture, and hence there is little autoecological information, and only scarce knowledge on natural distribution and toxin production. Research on *F. ruetzleri* from Belize, North Carolina and the United States coast of the Gulf of Mexico found CTX and CTX-like compounds (Holland et al., 2013; Lewis et al., 2016; Litaker et al., 2017; Pisapia et al., 2017). A study on *F. paulensis* from Australia, detected no CTX or CTX-related bioactivity, but a putative MTX analog, known as MTX-3 was found (Larsson et al., 2018), though this species has not been identified in Latin America.

Diarrhetic shellfish poisoning (DSP) is a human intoxication syndrome caused by consumption of shellfish contaminated with okadaic acid (OA) and/or structurally related dinophysistoxins (DTXs) (**Figure 3A**). The OA toxin group comprises lipophilic polyether toxins that can accumulate in suspension-feeding shellfish such as oysters, mussels, scallops, cockles and clams. Some known analogs, such as DTX3, are considered almost non-toxic in their natural form (Braga et al., 2016), but could be hydrolyzed after ingestion and thereby converted into more potent compounds, such as OA, DTX1 and DTX2 (Doucet et al., 2007). These toxins are potent inhibitors of the serine/threonine phosphatases PP1, PP2A, and PP2B (Bialojan and Takai, 1988; Hu et al., 2017). Since diarrheic episodes have not been explicitly confirmed as linked with PP inhibition, other modes of action could be involved in the intoxication process, probably related to a neuropeptide that protects against diarrhea by the inhibition of intestinal mobility and water and electrolyte secretion (Louzao et al., 2015).

On a global basis, production of DSP toxins and associated HAB events are usually associated with members of the phytoplankton community belonging to the genus *Dinophysis*, but these toxins are also produced by several species of benthic dinoflagellates of the genus *Prorocentrum* (Valdiglesias et al., 2013). Benthic *Prorocentrum* species known to produce OA and toxic DTX analogs include *P. borbonicum, P. caipirignum, P. cassubicum, P. concavum, P. cordatum, P. emarginatum, P. faustiae, P. hoffmannianum, P. lima, P. maculosum, P. mexicanum,* and *P. texanum* (Moestrup et al., 2009). Even though many of these toxigenic species have been reported from Latin American coasts (**Tables 1**, **2**), except for earlier work in Cuba (Delgado et al., 2002, 2005) and Mexico (Heredia-Tapia et al., 2002), few studies have been conducted to corroborate their toxicity in the region.

To our knowledge, there are no confirmed records of DSP caused by toxins associated with benthic dinoflagellates in Latin America, although there is mention of one case which could be attributable to such toxins (Heredia-Tapia et al., 2002). In general, there are no reliable data of incidence or etiology of DSP

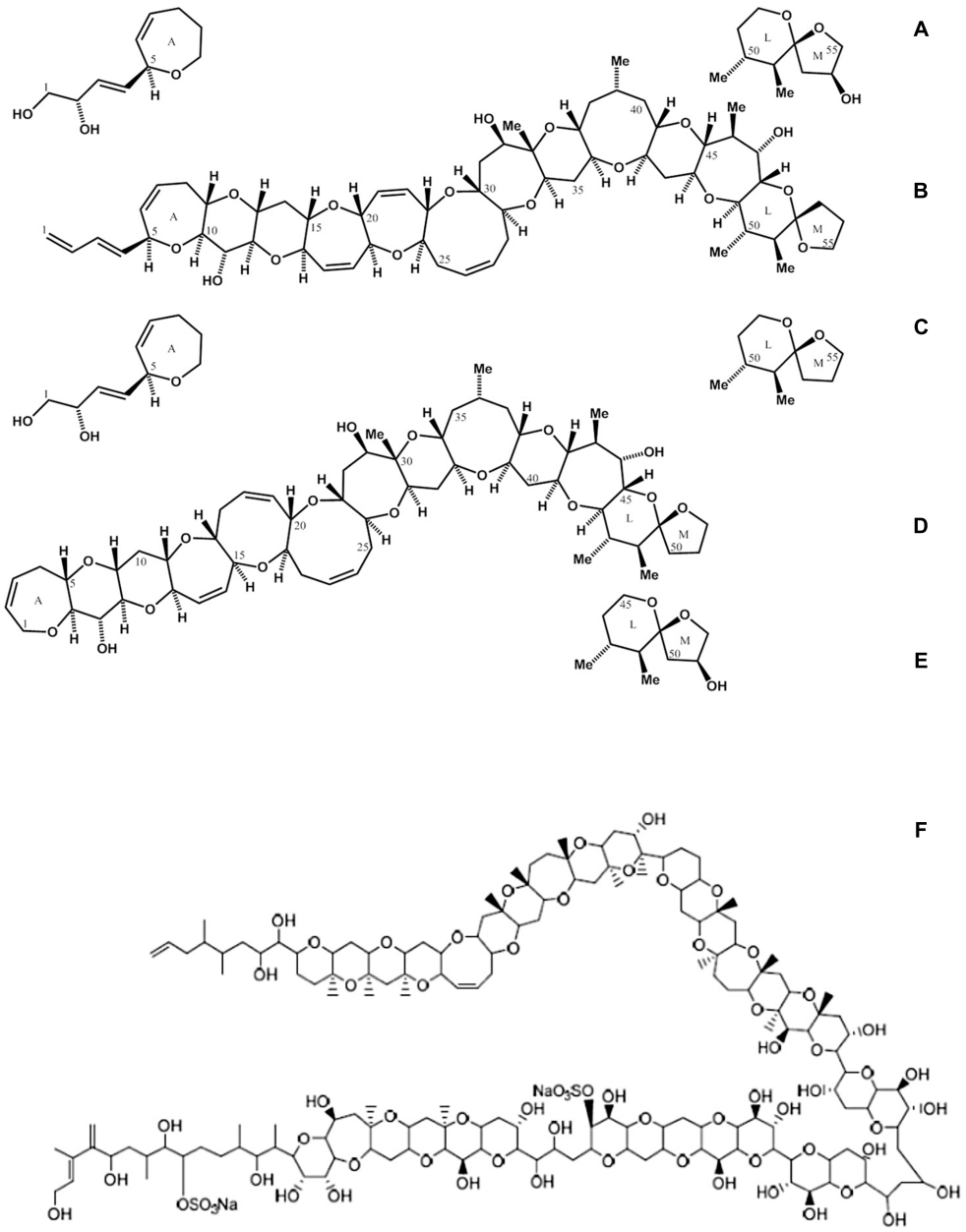
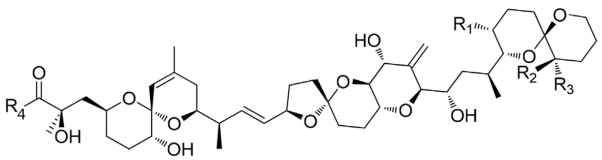


FIGURE 2 | Some toxins associated with ciguatera fish poisoning (CPF) and originating from members of the genus *Gambierdiscus* and/or *Fukuyoa*: **(A)** CTX1B; **(B)** CTX4A; **(C)** epideoxyCTX1B; **(D)** CTX3C; **(E)** 51OH-CTX3C; **(F)** MTX. Structures modified from Ajani et al. (2017) and Kato and Yasumoto (2017).

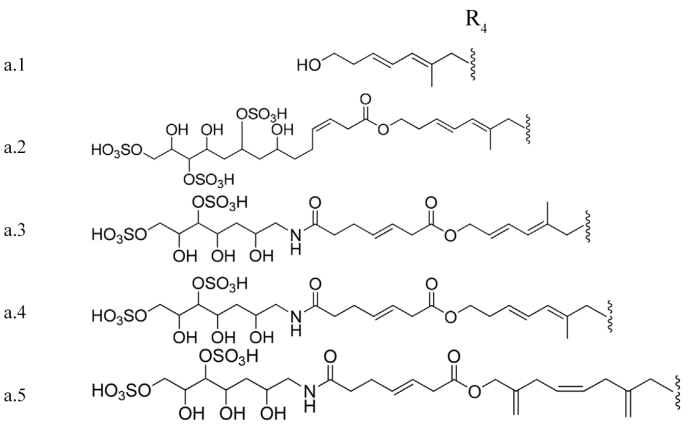
episodes in the region (Acuña, 2015). In most cases of putative DSP, affected people are not attended to by medical services or, even when this is the case, because the major DSP symptoms include diarrhea, abdominal pain, gastrointestinal distress and sometimes fever (James et al., 2010), they are easily confused with other food-borne gastrointestinal syndromes.

Bioactive cyclic imine compounds, known as prorocentrolides, may be produced by *P. lima* (**Figure 3B**), but the human health risk has not been established. Members of this family of macrocyclic imines are known as "fast-acting toxins" due to the rapid onset of neurological symptoms, followed by paralysis and death after intraperitoneal injection into mice (Amar et al., 2018). At least one benthic dinoflagellate species found in Latin America, *P. caipirignum*, is capable of producing both OA and prorocentrolides (Nascimento et al., 2017). Recent studies including competition-binding assays, electrophysiological techniques and *in silico* simulations have been performed with prorocentrolide-A to determine potency and biological activity (Amar et al., 2018). Such cyclic imines have been reported to be antagonists of muscle and neuronal

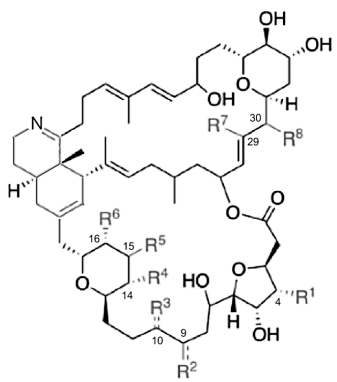
A



	R_1	R_2	R_3	R_4
Okadaic acid	CH_3	H	H	OH
DTX1	CH_3	CH_3	H	OH
DTX2	H	H	CH_3	OH
OA C8-Diol	CH_3	H	H	a.1
DTX4	CH_3	H	H	a.2
DTX5a	CH_3	H	H	a.3
DTX5b	CH_3	H	H	a.4
DTX5c	CH_3	H	H	a.5



B



	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
Prorocentrolide-A	H	CH_2	H	OH	OH	H	CH_3	OH
30-sulfate prorocentrolide	H	CH_2	H	OH	OH	H	CH_3	OSO_3H
4-hydroxy prorocentrolide	OH	CH_2	H	OH	OH	H	CH_3	OH
9,51-dihydro prorocentrolide	OH	CH_3	H	OH	OH	H	CH_3	OH
14-*O*-acetyl-4-hydroxy prorocentrolide	OH	CH_2	H	$OC(=O)CH_3$	OH	H	CH_3	OH
Prorocentrolide-B	OSO_3H	CH_3	CH_2	OH	H	OH	H	OH

FIGURE 3 | (A) Okadaic acid (OA) and dinophysistoxins (DTXs) produced by benthic members of the genus *Prorocentrum* (Twiner et al., 2016); a.1 to a.5 C8-diol ester and sulfated diesters of OA. **(B)** Prorocentrolides and analogs reported to date. Modified from Amar et al. (2018).

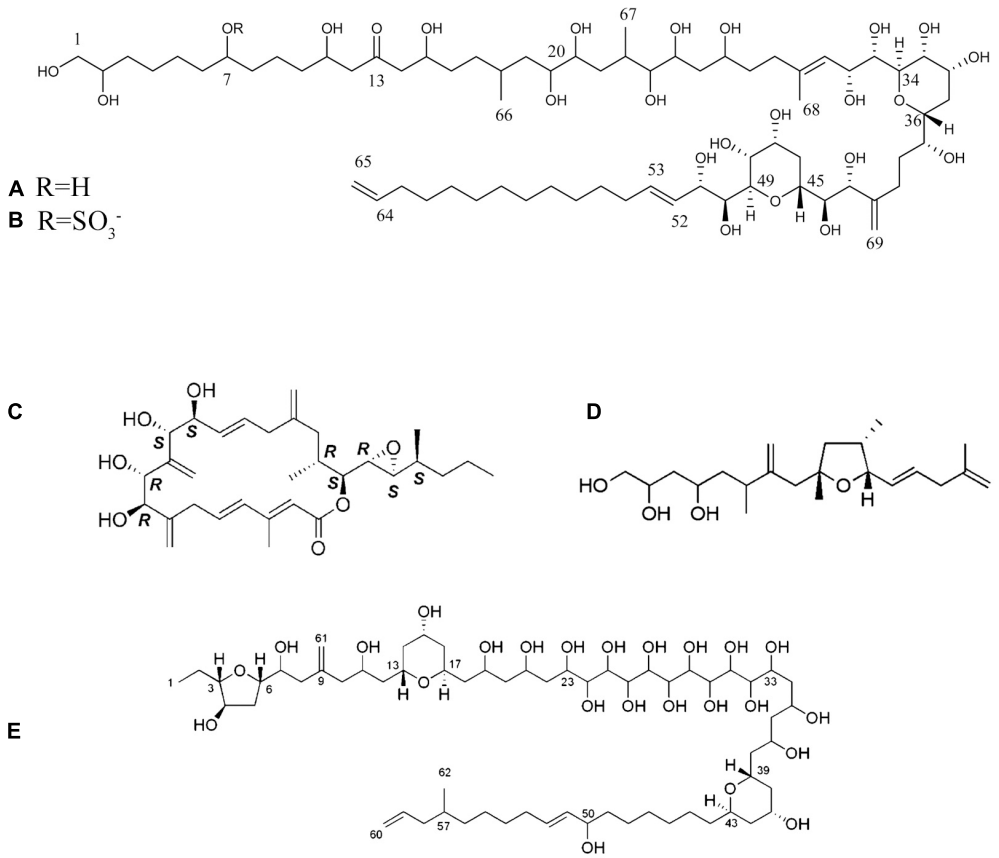


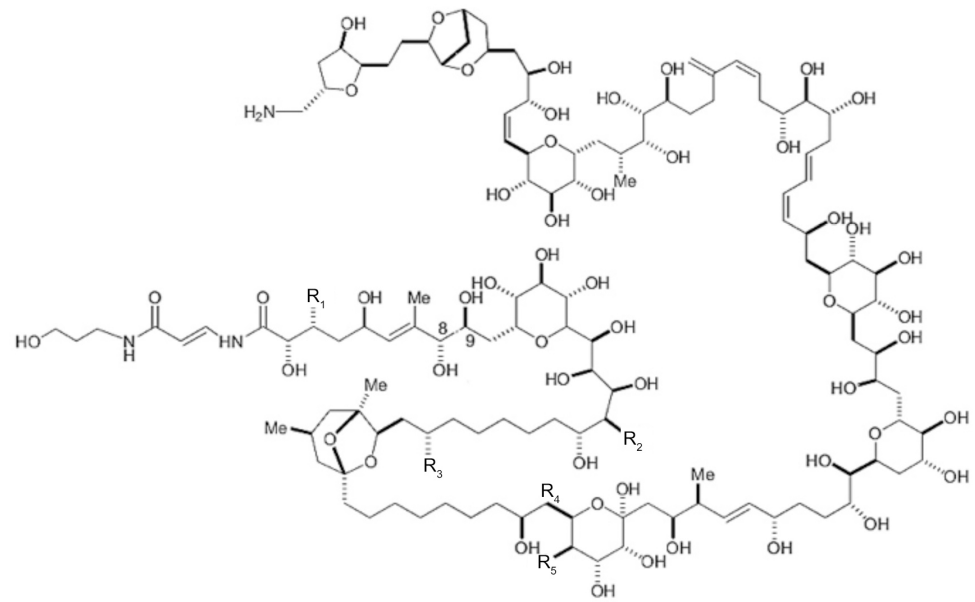
FIGURE 4 | (A,B) Amphidinols A and B; **(C)** Amphidinolide A; **(D)**. Amphidinin; **(E)** Amphezonol (Kobayashi and Kubota, 2007; Cutignano et al., 2017). Reprinted with permission from Kobayashi and Kubota (2007).

types of nicotinic acetylcholine receptors (nAChRs) (Molgó et al., 2017), but their exact mode of action remains unknown.

Cytotoxic macrolides produced by members of the primarily benthic dinoflagellate genus *Amphidinium*, and hence called amphidinolides (**Figures 4A–E**), have shown hemolytic activity (Yasumoto et al., 1987), as well as evidence of cytotoxicity against cancer cell lines, and antifungal properties (Kobayashi et al., 1991; Bauer et al., 1995a,b; Kobayashi et al., 2002; Tsuda et al., 2007; Espiritu et al., 2017). Within the last decade, interest in the toxigenicity of this genus has increased because some species, namely the type species *A. carterae* Hulburt 1957 and members of the *A. operculatum* Claparède & Lachmann, 1859 species complex, are known to produce ichthyotoxic substances (Kobayashi et al., 1991; Satake et al., 1991; Tsuda et al., 1994; Bauer et al., 1995a,b). In most cases, bioactivity (and in higher doses toxicity) is assumed to be related to various polyketides and macrolides isolated from different species of the genus (Kobayashi et al., 1991; Bauer et al., 1995a,b; Kobayashi et al., 2002; Tsuda et al., 2007; Espiritu et al., 2017). Nevertheless, many of the pioneering studies lacked sophisticated chemical analysis for structural elucidation and toxin potency confirmation was often conducted only on semi-purified material.

No human illnesses or marine faunal mortalities have been directly linked to bHABs of *Amphidinium* or via toxins transferred in marine food webs (Holmes et al., 2014), but some fish kills have been circumstantially attributed to *Amphidinium.* If confirmed, such cases are probably due to amphidinol and its analogs, because they are structurally similar to karlotoxins, known ichthyotoxins produced by planktonic *Karlodinium* species (Place et al., 2012). In any case, to date there are no published data on the toxin composition or associated ichthyotoxicity of cultured isolates or natural populations of *Amphidinium* species found in Latin America.

Members of the genus *Coolia* are known to produce bioactive secondary metabolites (and putative toxins), but there is still confusion about both their toxigenic potency and taxonomic affinities within the genus. The genus is undergoing rapid taxonomic revisions, and two new toxic species have been recently added to the list: *C. palmyrensis* and *C. santacroce* (Karafas et al., 2015; Karafas and Tomas, 2015); it is also likely that certain *Amphidinium* species have been previously misidentified as *C. monotis* and *C. malayensis.* According to the IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae (Moestrup et al., 2009), *C. tropicalis* is the only confirmed toxin producing species among of the five *Amphidinium* species



	R_1	R_2	R_3	R_4	R_5
Palytoxin	Me	OH	Me	H	OH
Ostreocin-D	H	H	H	OH	H

FIGURE 5 | Structures of palytoxin (PLTX) and ostreocin-D, produced by certain benthic members of the genus *Ostreopsis* (Amzil et al., 2012).

on the list (*C. monotis, C. tropicalis, C. areolata, C. canariensis*, and *C. malayensis*). Most members of the genus are apparently non-toxigenic, although in any early report (Holmes et al., 1995) based on a mass spectrometric analysis, *C. monotis* was found to produce cooliatoxin, a probable monosulfated analog of yessotoxin. Extracts and supernatants of cultured *C. monotis* have showed hemolytic activity, toxicity to mice, and sodium channel activity (Rhodes and Thomas, 1997), but the specific compounds responsible for such bioactivity have not been structurally elucidated. Until now, no human intoxications caused by metabolites produced by members of the genus *Coolia* have been reported, and many studies have failed to detect toxicity in any species (Holmes et al., 2014). There is no published research on bioactive metabolites (including toxins) produced by this genus in Latin America.

Palytoxins (PLTXs) are complex polyhydroxylated polyketides with both lipophilic and hydrophilic moieties (**Figure 5**; Ramos and Vasconcelos, 2010); PLTX is considered to be among the most potent non-proteinaceous toxins, second only to MTX with respect to intraperitoneal toxicity in mice. Recently, members of the genus *Ostreopsis* have been found to produce PLTX, and almost 20 analogs are known to date (Ajani et al., 2017). Four structural sub-groups of these toxins have been defined: palytoxins, ostreocins, ovatoxins and mascarenotoxins (Tubaro et al., 2012), but toxigenicity of most individual analogs remains undetermined. Blooms of *Ostreopsis* are now well known for causing respiratory distress in humans in coastal areas, particularly in southern Europe and the Mediterranean Sea, because of toxic aerosol formation, but the problem has not yet become evident in Latin America. Isolates of the *O.* cf. *ovata* complex from the coast of Ecuador were judged to be non-toxic according to hemolytic cell assays, and the absence of PLTX-like compounds was confirmed by liquid chromatography-mass spectrometry (LC-MS/MS) (Carnicer et al., 2016). Whether lack of human toxic events associated with *Ostreopsis* in Latin America is primarily due to the relatively low magnitude of the blooms, hydrodynamic factors associated with aerosol formation, lack of human exposure in bHABs affected areas or to the non-toxigenicity of local populations remains to be established.

Studies on benthic dinoflagellates in Latin America have traditionally relied on microscopic taxonomic analysis of samples collected from benthic or epiphytic substrates; if species known to be toxic elsewhere are detected and morphologically confirmed, local populations are often assumed to be toxigenic as well. Such populations are then considered to be the causative agent of toxic events associated with bHAB toxins found nearby, e.g., in reef fish associated with ciguatera fish poisoning (CFP) even without proving toxicity of the local strains (Delgado-Miranda, 2005). Ciguatera chemistry in Latin America is focused mostly on public safety, e.g., fish tissues are analyzed or assayed by governmental laboratories. Toxin chemistry research is scarce,

TABLE 2 | Toxic benthic dinoflagellates and evidence of toxigenicity.

Species	Original species description	Toxinological source	Toxins produced
Amphidinium			
A. carterae	Hulburt 1957	Yasumoto et al., 1987; Echigoya et al., 2005; Morsy et al., 2006; Meng et al., 2010; Rhodes et al., 2010	Polyketides with antifungal, hemolytic and/or ichthyotoxic activity
A. operculatum	Clap. & J. Lachm, 1859	Maranda and Shimizu, 1996	Cytotoxic compounds
Coolia			
C. palmyrensis	Karafas, Tomas & York 2015	Karafas et al., 2015	Cytotoxic compounds
C. santacroce	Karafas, Tomas & York 2015	Karafas et al., 2015	Cytotoxic compounds
C. tropicalis	M.A. Faust, 1995	Holmes et al., 1995; Mohammad-Noor et al., 2013	Cooliatoxin
Fukuyoa			
F. yasumotoi	(M.J. Holmes) F. Gómez, D. Qiu, R.M. Lopes & S. Lin, 2015	Litaker et al., 2017	CTX
Gambierdiscus			
G. belizeanus	M.A. Faust, 1995	Chinain et al., 1999, 2010a; Kohli et al., 2015	CTX and MTX
G. caribaeus	Vandersea, Litaker, Faust, Kibler, Holland & Tester, 2009	Holland et al., 2013	MTX
G. carolinianus	Litaker, Vandersea, Faust, Kibler, Holland & Tester, 2009	Holland et al., 2013	MTX
G. carpenteri	Kibler, Litaker, Faust, Holland, Vandersea & Tester, 2009	Holland et al., 2013	MTX
G. toxicus	Adachi & Fukuyo, 1979	Chinain et al., 2010a; Kohli et al., 2015	CTX and MTX
Ostreopsis			
O. lenticularis	Fukuyo 1981	Tindall et al., 1990	PLTX, ostreotoxin-1 and 3
O. ovata	Fukuyo 1981	Nakajima et al., 1981; Penna et al., 2005; Honsell et al., 2013	Ovatoxins and other PLTX analogs
O. siamensis	Schmidt 1901	Ukena et al., 2001; Ciminiello et al., 2013, 2014	PLTX, ostreocin-B, ostreocin-D
Prorocentrum			
P. arenarium	M.A. Faust, 1994	Murakami et al., 1982; Bialojan and Takai, 1988; Morton and Bomber, 1994; Nascimento et al., 2005; Napolitano et al., 2009; An et al., 2010; Dominguez et al., 2010; Ferron et al., 2014; Braga et al., 2016; Twiner et al., 2016; Hu et al., 2017; Nascimento et al., 2017	Benthic *Prorocentrum* species may produce: OA and/or ethyl-, methyl-, deoxy-, diol ester-OA analogs; DTX1-6 analogs; prorocentrolides and related macrolides and other non-characterized toxins
P. belizeanum	M.A. Faust 1993		
P. concavum	Fukuyo 1981		
P. emarginatum	Fukuyo 1981		
P. hoffmannianum	M.A. Faust 1990		
P. lima	(Ehrenb.) F. Stein 1878		
P. maculosum	M.A. Faust 1993		
P. mexicanum	B.F. Osorio, 1942		
P. rhathymum	A.R. Loebl., Sherley & R.J. Schmidt, 1979		

Note that listing in this table does not imply that all strains and global populations produce toxins and many earlier reports of toxicity remain unconfirmed. CTX, ciguatoxin; MTX, maitotoxins; PLTX, palytoxin; OA, okadaic acid; DTX, dinophysistoxin.

and most scientific data are presented only at local or national conferences (Barón-Campis et al., 2014; Ley-Martinez et al., 2014; Núñez-Vázquez et al., 2018).

The necessary methods for the determination of toxin composition, most notably LC-MS/MS, are not widely applied for regulatory toxin surveillance in seafood or in research programs within Latin America. Many known toxins have not yet been confirmed from benthic dinoflagellates or food web compartments and seafood from the region. In some cases, such as for the CTXs, neither certified analytical standards nor reliable calibration reference materials are available for these assays and analyses (Dechraoui-Bottein and Clausing, 2017). Nevertheless, several Latin American countries, namely Brazil, Chile, Argentina and Mexico among others, have recently established LC-MS/MS facilities for phycotoxin analysis, including certain lipophilic analogs of highest relevance for toxigenic benthic dinoflagellates and contaminated seafood. These are now being applied to both research questions and toxin monitoring activities on a limited basis.

In most of Latin America, the conventional method for quick decisions on fisheries and aquaculture closures for public health protection remains the mouse bioassay (MBA). Variations of the standardized AOAC MBA for hydrophilic and lipophilic toxins, respectively, are widely applied in laboratories for seafood quality control throughout Latin America. In certain countries, the MBA has been largely replaced by chemical analytical methods for hydrophilic toxins, in particular by liquid chromatography coupled to ultraviolet detection [LC-UV] for domoic acid, and by LC coupled with fluorescence detection (LC-FD) for PSTs. Alternative diagnostic techniques, such as the phosphatase inhibition assay for DSTs or immunoassays for PSTs and domoic acid are often available, but they are typically applied for research

or limited toxin screening purposes and are not common in regulatory protocols (Ministerio de Salud, 2008; NOM-242-SSA1, 2009; Secretaría de Salud, 2015; Hardison et al., 2016; Lewis et al., 2016). The MBA allows for rapid results to decide on closures for shellfish and finfish harvesting but has a high detection limit and is rather imprecise regarding toxicity.

Biochemical and functional biological assay methods are primarily useful for semi-quantitative screening for specific toxin groups. For example, *in vitro* biological assays such as the neuroblastoma (N2A) assay, and the radioligand/fluorescent receptor-binding assay (RBA) have been successfully applied for detection of certain lipophilic neurotoxins. The latter technique has been developed for screening the Na_V site 5 specific neurotoxins, including the brevetoxins (PbTxs) and CTXs (Dechraoui-Bottein and Clausing, 2017). In Cuba, an optimized receptor binding assay (RBA) has been used to detect CTXs (Díaz-Asencio et al., 2016), and the protocol supports the validation of the assay. This will allow the implementation of a monitoring program for CTXs in this country, but to date such technological approaches have not been widely deployed in Latin America (Cuellar-Martinez et al., 2018). In any case, in addition to the drawback of the lack of certified reference materials for many toxin analogs, none of these assay methods are adequate for full qualitative and quantitative determination of lipophilic bHAB toxin analogs in either plankton or seafood matrices.

Among the phycotoxin syndromes associated with benthic dinoflagellates in Latin America, human illnesses linked to CFP are clearly the most acute and socio-economically relevant, but little etiological information is available on a regional scale. Within Mexico, 25 relatively well documented cases of CFP have been reported between 1979 and 2014 (Cuellar-Martinez et al., 2018), and CTX has been found in tissues of several fish species in the Caribbean (Ley-Martinez et al., 2014). Ciguateric areas and fishes are well-known by inhabitants of Caribbean coasts, in particular, through experience from historical and recent cases of CFP. Ciguatoxins are accumulated through the food webs in many tropical marine ecosystems, but to date little research has been conducted on the kinetics and mechanisms of toxin transfer from benthic dinoflagellates to carnivorous fish in Latin America and metabolism and toxin composition in various ecological compartments remains poorly understood. Registration of human cases and confirmed CFP intoxications is inconsistent in the region; in the Caribbean it has been estimated that almost half of the cases are not reported to medical facilities (Olsen et al., 1984), but public health surveillance and awareness are gradually improving.

In the absence of reliable diagnostic procedures for toxin detection, in many cases, fish that are known to be potentially toxic based upon historical medical or anecdotal data are simply avoided or banned for human consumption, such as the barracuda (*Sphyraena* spp.) in the Mexican Caribbean state of Quintana Roo (Olsen et al., 1984; García-Mendoza et al., 2016). Traditionally, fish are sold directly to the consumers by fisherman, or through cooperatives and fish markets; fish purchase is considered a hazardous activity, such that even hotels and restaurants in the Virgin Islands have refused to serve local fish (Olsen et al., 1984).

A key study carried out in Cuba on three coastal fishing communities showed a circumstantial link between macroalgal biofouling enhanced by thermal pollution that caused coral bleaching, and ciguatera cases (Morrison et al., 2008). More importantly, the social–ecological resilience of the community was demonstrated by linking through the local fishermen organizations via communication related to ciguatera and ciguatoxic fish in the fishery areas, and the associated CFP cases. In a well-organized fishing community, where this information was provided and shared, the number of CFP cases remained low, whereas in a larger non-organized community, where the relevant information scarcely reached the fishermen, intoxication cases increased during the same time period. This highlights the critical importance of local education, networking among seafood producers, regulatory authorities and public health personnel, and information exchange in ciguateric areas.

CURRENT AND FUTURE PERSPECTIVES ON BENTHIC HARMFUL ALGAL BLOOMS AND GLOBAL CHANGE

On a global scale, HABs and their environmental and human health consequences appear to be more frequent, intense and widespread within the last several decades (Hallegraeff, 2010). Toxic incidents caused by polyether phycotoxins are increasing worldwide, with an apparent expansion in the distributional range of toxin-producing benthic dinoflagellates, perhaps driven by climatic shifts and anthropogenic factors. Current assumptions are that this apparent increase is primarily related to global climate change, anthropogenic impacts in the coastal zones and enhanced public and scientific awareness via improved toxin and bloom surveillance programs (Accoroni et al., 2011). Evidence for the "global spreading hypothesis" has been acquired primarily for planktonic HABs, as they have historically received more research attention than bHABs, which tend to be cryptic and less spectacular. Recent studies, however, have shown that marine benthic and epiphytic dinoflagellates are also expanding their biogeographical ranges, with frequent species occurrences at latitudes where they were formerly unknown or uncommon (Shears and Ross, 2009). Benthic and epiphytic toxic dinoflagellates are now emerging as a critical issue due to their range expansion from tropical and subtropical waters to temperate coasts (Aligizaki, 2009; Shears and Ross, 2009).

This global spreading phenomenon was noted several decades ago for phytoplankton (Smayda, 1990; Hallegraeff, 2010), and more recently for species associated with HAB events linked to benthic systems (GEOHAB, 2012; Kibler et al., 2015). In particular, whereas bHABs were often previously considered to be restricted to discrete zones (e.g., coral reefs, mangrove coastal waters) of tropical ecosystems there have been increasing reports of toxigenic benthic species and bHAB events in subtropical

and even warm temperate regions (GEOHAB, 2012; Kibler et al., 2015).

The implementation of more effective and comprehensive surveillance programs for detection of putatively toxigenic species and better linkages to environmental and human health consequences undoubtedly accounts for some of this apparent increase in the magnitude, frequency and biogeographical distribution of bHABs. Nevertheless, there is now empirical evidence of shifts in distributional range of bHAB-associated taxa that can be circumstantially linked to global change and the mesoscale consequences in local and regional ecosystems. For example, expansion into temperate waters by members of the genus *Gambierdiscus* has been reported (Jeong et al., 2012). The associated risk of exposure to CFP is usually higher in the tropics (Lewis, 1986), but as sea surface temperatures continue to rise, it is likely to become more prevalent in temperate regions (Kibler et al., 2015). The positive correlation between water temperature and abundance of *Gambierdiscus* cells could lead to expansion into areas where this genus was never or rarely previously reported.

Blooms of *Ostreopsis* have emerged within the last decade as a major problem in southern Europe, particularly in the Mediterranean Sea, where they are associated with palytoxins and toxic aerosols causing respiratory problems in humans in affected coastal areas (Lemée et al., 2012). Closely related, if not identical *Ostreopsis* species, are commonly found in Latin America but not yet associated with toxic events. Knowledge of toxigenicity of *Ostreopsis* populations in Latin America is also lacking and this must be regarded as a critical issue to be addressed in the near future. Sudden expansion of bloom magnitude within local areas and/or range extension into more temperate waters could yield severe impacts to the tourist industry and shellfish production in Latin America.

Global climate change is frequently invoked to explain shifts in biogeographical distribution of marine microeukaryotes, with increased temperatures considered the primary driver of range expansions. Correlations between climate variability and CFP incidence has led to preliminary conclusions that climate change is already responsible for global increases in CFP cases and that this trend will continue in the future (Tester et al., 2010; Gingold et al., 2014). In addition to the caveat that correlation is not necessarily causality, the assumption that climate-dependent biogeographical shifts in bHAB species distribution is a direct function of sea surface temperature increases is clearly an oversimplification and perhaps not generally valid. Furthermore, although higher water temperatures at benthic interfaces should lead to slightly higher growth rates and range expansion within temperature limits, evidence that such temperature regimes will selectively favor genotypes of bHAB dinoflagellates over other members of benthic communities is lacking. Global change scenarios for consequences on bHAB biodiversity and biogeography must consider not only direct temperature effects, but also anthropogenic consequences due to deforestation, destruction of mangroves, urbanization and altered land use strategies in coastal zones, which can contribute to nutrient loading (eutrophication), organic and heavy metal pollution, ocean acidification, alteration of run-off patterns and volumes, changes in sediment deposition and coastal erosion processes. Anthropogenic disturbances, such as pollution, dredging and construction activities close to reefs have already had a dramatic impact on the health and diversity of reef ecosystems and presumably could influence the pattern and intensity of bHAB events (Chinain et al., 2010b; Rongo and van Woesik, 2011). In addition to these direct anthropogenic environmental stressors, extreme weather events (hurricanes, typhoons, tornados, etc.), whether they are linked to global climate change or not, creates short-term habitat disruption and hydrodynamic shifts that could account for permanent changes in bHAB species biogeography.

The integration of all these oceanographic processes leads to a complex expression of regime shifts yielding changes in the dynamics and distribution of bHABs. Such challenges must be addressed at the appropriate temporal and spatial level, e.g., submesoscale. Perhaps, it is premature to consider constructing predictive models of bHAB dynamics in Latin America, given the current lack of the knowledge of the functional ecology of these complex benthic ecosystems. Nevertheless, it is not too soon to generate testable hypotheses on the interactions among bHAB dinoflagellates and their shifting environmental regime, and the extent to which growth, survival and toxigenesis are determined by external "bottom-up" abiotic mechanisms versus internally regulated acclimation and adaptive responses. Warning signs are required to improve surveillance and research on these bHABs and addressing these hypotheses will generate knowledge to support risk assessment and bHAB dynamic modeling.

The urgency of the requirements for enhanced research and monitoring strategies is underscored by the fact that thousands of cases of human illnesses linked to seafood consumption, primarily CFP caused by transfer of phycotoxins into fish, but also other toxin syndromes originating from benthic dinoflagellates occur annually and often in tropical regions with poorly developed human health and research infrastructure. In Latin America the socio-economic impacts of bHABs, including increases in public health expenses, reduced productivity, lost tourism revenue, the need to seek alternative seafood resources, increases in the cost of monitoring bHAB events and toxins (Berdalet et al., 2017) are often not taken into account and are rarely systematically addressed. Collaborative work among public health authorities, fishermen, aquaculturists and scientists is required to address the challenges posed by bHABs at the local and regional level within Latin America. On a global scale, the integrated research and observational program GlobalHAB has been established to enhance research on these harmful algal species, to understand their relationships within the ecosystem and to determine their future trends in a changing world (Berdalet et al., 2017). Such international programs will provide detailed knowledge about toxins, public health risks, microalgal metabolism, and oceanographic processes and mechanisms related to HAB dynamics, with the ultimate goal of developing strategies for decreasing the impacts of HABs on human health and wellbeing (Berdalet et al., 2016).

In the Latin American context, it is encouraging that bHABs are considered as a major focus of the international research agenda. With the exception of the Gulf of Mexico and the Florida coasts of the United States, and a few isolated islands in the Caribbean region, biogeography and biodiversity of toxigenic benthic dinoflagellates in the Americas is virtually unexplored and poorly understood. In Latin America, the current lack of knowledge on bHABs is at least partially attributable to the paucity of advanced technology (e.g., gene sequencing facilities for phylogenomics and molecular taxonomy; liquid chromatography-mass spectrometry for toxin analysis) and trained personnel applied to biodiversity studies. Yet such capacity building and technology transfer is now underway and successful research initiatives on biogeography and biodiversity will assist in designing effective management strategies for bHAB events in Latin America.

AUTHOR CONTRIBUTIONS

All authors LD-R, AC, and YO contributed actively in reviewing the literature and interpreting the experimental aspects cited in this review and co-wrote the manuscript with particular focus on their areas of scientific expertise, i.e., toxin chemistry, chemical ecology, ecophysiology and bloom dynamics, and taxonomy, respectively, of marine dinoflagellates.

FUNDING

Financial contribution for the preparation of this manuscript and associated research activities was provided to AC via the PACES II Research Program (Topic II Coast: WP3) of the Alfred-Wegener-Institut, Helmholtz Zentrum für Polar- und Meeresforschung under the research theme Earth and Environment, Helmholtz Gemeinschaft, Germany.

ACKNOWLEDGMENTS

The authors thank Mario Fernando Sánchez-Bernal for the graphic design. All authors are members of RedFAN and SOMEFAN, national scientific societies of Mexico for the study of harmful algal blooms.

REFERENCES

Accoroni, S., Glibert, P. M., Pichierri, S., Romagnoli, T., Marini, M., and Totti, C. (2015). A conceptual model of annual *Ostreopsis* cf. *ovata* blooms in the northern Adriatic Sea based on the synergic effects of hydrodynamics, temperature, and the N:P ratio of water column nutrients. *Harmful Algae* 45, 14–25. doi: 10.1016/j.hal.2015.04.002

Accoroni, S., Romagnoli, T., Colombo, F., Pennesi, C., Di Camillo, C. G., Marini, M., et al. (2011). *Ostreopsis* cf. *ovata* bloom in the northern Adriatic Sea during summer 2009: ecology, molecular characterization and toxin profile. *Mar. Poll. Bull.* 62, 2512–2519. doi: 10.1016/j.marpolbul.2011.08.003

Accoroni, S., and Totti, C. (2016). The toxic benthic dinoflagellates of the genus *Ostreopsis* in temperate areas: a review. *Adv. Oceanogr. Limnol.* 7, 1–15. doi: 10.4081/aiol.2016.5591

Acuña, R. (2015). Diarrea aguda. *Revista Médica Clínica Las Condes* 26, 676–686. doi: 10.1016/j.rmclc.2015.09.010

Aguilar-Trujillo, A. C. (2017). *Variación de grupos funcionales de dinoflagelados epifíticos/bentónicos en la costa norte de la Península de Yucatán*. Ph.D. Thesis, CINVESTAV-IPN Unidad Mérida, Mérida.

Aguilar-Trujillo, A. C., Okolodkov, Y. B., Herrera-Silveira, J. A., Merino-Virgilio, F. D. C., and Galicia-García, C. (2017). Taxocoenosis of epibenthic dinoflagellates in the coastal waters of the northern Yucatan Peninsula before and after the harmful algal bloom event in 2011–2012. *Mar. Poll. Bull.* 119, 396–406. doi: 10.1016/j.marpolbul.2017.02.074

Aguilar-Trujillo, A. C., Okolodkov, Y. B., Merino-Virgilio, F., Osorio-Moreno, I., and Herrera-Silveira, J. (2014). "Variación espacial de dinoflagelados bentónicos/epifíticos en aguas costeras del norte de Yucatán (agosto de 2011)," in *Golfo de México. Contaminación, Impacto Ambiental, Diagnóstico y Tendencias*, 3 Edn, eds A. V. Botello, J. Rendón Von Osten, J. A. Benítez, and G. Gold-Bouchot (México: UNAM-ICMYL), 147–160.

Ajani, P., Harwood, D. T., and Murray, S. A. (2017). Recent trends in marine phycotoxins from Australian coastal waters. *Mar. Drugs* 15:33. doi: 10.3390/md15020033

Aligizaki, K. (2009). "Spread of potentially toxic benthic dinoflagellates in the Mediterranean Sea: A response to climate change?," in *Phytoplankton Response to Mediterranean Environmental Change*, ed. F. Briand (Paris: CIESM), 57–61.

Almazán-Becerril, A. (2000). *Estudio taxonómico de algunos dinoflagelados potencialmente tóxicos en el Caribe Mexicano*. M.Sc. Thesis, Universidad Nacional Autónoma de México, Ciudad Universitaria, México.

Almazán-Becerril, A., Escobar-Morales, S., Rosiles-González, G., and Valadez, F. (2015). Benthic-epiphytic dinoflagellates from the northern portion of the Mesoamerican Reef System. *Bot. Mar.* 58, 115–128.

Amar, M., Aráoz, R., Iorga, B. I., Yasumoto, T., Servent, D., and Molgó, J. (2018). Prorocentrolide-A from cultured *Prorocentrum lima* dinoflagellates collected in Japan blocks sub-types of nicotinic acetylcholine receptors. *Toxins* 10:97. doi: 10.3390/toxins10030097

Amzil, Z., Sibat, M., Chomerat, N., Grossel, H., Marco-Miralles, F., Lemee, R., et al. (2012). Ovatoxin-a and palytoxin accumulation in seafood in relation to *Ostreopsis* cf. *ovata* blooms on the French Mediterranean coast. *Mar. Drugs* 10, 477–496. doi: 10.3390/md10020477

An, T., Winshell, J., Scorzetti, G., Fell, J. W., and Rein, K. S. (2010). Identification of okadaic acid production in the marine dinoflagellate *Prorocentrum rhathymum* from Florida Bay. *Toxicon* 55, 653–657. doi: 10.1016/j.toxicon.2009.08.018

Arbelaez, N., Pineda, J. E. M., and Reguera, B. (2017). Dinoflagelados epífitos de *Thalassia testudinum* en dos sistemas costeros del Caribe colombiano. *Bol. Invest. Mar. Cost.* 46, 9–40. doi: 10.25268/bimc.invemar.2017.46.2.725

Balech, E. (1956). Étude des Dinoflagellés du sable de Roscoff. *Revue Algologique, Nouvelle Serie* 2, 29–52.

Barón-Campis, S. A., Okolodkov, Y. B., Ríos-Lara, G. V., Vázquez-Gómez, N., Rubio-Sánchez, V., Arce-Rocha, G., et al. (2014). "Dinoflagelados nocivos y ciguatoxinas en la costa norte de Yucatán: un riesgo para el futuro desarrollo de la maricultura," in *Proceedings of the XXI Congreso Nacional de Ciencia y Tecnología del Mar*, México City.

Bauer, I., Maranda, L., Young, K. A., Shimizu, Y., Fairchild, C., Cornell, L., et al. (1995a). Isolation and structure of caribenolide I, a highly potent antitumor macrolide from a cultured free-swimming Caribbean dinoflagellate, *Amphidinium* sp. S1-36-5. *J. Org. Chem.* 60, 1084–1086. doi: 10.1021/jo00109a050

Bauer, I., Maranda, L., Young, K. A., Shimizu, Y., and Huang, S. (1995b). The isolation and structures of unusual 1, 4-polyketides from the dinoflagellate, *Amphidinium* sp. *Tetrahedron Lett.* 36, 991–994.

Ben-Gharbia, H., Yahia, O. K.-D., Amzil, Z., Chomérat, N., Abadie, E., Masseret, E., et al. (2016). Toxicity and growth assessments of three thermophilic benthic dinoflagellates (*Ostreopsis* cf. *ovata*, *Prorocentrum lima* and *Coolia monotis*) developing in the southern Mediterranean basin. *Toxins* 8:297.

Berdalet, E., Fleming, L. E., Gowen, R., Davidson, K., Hess, P., Backer, L. C., et al. (2016). Marine harmful algal blooms, human health and wellbeing: challenges

and opportunities in the 21st century. *J. Mar. Biol. Assoc.* 96, 61–91. doi: 10.1017/S0025315415001733

Berdalet, E., Tester, P. A., Chinain, M., Fraga, S., Lemée, R., Litaker, W., et al. (2017). Harmful algal blooms in benthic systems: recent progress and future research. *Oceanography* 30, 36–45. doi: 10.5670/oceanog.2017.108

Bialojan, C., and Takai, A. (1988). Inhibitory effect of a marine-sponge toxin, okadaic acid, on protein phosphatases. specificity and kinetics. *Biochem. J.* 256, 283–290. doi: 10.1042/bj2560283

Blanco, J., Moroño, Á., and Fernández, M. L. (2005). Toxic episodes in shellfish, produced by lipophilic phycotoxins: an overview. *Galician J. Mar. Resour.* 1, 1–70.

Boisnoir, A., Pascal, P.-Y., Cordonnier, S., and Lemée, R. (2018). Depth distribution of benthic dinoflagellates in the Caribbean Sea. *J. Sea Res.* 135, 74–83. doi: 10.1016/j.seares.2018.02.001

Boisnoir, A., Pascal, P.-Y., Cordonnier, S., and Lemée, R. (2019). Spatio-temporal dynamics and biotic substrate preferences of benthic dinoflagellates in the Lesser Antilles, Caribbean sea. *Harmful Algae* 81, 18–29. doi: 10.1016/j.hal.2018.11.012

Bomber, J. W., Morton, S. L., Babinchak, J. A., Norris, D. R., and Morton, J. G. (1988). Epiphytic dinoflagellates of drift algae—another toxigenic community in the ciguatera food chain. *Bull. Mar. Sci.* 43, 204–214.

Bomber, J. W., Rubio, M. G., and Norris, D. R. (1989). Epiphytism of dinoflagellates associated with the disease ciguatera: substrate specificity and nutrition. *Phycologia* 28, 360–368. doi: 10.2216/i0031-8884-28-3-360.1

Braga, A. C., Alves, R. N., Maulvault, A. L., Barbosa, V., Marques, A., and Costa, P. R. (2016). *In vitro* bioaccessibility of the marine biotoxin okadaic acid in shellfish. *Food Chem. Toxicol.* 89, 54–59. doi: 10.1016/j.fct.2016.01.008

Bravo, I., Figueroa, R. I., and Fraga, S. (2014). Cellular and nuclear morphological variability within a single species of the toxigenic dinoflagellate genus *Gambierdiscus*: relationship to life-cycle processes. *Harmful Algae* 40, 1–8. doi: 10.1016/j.hal.2014.09.009

Calado, A. J., and Moestrup, Ø (2005). On the freshwater dinoflagellates presently included in the genus *Amphidinium*, with a description of *Prosoaulax* gen. nov. *Phycologia* 44, 112–119. doi: 10.2216/0031-8884(2005)44[112:OTFDPI]2.0.CO;2

Carlson, R. D., and Tindall, D. R. (1985). "Distribution and periodicity of toxic dinoflagellates in the Virgin Islands," in *Toxic dinoflagellates*, eds D. M. Anderson, A. W. White, and D. G. Baden (New York, NY: Elsevier Sci), 171–176.

Carnicer, O., García-Altares, M., Andree, K. B., Diogène, J., and Fernández-Tejedor, M. (2016). First evidence of *Ostreopsis* cf. *ovata* in the eastern tropical Pacific Ocean, Ecuadorian coast. *Bot. Mar.* 59, 267–274. doi: 10.1515/bot-2016-0022

Chinain, M., Darius, H. T., Ung, A., Cruchet, P., Wang, Z., Ponton, D., et al. (2010a). Growth and toxin production in the ciguatera-causing dinoflagellate *Gambierdiscus polynesiensis* (Dinophyceae) in culture. *Toxicon* 56, 739–750. doi: 10.1016/j.toxicon.2009.06.013

Chinain, M., Darius, H. T., Ung, A., Fouc, M. T., Revel, T., Cruchet, P., et al. (2010b). Ciguatera risk management in French Polynesia: the case study of Raivavae Island (Australes Archipelago). *Toxicon* 56, 674–690. doi: 10.1016/j.toxicon.2009.05.032

Chinain, M., Faust, M. A., and Pauillac, S. (1999). Morphology and molecular analyses of three toxic species of *Gambierdiscus* (Dinophyceae): *G. pacificus*, sp. nov., *G. australes*, sp. nov., and *G. polynesiensis*, sp. nov. *J. Phycol.* 35, 1282–1296. doi: 10.1046/j.1529-8817.1999.3561282.x

Ciminiello, P., Dell'Aversano, C., Iacovo, E. D., Fattorusso, E., Forino, M., Tartaglione, L., et al. (2013). Investigation of toxin profile of Mediterranean and Atlantic strains of *Ostreopsis* cf. *siamensis* (Dinophyceae) by liquid chromatography–high resolution mass spectrometry. *Harmful Algae* 23, 19–27. doi: 10.1016/j.hal.2012.12.002

Ciminiello, P., Dell'Aversano, C., Iacovo, E. D., Fattorusso, E., Forino, M., Tartaglione, L., et al. (2014). First finding of *Ostreopsis* cf. *ovata* toxins in marine aerosols. *Environ. Sci. Technol.* 48, 3532–3540. doi: 10.1021/es405617d

Cortés-Altamirano, R., Sierra-Beltrán, A., and Cortés-Lara, M. C. (2011). "Ultraestructura de *Ostreopsis siamensis* y *O. ovata* (Dinophyceae): referencia especial a la placa 2′, el poro apical (Po) y poros tecales," in *Proceedings of the XIV Congreso Latino-Americano de Ciências de Mar, COLACMAR*, Camboriú.

Cortés-Altamirano, R., and Sierra-Beltrán, A. P. (2003). Morphology and taxonomy of *Prorocentrum mexicanum* and reinstatement of *Prorocentrum rhathymum* (Dinophyceae). *J. Phycol.* 39, 221–225. doi: 10.1046/j.1529-8817.2003.01112.x

Cortés-Lara, M. C., Cortés-Altamirano, R., Sierra-Beltrán, A., and Reyes-Juárez, A. (2005). *Ostreopsis siamensis* (Dinophyceae) a new tychoplanktonic record from Isabel Island National Park, Pacific Mexico. *Harmful Algae News* 28, 4–5.

Cuellar-Martinez, T., Ruiz-Fernández, A. C., Alonso-Hernández, C., Amaya-Monterrosa, O., Quintanilla, R., Carrillo-Ovalle, H. L., et al. (2018). Addressing the problem of harmful algal blooms in Latin America and the Caribbean-a regional network for early warning and response. *Front. Mar. Sci.* 5:409. doi: 10.3389/fmars.2018.00409

Cutignano, A., Nuzzo, G., Sardo, A., and Fontana, A. (2017). The missing piece in biosynthesis of amphidinols: first evidence of glycolate as a starter unit in new polyketides from *Amphidinium carterae*. *Mar. Drugs* 15:157. doi: 10.3390/md15060157

Dechraoui-Bottein, M. Y., and Clausing, R. J. (2017). Receptor-binding assay for the analysis of marine toxins: detection and mode of action. *Comp. Anal. Chem.* 78, 277–301. doi: 10.1016/bs.coac.2017.08.004

Delgado, G., Popowski, G., García, C., Lagos, N., and Lechuga-Devéze, C. H. (2005). Presence of DSP-toxins in *Prorocentrum lima* (Ehrenberg) Dodge in Cuba. *Rev. Invest. Mar.* 26, 229–234.

Delgado, G., Popowski, G., and Pombo, M. D. C. (2002). Nuevos registros de dinoflagelados tóxicos epibenticos en Cuba. *Rev. Invest. Mar.* 23, 229–232.

Delgado-Miranda, G. (2005). *Dinoflagelados bentónicos tóxicos asociados a la ciguatera: abundancia, toxicidad y relación con los factores ambientales en un área de litoral noroccidental de Cuba*. Ph. D. Thesis, Centro de Investigaciones Biológicas del Noroeste, La Paz, B.C.S., Mexico.

Díaz-Asencio, L., Chamero-Lago, D., Gómez-Batista, M., Vandersea, M. W., García-Chamero, A., and Moreira-González, A. (2016). "Progress on ciguatera risk assessment in South Central Cuba," in *Proceedings of the Abstract Book of the 17th International Conference on Harmful Algae*, Florianópolis.

Dodge, J. D. (1982). *Marine Dinoflagellates of the British Isles*. London: Her Majesty's Stationery Office.

Dominguez, H. J., Paz, B., Daranas, A. H., Norte, M., Franco, J. M., and Fernández, J. J. (2010). Dinoflagellate polyether within the yessotoxin, pectenotoxin and okadaic acid toxin groups: characterization, analysis and human health implications. *Toxicon* 56, 191–217. doi: 10.1016/j.toxicon.2009.11.005

Doucet, E., Ross, N. N., and Quilliam, M. A. (2007). Enzymatic hydrolysis of esterified diarrhetic shellfish poisoning toxins and pectenotoxins. *Anal. Bioanal. Chem.* 389, 335–342. doi: 10.1007/s00216-007-1489-3

Echigoya, R., Rhodes, L., Oshima, Y., and Satake, M. (2005). The structures of five new antifungal and hemolytic amphidinol analogs from *Amphidinium carterae* collected in New Zealand. *Harmful Algae* 4, 383–389. doi: 10.1016/j.hal.2004.07.004

Espiritu, R. A., Tan, M. C. S., and Oyong, G. G. (2017). Evaluation of the anti-cancer potential of amphidinol 2, a polyketide metabolite from the marine dinoflagellate *Amphidinium klebsii*. *Jordan J. Biol. Sci.* 10, 297–302.

Estacion, M. (2000). "Ciguatera toxins: Mechanism of action and pharmacology of maitotoxin," in *Seafood and Freshwater toxins. Pharmacology, Physiology and Detection*, ed. L. Botana (New York, NY: Marcel Dekker), 473–504. doi: 10.1201/9780203909539.ch22

Estrada-Vargas, L., Okolodkov, Y., Aké-Castillo, J. A., Pérez-España, H., Morquecho-Escamilla, L., and Martínez-Serrano, I. (2017). "Dinoflagelados epibentónicos en la zona arrecifal de Punta Norte, Veracruz," in *4o Congreso Nacional de la Sociedad Mexicana para el Estudio de los Florecimientos Algales Nocivos A.C. y 2a Reunión de la Asociación Latinoamericana para el Estudio de Algas Nocivas: Sociedad Mexicana para el Estudio de los Florecimientos Algales Nocivos A.C*, Cancún.

Faust, M. A. (1990a). "Cysts of *Prorocentrum marinum* (Dinophyceae) in floating detritus at Twin Cays, Belize mangrove habitats," in *Proceedings of the IV International Conference on Toxic Marine Phytoplankton*, eds E. Granéli, B. Sundström, L. Edler, and D. M. Anderson (New York, NY: Elsevier), 138–143.

Faust, M. A. (1990b). Morphologic details of six benthic species of *Prorocentrum* (Pyrrophyta) from a mangrove island, Twin Cays, Belize, including

two new species. *J. Phycol.* 26, 548–558. doi: 10.1111/j.0022-3646.1990.00548.x

Faust, M. A. (1991). Morphology of ciguatera-causing *Prorocentrum lima* (Pyrrophyta) from widely differing sites. *J. Phycol.* 27, 642–648. doi: 10.1111/j.0022-3646.1991.00642.x

Faust, M. A. (1992). Observations on the morphology and sexual reproduction of *Coolia monotis* (Dinophyceae). *J. Phycol.* 28, 94–104. doi: 10.1111/j.0022-3646.1992.00094.x

Faust, M. A. (1993a). "Alternate asexual reproduction of *Prorocentrum lima* in culture," in *Toxic Phytoplankton Blooms in the Sea*, eds T. J. Smayda and Y. Shimizu (New York, NY: Elsevier Sci Publ), 115–120.

Faust, M. A. (1993b). "Ecology of toxic benthic, toxic dinoflagellates: an overview," in *Proceedings of the VI International Conference on Toxic Marine Phytoplankton*, eds P. Lassus, G. Arzul, E. Erard-Le Denn, P. Gentien, and C. Marcaillou-Le Baut (Paris: Intercept), 847–854.

Faust, M. A. (1993c). *Prorocentrum belizeanum*, *Prorocentrum elegans*, and *Prorocentrum caribbaeum*, three new benthic species (Dinophyceae) from a mangrove island, Twin Cays, Belize. *J. Phycol.* 29, 100–107. doi: 10.1111/j.1529-8817.1993.tb00287.x

Faust, M. A. (1993d). Three new benthic species of *Prorocentrum* (Dinophyceae) from Twin Cays, Belize: *P. maculosum* sp. nov., *P. foraminosum* sp. nov. and *P. formosum* sp. nov. *Phycologia* 32, 410–418. doi: 10.1111/j.1529-8817.2007.00450.x

Faust, M. A. (1994). Three new benthic species of *Prorocentrum* (Dinophyceae) from Carry Bow Cay, Belize: *P. sabulosum* sp. nov., *P. sculptile* sp. nov., and *P. arenarium* sp. nov. *J. Phycol.* 30, 755–763. doi: 10.1111/j.0022-3646.1994.00755.x

Faust, M. A. (1995). Observation of sand-dwelling toxic dinoflagellates (Dinophyceae) from widely differing sites, including two new species. *J. Phycol.* 31, 996–1003. doi: 10.1111/j.0022-3646.1995.00996.x

Faust, M. A. (1996). Dinoflagellates in a mangrove ecosystem, Twin Cays, Belize. *Nova Hedwigia* 112, 447–460.

Faust, M. A. (1997). Three new benthic species of *Prorocentrum* (Dinophyceae) from Belize: *P. norrisianum* sp. nov., *P. tropicalis* sp. nov., and *P. reticulatum* sp. nov. *J. Phycol.* 33, 851–858. doi: 10.1111/j.0022-3646.1997.00851.x

Faust, M. A. (1999). Three new *Ostreopsis* species (Dinophyceae): *O. marinus* sp. nov., *O. belizeanus* sp. nov., and *O. caribbeanus* sp. nov. *Phycologia* 38, 92–99. doi: 10.2216/i0031-8884-38-2-92.1

Faust, M. A. (2000). Dinoflagellate associations in a coral reef-mangrove ecosystem: pelican and associated Cays, Belize. *Atoll. Res. Bull.* 473, 135–147. doi: 10.5479/si.00775630.473.137

Faust, M. A. (2004). The dinoflagellates of Twin Cays, Belize: biodiversity, distribution and vulnerability. *Atoll. Res. Bull.* 514, 1–20. doi: 10.5479/si.00775630.514.1

Faust, M. A., and Gulledge, R. A. (1996). Associations of microalgae and meiofauna in floating detritus at a mangrove island, Twin Cays, Belize. *J. Exp. Mar. Biol. Ecol.* 197, 159–175. doi: 10.1016/0022-0981(95)00159-X

Faust, M. A., and Gulledge, R. A. (2002). Identifying harmful marine dinoflagellates. *Contr. U.S Natl. Herb.* 42, 1–144.

Faust, M. A., Larsen, J., and Moestrup, Ø (1999). "Potentially toxic phytoplankton. 3. Genus *Prorocentrum* (Dinophyceae)," in *ICES Identification Leaflets for Plankton*, ed. J. A. Lindley (Copenhagen: International Council for the Exploration of the Sea), 1–23.

Faust, M. A., Litaker, R. W., Vandersea, M. V., Kibler, S. R., and Tester, P. A. (2005). Dinoflagellate, diversity and abundance in two Belizean coral reef-mangrove lagoons: a test of Margalef's Mandala. *Atoll. Res. Bull.* 534, 105–131. doi: 10.5479/si.00775630.534.105

Faust, M. A., and Morton, S. L. (1996). Morphology and ecology of the marine dinoflagellate *Ostreopsis labens* sp. nov. (Dinophyceae). *J. Phycol.* 32, 456–463.

Faust, M. A., and Steidinger, K. A. (1998). *Bysmatrum* gen. nov.(Dinophyceae) and three new combinations for benthic scrippsielloid species. *Phycologia* 37, 47–52. doi: 10.2216/i0031-8884-37-1-47.1

Faust, M. A., Vandersea, M. W., Kibler, S. R., Tester, P. A., and Litaker, R. W. (2008). *Prorocentrum levis*, a new benthic species (Dinophyceae) from a mangrove island, Twin Cays, Belize. *J. Phycol.* 44, 232–240. doi: 10.1111/j.1529-8817.2007.00450.x

Ferron, P.-J., Hogeveen, K., Fessard, V., and Hégarat, L. L. (2014). Comparative analysis of the cytotoxic effects of okadaic acid-group toxins on human intestinal cell lines. *Mar. Drugs* 12, 4616–4634. doi: 10.3390/md12084616

Gallegos-Mendiola, V., Pérez-Cruz, B., Díaz-Ortiz, J. A., Morán-Vega, D. E., Huante-Catalán, R., and Gárate-Lizárraga, I. (2017). "Ocurrencia de *Ostreopsis ovata* en la playa Piedra de Tlalcoyunque en la región Costa Grande, Guerrero, México," in *4o Congreso Nacional de la Sociedad Mexicana para el Estudio de los Florecimientos Algales Nocivos A.C. y 2a Reunión de la Asociación Latinoamericana para el Estudio de Algas Nocivas*, Cancún, 96.

Gárate-Lizárraga, I. (2005). "Florecimientos algales nocivos," in *Perspectivas para el desarrollo acuacultural del sistema lagunar de Bahía Magdalena-Almejas, B.C.S., México*, ed. B. H. Anguas-Vélez (Mexico: Comité Editorial del Instituto Politécnico Nacional), 41–48.

Gárate-Lizárraga, I. (2012). Proliferation of *Amphidinium carterae* (Gymnodiniales: Gymnodiniaceae) in Bahía de La Paz, Gulf of California. *CICIMAR Oceánides* 27, 37–49.

Gárate-Lizárraga, I., Band-Schmidt, C. J., Verdugo-Díaz, G., Muñetón-Gómez, M. d. S., and Féliz-Pico, E. F. (2007). "Dinoflagelados (Dinophyceae) del sistema lagunar Magdalena-Almejas," in *Estudios Ecológicos de Bahía Magdalena*, eds R. Funes-Rodríguez, J. Gómez-Gutierrez, and R. Palomares-García (Mexico City: Instituto Politécnico Nacional), 145–174.

Gárate-Lizárraga, I., González-Armas, R., and Okolodkov, Y. B. (2018). Occurrence of *Ostreopsis lenticularis* (Dinophyceae: Gonyaulacales) from the Archipielago de Revillagigedo, Mexican Pacific. *Mar. Poll. Bull.* 128, 390–395. doi: 10.1016/j.marpolbul.2018.01.020

García-Mendoza, E., Núñez-Vázquez, E., and Almazán Becerril, A. (2016). Legislación sanitaria asociada al control de ficotoxinas marinas en México. *La Jornada Ecológica*. Available at: https://www.jornada.com.mx/2016/07/25/eco-i.html

GEOHAB (2012). "Global ecology and oceanography of harmful algal blooms," in *GEOHAB Core Research Project: HABs in Benthic Systems*, eds E. Berdalet, P. Tester, and A. Zingone, (Paris: IOC-UNESCO), 64.

Gingold, D. B., Strickland, M. J., and Hess, J. J. (2014). Ciguatera fish poisoning and climate change: analysis of National Poison Center data in the United States, 2001–2011. *Environ. Health Perspect.* 122, 580–586. doi: 10.1289/ehp.1307196

Gómez, F., Qiu, D., and Lin, S. (2017a). The synonymy of the toxic dinoflagellates *Prorocentrum mexicanum* and *P. rhathymum* and the description of *P. steidingerae* sp. nov. (Prorocentrales, Dinophyceae). *J. Eukaryot Microbiol.* 64, 668–677. doi: 10.1111/jeu.12403

Gómez, F., Qiu, D., Lopes, R. M., and Lin, S. (2017b). Morphological and molecular characterization of the toxic dinoflagellate *Ostreopsis* cf. *ovata* (Gonyaulacales: Dinophyceae) from Brazil (South Atlantic Ocean). *Rev. Biol. Trop.* 65, 1022–1032. doi: 10.15517/rbt.v65i3.29436

Gómez, F., Qiu, D., Lopes, R. M., and Lin, S. (2015). *Fukuyoa paulensis* gen. et sp. nov., a new genus for the globular species of the dinoflagellate *Gambierdiscus* (Dinophyceae). *PLoS One* 10:e0119676. doi: 10.1371/journal.pone.0119676

Hallegraeff, G. M. (2010). Ocean climate change, phytoplankton community responses, and harmful algal blooms: a formidable predictive challenge. *J. Phycol.* 46, 220–235. doi: 10.1111/j.1529-8817.2010.00815.x

Hardison, D. R., Holland, W. C., Mccall, J. R., Bourdelais, A. J., Baden, D. G., Darius, H. T., et al. (2016). Fluorescent receptor binding assay for detecting ciguatoxins in fish. *PLoS One* 11:e0153348. doi: 10.1371/journal.pone.0153348

Heredia-Tapia, A., Arredondo-Vega, B., Nuñez-Vázquez, E., Yasumoto, T., Yasuda, M., and Ochoa, J. (2002). Isolation of *Prorocentrum lima* (Syn. *Exuviaella lima*) and diarrhetic shellfish poisoning (DSP) risk assessment in the Gulf of California, Mexico. *Toxicon* 40, 1121–1127. doi: 10.1016/S0041-0101(02)00111-3

Hernández-Becerril, D., and Almazán Becerril, A. (2004). Especies de dinoflagelados del género *Gambierdiscus* (Dinophyceae) del Mar Caribe mexicano. *Rev. Biol. Trop.* 52, 77–87.

Herrera-Sepúlveda, A., Medlin, L. K., Murugan, G., Sierra-Beltrán, A. P., Cruz-Villacorta, A. A., and Hernández-Saavedra, N. Y. (2015). Are *Prorocentrum hoffmannianum* and *Prorocentrum belizeanum* (Dinophyceae, Prorocentrales), the same species? An integration of morphological and molecular data. *J. Phycol.* 51, 173–188. doi: 10.1111/jpy.12265

Holland, W. C., Litaker, R. W., Tomas, C. R., Kibler, S. R., Place, A. R., Davenport, E. D., et al. (2013). Differences in the toxicity of six

Gambierdiscus (Dinophyceae) species measured using an in vitro human erythrocyte lysis assay. *Toxicon* 65, 15–33. doi: 10.1016/j.toxicon.2012.12.016

Holmes, M. J., Brust, A., and Lewis, R. J. (2014). "Dinoflagellate toxins: an overview," in *Seafood and Freshwater Toxins: Pharmacology, Physiology, and Detection*, ed. L. Botana (Boca Raton, FL: CRC Press), 3–38.

Holmes, M. J., Lewis, R. J., Jones, A., and Hoy, A. W. W. (1995). Cooliatoxin, the first toxin from *Coolia monotis* (Dinophyceae). *Nat. Toxins* 3, 355–362. doi: 10.1002/nt.2620030506

Honsell, G., Bonifacio, A., De Bortoli, M., Penna, A., Battocchi, C., Ciminiello, P., et al. (2013). New insights on cytological and metabolic features of *Ostreopsis* cf. *ovata* Fukuyo (Dinophyceae): a multidisciplinary approach. *PLoS One* 8:e57291. doi: 10.1371/journal.pone.0057291

Hoppenrath, M., Chomérat, N., Horiguchi, T., Schweikert, M., Nagahama, Y., and Murray, S. (2013). Taxonomy and phylogeny of the benthic *Prorocentrum* species (Dinophyceae)—A proposal and review. *Harmful Algae* 27, 1–28. doi: 10.1016/j.hal.2013.03.006

Hoppenrath, M., Elbrächter, M., and Drebes, G. (eds) (2009). *Marine phytoplankton. Selected microphytoplankton species from the North Sea around Helgoland and Sylt*. Stuttgart: E. Schweizerbart'sche Verlagsbuchhandlung.

Hoppenrath, M., Murray, S. A., Chomérat, N., and Horiguchi, T. (2014). *Marine Benthic Dinoflagellates-unveiling Their Worldwide Biodiversity*. Stuttgart: E. Schweizerbart'sche Verlagsbuchhandlung.

Hu, T., Leblanc, P., Burton, I. W., Walter, J. A., Mccarron, P., Melanson, J. E., et al. (2017). Sulfated diesters of okadaic acid and DTX-1: self-protective precursors of diarrhetic shellfish poisoning (DSP) toxins. *Harmful Algae* 63, 85–93. doi: 10.1016/j.hal.2017.01.012

Irola-Sansores, E. D., Delgado-Pech, B., Garcia-Mendoza, E., Núñez-Vázquez, E. J., Olivos-Ortiz, A., and Almazán-Becerril, A. (2018). Population dynamics of benthic-epiphytic dinoflagellates on two macroalgae from coral reef systems of the northern Mexican Caribbean. *Front. Mar. Sci.* 5:487. doi: 10.3389/fmars.2018.00487

James, K., Carey, B., O'halloran, J., and Škrabáková, Z. (2010). Shellfish toxicity: human health implications of marine algal toxins. *Epidemiol. Infect.* 138, 927–940. doi: 10.1017/S0950268810000853

Jeong, H. J., Lim, A. S., Jang, S. H., Yih, W. H., Kang, N. S., Lee, S. Y., et al. (2012). First report of the epiphytic dinoflagellate *Gambierdiscus caribaeus* in the temperate waters off Jeju Island, Korea: morphology and molecular characterization. *J. Eukaryot Microbiol.* 59, 637–650. doi: 10.1111/j.1550-7408.2012.00645.x

Jørgensen, M. F., Murray, S., and Daugbjerg, N. (2004). *Amphidinium* revisited. I. Redefinition of *Amphidinium* (Dinophyceae) based on cladistic and molecular phylogenetic analyses. *J. Phycol.* 40, 351–365. doi: 10.1111/j.1529-8817.2004.03131.x

Karafas, S., Teng, S. T., Leaw, C. P., and Alves-De-Souza, C. (2017). An evaluation of the genus *Amphidinium* (Dinophyceae) combining evidence from morphology, phylogenetics, and toxin production, with the introduction of six novel species. *Harmful Algae* 68, 128–151. doi: 10.1016/j.hal.2017.08.001

Karafas, S., York, R., and Tomas, C. (2015). Morphological and genetic analysis of the *Coolia monotis* species complex with the introduction of two new species, *Coolia santacroce* sp. nov. and *Coolia palmyrensis* sp. nov. (Dinophyceae). *Harmful Algae* 46, 18–33. doi: 10.1016/j.hal.2015.05.002

Karafas, S. J., and Tomas, C. R. (2015). Further observations on the genetics and morphometrics of *Coolia santacroce* (Dinophyceae). *Algae* 30, 275–280. doi: 10.4490/algae.2015.30.4.275

Kato, T., and Yasumoto, T. (2017). Quantification of representative ciguatoxins in the Pacific using quantitative nuclear magnetic resonance spectroscopy. *Mar. Drugs* 15:309. doi: 10.3390/md15100309

Kibler, S. R., Tester, P. A., Kunkel, K. E., Moore, S. K., and Litaker, R. W. (2015). Effects of ocean warming on growth and distribution of dinoflagellates associated with ciguatera fish poisoning in the Caribbean. *Ecol. Model.* 316, 194–210. doi: 10.1016/j.ecolmodel.2015.08.020

Kobayashi, J. I., and Kubota, T. (2007). Bioactive macrolides and polyketides from marine dinoflagellates of the genus *Amphidinium*. *J. Nat. Prod.* 70, 451–460. doi: 10.1021/np0605844

Kobayashi, J. I., Shimbo, K., Sato, M., and Tsuda, M. (2002). Amphidinolides H2- H5, G2, and G3, new cytotoxic 26-and 27-membered macrolides from dinoflagellate *Amphidinium* sp. *J. Org. Chem.* 67, 6585–6592. doi: 10.1021/jo016222c

Kobayashi, J. I., Tsuda, M., Ishibashi, M., Shigemori, H., Yamasu, T., Hirota, H., et al. (1991). Amphidinolide F, a new cytotoxic macrolide from the marine dinoflagellate *Amphidinium* sp. *J. Antibiot.* 44, 1259–1261. doi: 10.7164/antibiotics.44.1259

Kohli, G. S., Farrell, H., and Murray, S. A. (2015). "*Gambierdiscus*, the cause of ciguatera fish poisoning: An increased human health threat influenced by climate change," in *Climate Change and Marine and Freshwater Toxins*, eds L. Botana, C. Louzao, and N. Vilariño (Boston, MA: Walter de Gruyter GmbH), 273–312.

Kohli, G. S., Papiol, G. G., Rhodes, L. L., Harwood, D. T., Selwood, A., Jerrett, A., et al. (2014). A feeding study to probe the uptake of maitotoxin by snapper (*Pagrus auratus*). *Harmful Algae* 37, 125–132. doi: 10.1016/j.hal.2014.05.018

Kumar-Roiné, S., Matsui, M., Chinain, M., Laurent, D., and Pauillac, S. (2008). Modulation of inducible nitric oxide synthase gene expression in RAW 264.7 murine macrophages by Pacific ciguatoxin. *Nitric Oxide* 19, 21–28. doi: 10.1016/j.niox.2008.03.001

Larsson, M. E., Harwood, T. D., Lewis, R. J., Swa, H., and Doblin, M. A. (2018). Toxicological characterization of *Fukuyoa paulensis* (Dinophyceae) from temperate Australia. *Phycol. Res.* 67, 65–71. doi: 10.1111/pre.12349

Lassus, P., Chomérat, N., Hess, P., and Nézan, E. (2016). *Toxic and Harmful Microalgae of the World Ocean–Micro-Algues Toxiques et Nuisibles de L'océan Mondial*. Copenhagen: Intergovernmental Oceanographic Commission of UNESCO.

Le Gac, M., Metegnier, G., Chomérat, N., Malestroit, P., Quéré, J., Bouchez, O., et al. (2016). Evolutionary processes and cellular functions underlying divergence in *Alexandrium minutum*. *Mol. Ecol.* 25, 5129–5143. doi: 10.1111/mec.13815

Leaw, C. P., Lim, P. T., Cheng, K. W., Ng, B. K., and Usup, G. (2010). Morphology and molecular characterization of a new species of thecate benthic dinoflagellate, *Coolia malayensis* sp. nov. (Dinophyceae). *J. Phycol.* 46, 162–171. doi: 10.1111/j.1529-8817.2009.00778.x

Lehane, L. (2000). Ciguatera update. *Med. J. Aust.* 172, 176–179.

LeJeunesse, T. C., Parkinson, J. E., Gabrielson, P. W., Jeong, H. J., Reimer, J. D., Voolstra, C. R., et al. (2018). Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. *Curr. Biol.* 28, 2570–2580. doi: 10.1016/j.cub.2018.07.008

Lemée, R., Mangialajo, L., Cohu, S., Amzil, Z., Blanfune, A., Chomerat, N., et al. (2012). Interactions between scientists, managers and policy makers in the framework of the French MediOs project on *Ostreopsis* (2008–2010). *Cryptogam. Algol.* 33, 137–142. doi: 10.7872/crya.v33.iss2.2011.137

Lewis, N. D. (1986). Disease and development: ciguatera fish poisoning. *Soc. Sci. Med.* 23, 983–993. doi: 10.1016/0277-9536(86)90255-8

Lewis, R. J., Inserra, M., Vetter, I., Holland, W. C., Hardison, D. R., Tester, P. A., et al. (2016). Rapid extraction and identification of maitotoxin and ciguatoxin-like toxins from Caribbean and Pacific *Gambierdiscus* using a new functional bioassay. *PLoS One* 11:e0160006. doi: 10.1371/journal.pone.0160006

Ley-Martinez, T. C., Nuñez-Vázquez, E. J., Almazán Becerril, A., Barón-Campis, S. A., Ramírez-Camarena, C., Caballero-Vázquez, J. A., et al. (2014). "Bioprospección de toxinas tipo ciguatoxinas en peces carnívoros del Caribe mexicano y aguas adyacentes," in *XXI Congreso Nacional de Ciencia y Tecnología del Mar*. Quintana Roo: Dirección General de Educación en Ciencia y Tecnología del Mar 1–13.

Lim, A. S., Jeong, H. J., Jang, T. Y., Kang, N. S., Lee, S. Y., Du Yoo, Y., et al. (2013). Morphology and molecular characterization of the epiphytic dinoflagellate *Prorocentrum* cf. *rhathymum* in temperate waters off Jeju Island, Korea. *Ocean Sci. J.* 48, 1–17. doi: 10.1007/s12601-013-0001-6

Litaker, R. W., Holland, W. C., Hardison, D. R., Pisapia, F., Hess, P., Kibler, S. R., et al. (2017). Ciguatoxicity of *Gambierdiscus* and *Fukuyoa* species from the Caribbean and Gulf of Mexico. *PLoS One* 12:e0185776. doi: 10.1371/journal.pone.0185776

Litaker, R. W., Vandersea, M. W., Faust, M. A., Kibler, S. R., Chinain, M., Holmes, M. J., et al. (2009). Taxonomy of *Gambierdiscus* including four new species, *Gambierdiscus caribaeus, Gambierdiscus carolinianus, Gambierdiscus carpenteri* and *Gambierdiscus ruetzleri* (Gonyaulacales, Dinophyceae). *Phycologia* 48, 344–390. doi: 10.3390/md16010007

Litaker, R. W., Vandersea, M. W., Faust, M. A., Kibler, S. R., Nau, A. W., Holland, W. C., et al. (2010). Global distribution of ciguatera causing dinoflagellates in the genus *Gambierdiscus*. *Toxicon* 56, 711–730. doi: 10.1016/j.toxicon.2010.05.017

López, C. (2018). *COFEPRIS mantiene alerta por venta de barracuda. La Jornada Maya*, domingo 8 de abril de 2018. Available at: https://www.lajornadamaya.mx/2018-04-08/Cofepris-mantiene-alerta-por-venta-de-barracuda

Louzao, M. C., Fernández, D. A., Abal, P., Fraga, M., Vilariño, N., Vieytes, M. R., et al. (2015). Diarrhetic effect of okadaic acid could be related with its neuronal action: changes in neuropeptide Y. *Toxicol. Lett.* 237, 151–160. doi: 10.1016/j.toxlet.2015.06.004

Mancera-Pineda, J. E., Montalvo-Talaigua, M., and Gavio, B. (2014). Dinoflagelados potencialmente tóxicos asociados a material orgánico flotante (drift) en San Andrés Isla, Reserva Internacional de la Biosfera-Seaflower: potentially toxic dinoflagellates associated to drift in San Andrés Island, International Biosphere Reservation-Seaflower. *Caldasia* 36, 139–156. doi: 10.15446/caldasia.v36n1.43896

Maranda, L., and Shimizu, Y. (1996). *Amphidinium operculatum* var. nov. *gibbosum* (Dinophyceae), a free-swimming marine species producing cytotoxic metabolites. *J. Phycol.* 32, 873–879. doi: 10.1111/j.0022-3646.1996.00873.x

Marchan-Álvarez, J. G., Valerio-González, L., Troccoli-Ghinaglia, L., and López-Monroy, F. (2017). Dinoflagelados bentónicos nocivos, asociados con el sedimento arenoso en dos playas de la isla de Margarita, Venezuela. *Rev. Biodivers. Neotrop.* 7, 156–168.

Martínez-Cruz, M. E., Okolodkov, Y. B., Aguilar-Trujillo, A. C., and Herrera-Silveira, J. A. (2016). Epiphytic dinoflagellates on the seagrass *Thalassia testudinum* at Dzilam, southeastern Gulf of Mexico. *Cymbella* 1, 2–9.

Meng, Y., Van Wagoner, R. M., Misner, I., Tomas, C., and Wright, J. L. (2010). Structure and biosynthesis of amphidinol 17, a hemolytic compound from *Amphidinium carterae*. *J. Nat. Prod.* 73, 409–415. doi: 10.1021/np900616q

Ministerio de Salud, G. D. C. (2008). *Programa Nacional de Vigilancia y Control de las Intoxicaciones Por Fenómenos Algales Nocivos ("marea roja")*. Santiago: Departamento de Alimentos y Nutrición.

Mitchell, L. E. (1985). *Ecological Studies of Benthic Dinoflagellates Associated with Ciguatera in the Florida Keys: the 0-38 Micrometer Size Fraction*. MSc, Florida Institute of Technology, Florida.

Moestrup, Ø, Akselmann, R., Fraga, S., Hoppenrath, M., Iwataki, M., Komárek, J., et al. (2009). *IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae*. Available at: http://www.marinespecies.org/hab/aphia.php?p=taxdetails&id=531446

Moestrup, Ø, and Calado, A. J. (2018). *Süßwasserflora von Mitteleuropa, Bd. 6-Freshwater Flora of Central Europe, Dinophyceae*, Vol. 6. Berlin: Springer-Verlag GmbH. doi: 10.1007/978-3-662-56269-7

Mohammad-Noor, N., Moestrup, Ø, Lundholm, N., Fraga, S., Adam, A., Holmes, M. J., et al. (2013). Autecology and phylogeny of *Coolia tropicalis* and *Coolia malayensis* (Dinophyceae), with emphasis on taxonomy of *C. tropicalis* based on light microscopy, scanning electron microscopy and LSU rDNA. *J. Phycol.* 49, 536–545. doi: 10.1111/jpy.12062

Molgó, J., Marchot, P., Aráoz, R., Benoit, E., Iorga, B. I., Zakarian, A., et al. (2017). Cyclic imine toxins from dinoflagellates: a growing family of potent antagonists of the nicotinic acetylcholine receptors. *J. Neurochem.* 142, 41–51. doi: 10.1111/jnc.13995

Moreira-González, A. R., Felicio-Fernandes, L., Peraza-Escarrá, R., Díaz-Asencio, L., Rodriguez, F., Riobó, P., et al. (2016). "Notes on morphology, phylogeny and toxicity of a dominant community of toxic benthic dinoflagellates from southern-central coast of Cuba," in *Proceedings of the 17th International Conference on Harmful Algae*, eds L. A. O. Proença and G. M. Hallegraeff (Florianopolis: International Society for the Study of Harmful Algae), 88.

Morquecho-Escamilla, L., Reyes-Salinas, A., and Okolodkov, Y. (2016). *Illustrated Taxonomic Guide of the Marine Dinoflagellate Collection (CODIMAR) Guía Taxonómica Ilustrada de la Colección de Dinoflagelados Marinos (CODIMAR)*. Mexico: Centro de Investigaciones Biológicas del Noreste, 147.

Morquecho-Escamilla, L., Reyes-Salinas, A., Okolodkov, Y. B., Gárate-Lizárraga, I., Mazariegos-Villarreal, A., and Galicia-García, C. (2017). "Aislamiento y caracterización taxonómica de dinoflagelados epífitos en Bahía de la Paz e Isla San José, Golfo de California," in *4o Congreso Nacional de la Sociedad Mexicana para el Estudio de los Florecimientos Algales Nocivos A.C. y 2a Reunión de la Asociación Latinoamericana para el Estudio de Algas Nocivas*, Cancún.

Morrison, K., Prieto, P. A., Domínguez, A. C., Waltner-Toews, D., and Fitzgibbon, J. (2008). Ciguatera fish poisoning in La Habana, Cuba: a study of local social–ecological resilience. *EcoHealth* 5, 346–359. doi: 10.1007/s10393-008-0188-7

Morsy, N., Houdai, T., Matsuoka, S., Matsumori, N., Adachi, S., Oishi, T., et al. (2006). Structures of new amphidinols with truncated polyhydroxyl chain and their membrane-permeabilizing activities. *Bioorg. Med. Chem.* 14, 6548–6554. doi: 10.1016/j.bmc.2006.06.012

Morton, S. L., and Bomber, J. W. (1994). Maximizing okadaic acid content from *Prorocentrum hoffmannianum* Faust. *J. Appl. Phycol.* 6, 41–44. doi: 10.1007/BF02185903

Morton, S. L., and Faust, M. A. (1997). Survey of toxic epiphytic dinoflagellates from the Belizean barrier reef ecosystem. *J. Mar. Sci.* 61, 899–906.

Murakami, Y., Oshima, Y., and Yasumoto, T. (1982). Identification of okadaic acid as a toxic component of a marine dinoflagellate *Prorocentrum lima*. *Nippon Suisan Gakkaishi* 48, 69–72. doi: 10.2331/suisan.48.69

Murray, S., Flø Jørgensen, M., Daugbjerg, N., and Rhodes, L. (2004). *Amphidinium* revisited. II. Resolving species boundaries in the *Amphidinium operculatum* species complex (Dinophyceae), including the descriptions of *Amphidinium trulla* sp. nov. and *Amphidinium gibbosum*. comb. nov. *J. Phycol.* 40, 366–382. doi: 10.1046/j.1529-8817.2004.03132.x

Nakajima, I., Oshima, Y., and Yasumoto, T. (1981). Toxicity of benthic dinoflagellates found in coral reef-II. Toxicity of benthic dinoflagellates in Okinawa. *Nippon Suisan Gakkaishi* 47, 1029–1033. doi: 10.2331/suisan.47.1029

Napolitano, J. G., Norte, M., Padrón, J. M., Fernández, J. J., and Hernández Daranas, A. (2009). Belizeanolide, a cytotoxic macrolide from the dinoflagellate *Prorocentrum belizeanum*. *Angew. Chem. Int. Ed.* 48, 796–799. doi: 10.1002/anie.200804023

Nascimento, S. M., Mendes, M. C. Q., Menezes, M., Rodríguez, F., Alves-De-Souza, C., Branco, S., et al. (2017). Morphology and phylogeny of *Prorocentrum caipirignum* sp. nov.(Dinophyceae), a new tropical toxic benthic dinoflagellate. *Harmful Algae* 70, 73–89. doi: 10.1016/j.hal.2017.11.001

Nascimento, S. M., Purdie, D. A., and Morris, S. (2005). Morphology, toxin composition and pigment content of *Prorocentrum lima* strains isolated from a coastal lagoon in southern UK. *Toxicon* 45, 633–649. doi: 10.1016/j.toxicon.2004.12.023

Navarro-Vargas, G., Díaz-Ramos, J. R., Troccoli-Ghinalgia, L., and Subero-Pino, S. (2014). Dinoflagelados epibentónicos presentes en diferentes sustratos en la Bahía Turpialito, golfo de Cariaco, Venezuela. *Bol. Inst. Oceanogr. Venezuela* 53, 161–170.

Nie, D., and Wang, C.-C. (1944). Dinoflagellata of the Hainan Region. VIII. On *Sinophysis microcephalus*, a new genus and species of Dinophysidae. *Sinensia* 15, 145–151.

NOM-242-SSA1 (2009). Norma Oficial Mexicana NOM-242-SSA1. *Diario Oficial de la Federación*. Available at: http://www.dof.gob.mx/normasOficiales/4295/salud2a/salud2a.htmNOM

Núñez-Vázquez, E. (2005). *Biotoxinas Marinas en Peces Comestibles de Baja California Sur, México: Origen, Identificación y Cuantificación*. México: Bachelor, Universidad Autónoma de Baja California Sur.

Núñez-Vázquez, E., Almazán-Becerril, A., Heredia-Tapia, A., Hernández-Becerril, D., Troccoli-Ghinaglia, L., Arredondo-Vega, B., et al. (2000). "Incidencia del envenenamiento por ciguatera en México," in *4a. Reunión de Expertos en Envenenamientos por Animales Ponzoñosos: Instituto de Biotecnología-UNAM, International Society on Toxicology-Sección Panamericana, Instituto Bioclon., Laboratorio Silanes*, Cuernavaca, 49–50.

Núñez-Vázquez, E. J., Poot-Delgado, C. A., Turner, A., Ley-Martínez, T. C., Domínguez-Solís, G., Cahuich-Sánchez, Y., et al. (2018). "Presencia de toxinas marinas en peces comerciales de las costas de Campeche, México," in *IX Foro Científico de Pesca Ribereña*, Mazatlán.

Okolodkov, Y. B., Campos-Bautista, G., Gárate-Lizárraga, I., González-González, J. A. G., Hoppenrath, M., and Arenas, V. (2007). Seasonal changes of benthic and epiphytic dinoflagellates in the Veracruz reef zone, Gulf of Mexico. *Aquat. Microb. Ecol.* 47, 223–237. doi: 10.3354/ame047223

Okolodkov, Y. B., and Gárate-Lizárraga, I. (2006). An annotated checklist of dinoflagellates (Dinophyceae) from the Mexican Pacific. *Acta Bot. Mex.* 74, 1–154. doi: 10.21829/abm74.2006.1008

Okolodkov, Y. B., Merino-Virgilio, F. D. C., Herrera-Silveira, J. A., Espinosa-Matías, S., and Parsons, M. L. (2009). *Gambierdiscus toxicus* in the southeastern Gulf of Mexico. *Harmful Algae News* 40, 12–14.

Okolodkov, Y. B., Virgilio, F. D. C. M., Castillo, J. A. A., Trujillo, A. C. A., Espinosa-Matías, S., and Silveira, A. H. (2014). Seasonal changes in epiphytic dinoflagellate assemblages near the northern coast of the Yucatan Peninsula, Gulf of Mexico. *Acta Bot. Mex.* 107, 121–151. doi: 10.21829/abm107.2014.204

Olsen, D. A., Nellis, D. W., and Wood, R. S. (1984). Ciguatera in the eastern Caribbean. *Mar. Fish Rev.* 46, 13–18.

Penna, A., Vila, M., Fraga, S., Giacobbe, M. G., Andreoni, F., Riobó, P., et al. (2005). Characterization of *Ostreopsis* and *Coolia* (Dinophyceae) isolates in the Western Mediterreanean Sea based on morphology, toxicity and internal transcribed spacer 5.8 *S rDNA sequences*. *J. Phycol.* 41, 212–225. doi: 10.1111/j.1529-8817.2005.04011.x

Pisapia, F., Sibat, M., Herrenknecht, C., Lhaute, K., Gaiani, G., Ferron, P.-J., et al. (2017). Maitotoxin-4, a novel MTX analog produced by *Gambierdiscus excentricus*. *Mar. Drugs* 15:220. doi: 10.3390/md15070220

Place, A. R., Bowers, H. A., Bachvaroff, T. R., Adolf, J. E., Deeds, J. R., and Sheng, J. (2012). *Karlodinium veneficum*—The little dinoflagellate with a big bite. *Harmful Algae* 14, 179–195. doi: 10.1016/j.hal.2011.10.021

Quintana-M, H., and Mercado-Gómez, J. (2017). Composición de dinoflagelados epífitos y forófitos en la Costa norte del golfo de Morrosquillo, Sucre, Colombia. *Rev. Colomb. Cienc. Anim.* 9, 129–140. doi: 10.24188/recia.v9.n2.2017.550

Quintanilla, C. R., and Amaya, O. (2016). "First report of the epiphytic genera *Gambierdiscus* and *Ostreopsis* from the coast of El Salvador, Eastern Tropical Pacific," in *Proceedings of the 17th International Conference on Harmful Algae*, eds L. A. O. Proença and G. M. Hallegraeff (Florianopolis: International Society for the Study of Harmful Algae), 80–82.

Rains, L. K., and Parsons, M. L. (2015). *Gambierdiscus* species exhibit different epiphytic behaviors toward a variety of macroalgal hosts. *Harmful Algae* 49, 29–39. doi: 10.1016/j.hal.2015.08.005

Ramos, V., and Vasconcelos, V. (2010). Palytoxin and analogs: biological and ecological effects. *Mar Drugs* 8, 2021–2037. doi: 10.3390/md8072021

Rhodes, L. L., Smith, K. F., Munday, R., Selwood, A. I., McNabb, P. S., Holland, P. T., et al. (2010). Toxic dinoflagellates (Dinophyceae) from Rarotonga, Cook Islands. *Toxicon* 56, 751–758. doi: 10.1016/j.toxicon.2009.05.017

Rhodes, L. L., and Thomas, A. E. (1997). *Coolia monotis* (Dinophyceae): a toxic epiphytic microalgal species found in New Zealand. *N. Z. J. Mar. Freshw. Res.* 31, 139–141. doi: 10.1080/00288330.1997.9516751

Richlen, M. L., and Lobel, P. S. (2011). Effects of depth, habitat, and water motion on the abundance and distribution of ciguatera dinoflagellates at Johnston Atoll, Pacific Ocean. *Mar. Ecol. Prog. Ser.* 421, 51–66. doi: 10.3354/meps08854

Rongo, T., and van Woesik, R. (2011). Ciguatera poisoning in Rarotonga, southern Cook islands. *Harmful Algae* 10, 345–355. doi: 10.1016/j.toxicon.2012.12.018

Satake, M., Murata, M., Yasumoto, T., Fujita, T., and Naoki, H. (1991). Amphidinol, a polyhydroxy-polyene antifungal agent with an unprecedented structure, from a marine dinoflagellate, *Amphidinium klebsii*. *J. Am. Chem. Soc.* 113, 9859–9861. doi: 10.1021/ja00026a027

Secretaría de Salud (2015). "Plan de contingencia para el control de biotoxinas marinas," in *Comisión Federal para la Protección contra Riesgos Sanitarios*, (Mexico City: COFEPRIS). Available at: https://www.gob.mx/cms/uploads/attachment/file/178793/Plan_de_contigencia_MB_ES_20151208_ESP.pdfq

Sepúlveda-Villarraga, M., Band-Schmidt, C. J., Leyva-Valencia, I., Hernández-Carmona, G., and Almazán-Becerril, A. (2017). "Dinoflagelados potencialmente tóxicos asociados a macroalgas en la Bahía de La Paz, B.C.S," in *4o Congreso Nacional de la Sociedad Mexicana para el Estudio de los Florecimientos Algales Nocivos A.C. y 2a Reunión de la Asociación Latinoamericana para el Estudio de Algas Nocivas*, Cancún, 83.

Shears, N. T., and Ross, P. M. (2009). Blooms of benthic dinoflagellates of the genus *Ostreopsis*; an increasing and ecologically important phenomenon on temperate reefs in New Zealand and worldwide. *Harmful Algae* 8, 916–925. doi: 10.1016/j.hal.2009.05.003

Sierra-Beltrán, A., Cruz, A., Nuñez, E., Del Villar, L., Cerecero, J., and Ochoa, J. (1998). An overview of the marine food poisoning in Mexico. *Toxicon* 36, 1493–1502. doi: 10.1016/S0041-0101(98)00139-1

Smayda, T. J. (1990). "Novel and nuisance phytoplankton blooms in the sea: evidence for a global epidemic," in *Toxic Marine Phytoplankton*, eds E. Granéli, B. Sundström, L. Edler, and D. M. Anderson (New York: Elsevier), 29–40.

Steidinger, K., and Tangen, K. (1997). "The Planktonic Marine Flagellates," in *Identifying Marine Phytoplankton*. ed. C. R. Tomas (San Diego, CA: Academic Press), 591–730.

Steidinger, K. A. (2018). "Chapter III. Toxic and potentially harmful dinoflagellates in the Gulf of Mexico," in *Guide to the Identification of Harmful Microalgae in the Gulf of Mexico*, eds K. A. Steidinger and M. E. Meave Del Castillo (St. Peterburg, FL: Florida Institute of Oceanography), 211–342.

Taylor, F., Hoppenrath, M., and Saldarriaga, J. F. (2008). Dinoflagellate diversity and distribution. *Biodivers. Conserv.* 17, 407–418. doi: 10.1007/s10531-007-9258-3

Tester, P. A., Feldman, R. L., Nau, A. W., Kibler, S. R., and Litaker, R. W. (2010). Ciguatera fish poisoning and sea surface temperatures in the Caribbean Sea and the West Indies. *Toxicon* 56, 698–710. doi: 10.1016/j.toxicon.2010.02.026

Tester, P. A., Vandersea, M. W., Buckel, C. A., Kibler, S. R., Holland, W. C., Davenport, E. D., et al. (2013). *Gambierdiscus* (Dinophyceae) species diversity in the Flower Garden Banks National Marine Sanctuary, Northern Gulf of Mexico, USA. *Harmful Algae* 29, 1–9. doi: 10.1016/j.hal.2013.07.001

Tindall, D., Miller, D., and Tindall, P. (1990). "Toxicity of *Ostreopsis lenticularis* from the British and United States Virgin Islands," in *Toxic Marine Phytoplankton*, eds E. Granéli, L. Edler, and D. M. Anderson (New York,NY: Elsevier), 424–429.

Tindall, D. R., and Morton, S. L. (1998). "Community dynamics and physiology of epiphytic/benthic dinoflagellates associated with ciguatera," in *Physiological Ecology of Harmful Algal Blooms*, eds D. A. Anderson, A. D. Cembella, and G. M. Hallegraeff (Berlin: Springer-Verlag), 293–314.

Tosteson, T., Ballantine, D., Tosteson, C., Bardales, A., Durst, H., and Higerd, T. (1986). Comparative toxicity of *Gambierdiscus toxicus, Ostreopsis* cf. *lenticularis*, and associated microflora. *Mar. Fish Rev.* 48, 57–60.

Totti, C., Accoroni, S., Cerino, F., Cucchiari, E., and Romagnoli, T. (2010). *Ostreopsis ovata* bloom along the Conero Riviera (northern Adriatic Sea): relationships with environmental conditions and substrata. *Harmful Algae* 9, 233–239. doi: 10.1016/j.hal.2009.10.006

Tsuda, M., Oguchi, K., Iwamoto, R., Okamoto, Y., Kobayashi, J. I., Fukushi, E., et al. (2007). Iriomoteolide-1a, a potent cytotoxic 20-membered macrolide from a benthic dinoflagellate *Amphidinium* species. *J. Org. Chem.* 72, 4469–4474. doi: 10.1021/jo070414b

Tsuda, M., Sasaki, T., and Kobayashi, J. (1994). Amphidinolide L, a new cytotoxic 27-membered macrolide from the cultured dinoflagellate *Amphidinium* sp. *J. Org. Chem.* 59, 3734–3737. doi: 10.3390/molecules16075422

Tubaro, A., Sosa, S., and Hungerford, J. (2012). "Toxicology and diversity of marine toxins," in *Veterinary Toxicology* (Second Edition), ed. R. C. Gupta (San Diego, CA: Elsevier), 896–934.

Twiner, M. J., Doucette, G. J., Pang, Y., Fang, C., Forsyth, C. J., and Miles, C. O. (2016). Structure–activity relationship studies using natural and synthetic okadaic acid/dinophysistoxin toxins. *Mar. Drugs* 14:207. doi: 10.3390/md14110207

Ukena, T., Satake, M., Usami, M., Oshima, Y., Naoki, H., Fujita, T., et al. (2001). Structure elucidation of ostreocin D, a palytoxin analog isolated from the dinoflagellate *Ostreopsis siamensis*. *Biosci. Biotech. Biochem.* 65, 2585–2588. doi: 10.1271/bbb.65.2585

Valdiglesias, V., Prego-Faraldo, M., Pásaro, E., Méndez, J., and Laffon, B. (2013). Okadaic acid: more than a diarrheic toxin. *Mar. Drugs* 11, 4328–4349. doi: 10.3390/md11114328

Valerio-González, L., and Díaz-Ramos, J. (2008). Distribución de dinoflagelados epifitos potencialmente tóxicos asociados a praderas de *Thalassia testudinum* en la isla la Tortuga, la Bahía de Mochima y Golfo de Cariaco, Venezuela. *Bol. Inst. Oceanogr. Venezuela* 47, 47–58.

Vargas-Montero, M., Morales, A., and Cortés, J. (2012). Primer informe del género *Gambierdiscus* (Dinophyceae) y otros dinoflagelados bentónicos en el Parque Nacional Isla del Coco, Costa Rica, Pacífico Tropical Oriental. *Rev. Biol. Trop.* 60, 187–199.

Villareal, T., Hanson, S., Qualia, S., Jester, E., Granade, H., and Dickey, R. (2007). Petroleum production platforms as sites for the expansion of ciguatera in the northwestern Gulf of Mexico. *Harmful Algae* 6, 253–259. doi: 10.1016/j.hal.2006.08.008

Wang, D. Z. (2008). Neurotoxins from marine dinoflagellates: a brief review. *Mar. Drugs* 6, 349–371. doi: 10.3390/md20080016

Yasumoto, T., and Murata, M. (1993). Marine toxins. *Chem. Rev.* 93, 1897–1909. doi: 10.1021/cr00021a011

Yasumoto, T., Seino, N., Murakami, Y., and Murata, M. (1987). Toxins produced by benthic dinoflagellates. *Biol. Bull.* 172, 128–131. doi: 10.2307/1541612

Yépez-Rendón, J. B., Keith, I., Ramírez-Sarmiento, A. K., Chávez-Rodríguez, N. F., and Carnicer-Castaño, O. (2018). Presencia de microalgas epibentónicas en el Pacífico este tropical. *Hallazgos* 21, 1–23.

Zhang, H., Li, Y., Cen, J., Wang, H., Cui, L., Dong, Y., et al. (2015). Morphotypes of *Prorocentrum lima* (Dinophyceae) from Hainan island, South China Sea: morphological and molecular characterization. *Phycologia* 54, 503–516. doi: 10.2216/15-8.1

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Durán-Riveroll, Cembella and Okolodkov. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Marine Science

ORIGINAL RESEARCH
published: 07 May 2019
doi: 10.3389/fmars.2019.00235

Marine Dinoflagellate Assemblage in the Galápagos Marine Reserve

Olga Carnicer[1], Patricia De La Fuente[2], Antonio Canepa[3], Inti Keith[4], Eduardo Rebolledo-Monsalve[1], Jorge Diogène[5] and Margarita Fernández-Tejedor[5]*

[1] Escuela de Gestión Ambiental, Pontificia Universidad Católica del Ecuador Sede Esmeraldas (PUCESE), Esmeraldas, Ecuador, [2] Institut de Ciències del Mar, Consejo Superior de Investigaciones Científicas, Barcelona, Spain, [3] Escuela Politécnica Superior, Universidad de Burgos, Burgos, Spain, [4] Charles Darwin Research Station, Charles Darwin Foundation, Galápagos, Ecuador, [5] Institut de Recerca i Tecnologia Agroalimentària (IRTA), Sant Carles de la Ràpita, Spain

It is likely that harmful algal blooms have increased in frequency, intensity, and geographic distribution in the last decades in response to anthropogenic activities. The Galápagos Islands are renowned for their exceptional biological diversity; however, marine dinoflagellate communities have not been represented in biodiversity assessments. Therefore, this study aims to provide key information about dinoflagellate diversity and abundances, with special attention to harmful species, during a weak La Niña event in the Galápagos Marine Reserve (GMR). The study was performed during March–April 2017 and four transects were conducted at four Islands (Santa Cruz, Santa Fé, Seymour, and Pinzón) representing the southern region of the GMR. Water net samples were collected at 2, 5, and 10 nautical miles (nm) from the coast, at a total of 48 sampling sites. The presence of toxic species, and their cell abundance was estimated in seven transects at 0, 15, and 30 m of depth. A total of 152 taxa belonging to 7 orders, 22 families, and 38 genera were registered. The number of taxa found is almost three times higher than the maximum observed in previous studies. Dinoflagellate species richness among stations ranged between 53 and 23 taxa and was higher in northern sites. From the applied cluster analysis, five dinoflagellate assemblages were identified as a discrete community structure, one was found only in Santa Fé Island, which is probably related to the presence of the Equatorial Undercurrent (EUC). Regarding cell abundance estimations, low abundances were registered throughout the sampling sites and no blooms were detected. Higher abundances were registered in the northern transects coinciding with one of the most productive areas of the archipelago, situated north of Santa Cruz. Among the identified taxa, 19 of them were potentially toxic, including epiphytic species, allowing the possibility of blooms in benthic areas. This study presents the first record of several dinoflagellate species in the area (both non-toxic and harmful species) and thus, emphasizing the need for the implementation of phytoplankton monitoring programs by the government to prevent potential ecological, sanitary and economic impacts in the GMR.

Keywords: harmful algal blooms, dinoflagellate assemblages, richness, spatial variability, CCA, Generalized Additive Models, environmental parameters

OPEN ACCESS

Edited by:
Marius Nils Müller, Federal University of Pernambuco, Brazil

Reviewed by:
Celeste López Abbate, Instituto Argentino de Oceanografía (IADO-CONICET), Argentina
Pietro Martins Barbosa Noga, Federal University of Bahia, Brazil

****Correspondence:***
Olga Carnicer
olgacarnicer@gmail.com

Specialty section:
This article was submitted to Marine Ecosystem Ecology, a section of the journal Frontiers in Marine Science

Received: *01 August 2018*
Accepted: *16 April 2019*
Published: *07 May 2019*

Citation:
Carnicer O, De La Fuente P, Canepa A, Keith I, Rebolledo-Monsalve E, Diogène J and Fernández-Tejedor M (2019) Marine Dinoflagellate Assemblage in the Galápagos Marine Reserve. Front. Mar. Sci. 6:235. doi: 10.3389/fmars.2019.00235

INTRODUCTION

Marine microalgae are primary producers and constitute key components of marine food webs: they fix carbon and produce nearly 50% of the oxygen on the planet (Field et al., 1998). Most phytoplankton communities are directly affected by human activities, especially through the excess inputs of organic matter and nutrients to the system (Hallegraeff, 2010), usually linked to high phytoplankton biomass in coastal areas (Davidson et al., 2014). Moreover, phytoplankton dynamics are affected by climate change, due to altering environmental conditions such as timing of large-scale climate events (Boyce et al., 2010) or sustained warmer temperatures that increase stratification and reduce the resupply of recycled nutrients to the upper mixed layer (Doney, 2006). Consequently, physiological responses of phytoplankton species, such as phenology, abundance and calcification rate may be affected (Poloczanska et al., 2016), leading to a reduction in ocean productivity (Behrenfeld et al., 2006) and changes in bloom timing and community structure (Henson et al., 2018). Thus, the study of phytoplankton communities and its response to environmental drivers is crucial to gain a better understanding of the functioning of the marine ecosystem.

Particularly, some species can form toxic proliferations of cells, thereby causing harm to aquatic ecosystems, including the resident plants and animals, and substantial economic losses and disturbances in touristic areas (Berdalet et al., 2015). These, so called "harmful algal blooms" (HABs), can affect humans through direct exposure by skin contact or inhalation of aerosols, or by ingestion of seafood contaminated by toxins that have bio-accumulated along the food web. Examples of this are well known, for instance in the digestive tract of shellfish (mussels, clams, oysters, scallops) or finfishes (Berdalet et al., 2015). Dinoflagellates are of particular interest among the harmful phytoplankton species because of their high species richness, morphological diversity and strategies for adaptation to thrive in different ecological niches (Smayda and Reynolds, 2003). Most dinoflagellates have cyst-forming stages, which are in a dormant state until environmental conditions, such as temperature, salinity, light intensity or turbulence, favor their growth, resulting in abundant proliferations in a short period of time (Delwiche, 2007). In addition, there is a high number of mixotrophic dinoflagellate species, whose predatory behavior enables them to increase their nutrient uptake, allowing them to survive under conditions that are unfavorable for strict autotrophs (Stoecker, 1999). From an ecotoxicological point of view, dinoflagellates are of vital importance since they show the highest representation among toxic phytoplankton with 99 species, in contrast with the number of diatom species (29), Haptophytes (8), Raphidophyceans (4), Dictyochophyceans (3), Pelagophyceans (2), and Cyanobacteria (35) (Moestrup et al., 2009; IOC-UNESCO Taxonomic Reference List of Harmful Microalgae). These features, mentioned above, make dinoflagellates crucial to marine ecosystems.

Most dinoflagellates' toxins are neurotoxic, but others cause specific poisoning syndromes including gastrointestinal disturbances (diarrhea, nausea, vomiting), muscle paralysis, or amnesia (Hallegraeff, 2003). The most frequent and studied syndromes caused by dinoflagellate toxins are Paralytic Shellfish Poisoning (PSP), caused by *Alexandrium* spp., *Gymnodinium catenatum*, and *Pyrodinium bahamense*; Diarrhetic Shellfish Poisoning (DSP) produced by *Dinophysis* spp. and *Prorocentrum* spp. Poisoning syndromes not only come from planktonic species, but also from benthic species. For example, *Gambierdiscus* spp., can cause ciguatera fish poisoning, a disease with worldwide impact, though mainly in tropical zones (Parsons et al., 2012), and *Ostreopsis* cf. *ovata* blooms that have been seen to affect human health directly by inhalation of marine aerosols that cause respiratory irritation (Vila et al., 2016).

Nowadays, there is a big concern about proliferations of toxic species that may be increasing in frequency, and expanding their biogeographic distribution under future global warming scenarios (Hallegraeff, 2010; Glibert et al., 2014; Wells et al., 2015). Thus, it is important to survey ecological hotspots such as the Galápagos Marine Reserve (GMR), which is one of the largest and most biologically diverse marine regions in the world (Schaeffer et al., 2008; Liu et al., 2014). Its great diversity derives from the situation of the GMR lying in a complex transition zone between tropical and subtropical waters and intense equatorial and local upwelling (Palacios, 2004; Liu et al., 2014). The GMR area is largely affected by oceanic–atmospheric perturbations such as the seasonal migration of the Intertropical Convergence Zone (ITCZ) and the El Niño Southern Ocean Oscillation (ENSO) (Palacios, 2004; Liu et al., 2014). During the dry season (July–December), the ITCZ is north of the equator, south trade winds increase, and sea surface temperature (SST) diminishes. In turn, in the warm, wet season (December–June), the ITCZ migrates southward (toward the equator), the northeast trade winds become prevalent and precipitation and SST increases. In general, during El Niño events, warmer surface conditions and the deepening of the thermocline inhibit the upwelling of cooler, nutrient-rich subsurface waters to the surface. As a consequence, during an ENSO, distributions of phytoplankton communities undergo large-scale disruptions and chlorophyll-a (Chl a) levels diminish (McCulloch, 2011). For example, in the equatorial Pacific Ocean, during the strong ENSO event in 1997/1998, Chl a reached a very low concentration (Chávez et al., 1999), which may have influenced higher trophic levels. During La Niña events, the cool ENSO phase, strong winds and cooling of ocean temperatures cause an elevation of the thermocline that increases nutrient supply and phytoplankton productivity along the equator (Ryan et al., 2002, 2006). In addition, there is some evidence that climate change may influence ENSO to some extent (Sachs and Ladd, 2002; Cai et al., 2014), resulting in unpredictable changes in phytoplankton assemblages and HAB frequencies.

There is little data regarding taxonomic characterization of phytoplankton assemblages in the coastal area of the Eastern Tropical Pacific (ETP), thus, the diversity of phytoplankton species remains poorly described. Regarding HABs, isolated studies have documented the prevalence of *Gymnodinium catenatum, Akashiwo sanguinea*, and *Alexandrium* spp., among others (Colombia – Giraldo et al., 2014; Costa Rica – Vargas-Montero et al., 2008; Calvo-Vargas et al., 2016; South of Mexico – Maciel-Baltazar, 2015). The Oceanographic Institute of the Ecuadorian Navy (INOCAR) has conducted sporadic cruises in

the GMR since 1968. Unfortunately, a reduced number of reports are available to the public and most are written in Spanish (Torres and Tapia, 2000, 2002; Torres, 2002; Tapia and Naranjo, 2012; Torres and Andrade, 2014; Naranjo and Tapia, 2015). Toxic species such as *Dinophysis* spp., *Prorocentrum mexicanum*, and *Ostreopsis* cf. *siamensis* have been registered, however, the number of species indicated within the reports is greater than the list of taxa supplied, thus, there is an under-representation of the presence of dinoflagellates in the GMR. Moreover, Torres (2015) reviewed fish mortality and algae proliferation events from 1968 to 2009 along the Ecuadorian coast and in the GMR, reporting *Prorocentrum gracile* in some bays of the GMR, associated with fish mortalities in 1980.

The absence of a systematic monitoring program leads to a lack of scientific data to evaluate phytoplankton communities and the future risk of HABs in the GMR (Kislik et al., 2017). Considering the unique features of dinoflagellates and their relevance among HAB species, the aim of this study is to give insights into the dinoflagellate community of the GMR, specifically focusing on the island of Santa Cruz and nearby islands, with special attention to toxic species, during a weak La Niña phase in the wet season (March–April, 2017).

MATERIALS AND METHODS

Study Area

The GMR is located in the ETP, ∼900 km west from the coast of Ecuador. The GMR area is influenced by cold and saltier Equatorial Surface Waters (ESW) with temperature (T) < 25°C and salinity (S) > 34, and by the warm and fresher Tropical Surface Waters (TSW) (T > 25°C, S < 34) (Fiedler and Talley, 2006; Sweet et al., 2007). The main currents affecting the GMR are the westward South Equatorial Current (SEC), which drives surface waters over the entire region around the Galápagos (Kessler, 2006; Schaeffer et al., 2008) and the upwelled waters of the EUC, developing a sub-surface (sub-thermocline) compensation against the westward SEC (Schaeffer et al., 2008). The collision of the subsurface iron and nutrient-carbon rich EUC with the Galápagos platform results in topographically induced local upwelling areas, which favor an enhanced production in many areas of the archipelago (Palacios, 2004; Schaeffer et al., 2008; Kislik et al., 2017).

The study took place in the southern area of the GMR (**Figure 1**) during the warm – wet season (March–April, 2017) coinciding with a weak La Niña phase. During this season, the EUC becomes stronger, shallower, and with higher salinity values than during the dry season (Sweet et al., 2007). Another source of nutrients in the GMR is partially attributed to the Island Mass Effect (IME) where iron provided by continental sediments, together with topographic upwellings, contribute to an increase in Chl a concentration (Kislik et al., 2017).

Sample Collection

Sampling was performed in four islands; Pinzón (27th March), Santa Fé (28th March), Santa Cruz (6th April), and Seymour (7th April). At each site, four transects were covered (A, B, C, D) and for each transect, three sampling stations were selected at 2, 5, and 10 nm from the shore (**Figure 1**). In every station, vertical net water samples (30 m) were collected in 200 mL plastic bottles using a 20 μm mesh plankton net, then samples were preserved in neutral Lugol's solution (final concentration

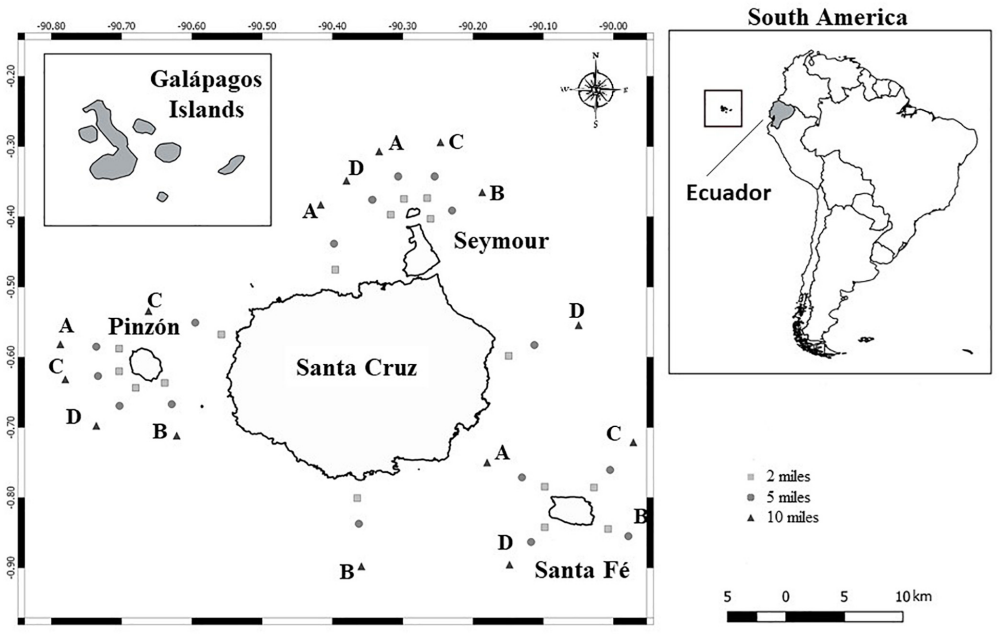


FIGURE 1 | GMR sampling sites: Pinzón, Santa Fé, Santa Cruz, and Seymour Islands. Four transects were performed in each Island at 2, 5, and 10 nm from the coast.

of 0.4%). These vertical net samples were used to identify the presence of dinoflagellates in each sampling site. In addition, seven transects (Santa Fé A, B; Pinzón D; Seymour A, B; Santa Cruz A, D) were selected based on the presence of toxic species found in net samples, for phytoplankton abundance estimation. To do this, at each sampling station (2, 5, and 10 nm) three water samples using a Van Dorn bottle were collected at surface, 15 and 30 m depth, then preserved in neutral Lugol's solution (final concentration of 0.4%).

Environmental Parameters

Vertical profiles were obtained using an EXO2 Multiparameter Sonde from Yellow Springs Instrument Company (YSI Inc.) equipped with sensors for temperature, conductivity, pressure, dissolved oxygen and pH, by deploying (at each station) from the surface down to 30 m depth. Only the downcast was selected for processing the data.

Phytoplankton Identification and Abundance Estimation

Species identification in vertical net samples was performed from 50 mL aliquots settled for 24 h and observed thoroughly under an inverted microscope (Nikon Eclipse TE2000-S).

Cell abundance estimation was performed from 50 mL of Van Dorn water samples settled in Utermöhl chambers (Utermöhl, 1958) during 24 h. Cell counting was performed under an inverted microscope (Nikon Eclipse TE2000-S), where the entire bottom of the chamber was examined at 200× magnification. To enumerate the small and more abundant organisms, one or two transects were counted at 200× or five/ten fields randomly chosen at 400× until reaching 100 cells.

The Utermöhl method is widely used, it enables identification and quantification of phytoplankton samples (Intergovernmental Oceanographic Commission [IOC], 2010), and it is recommended for phytoplankton samples with low abundance (Hallegraeff, 2003). The method has been standardized (CEN, 2006) and it is frequently used in research and monitoring programs for quantitative phytoplankton analysis (e.g., Reguera et al., 2016; Wasmund, 2017).

Species identification was based on Steidinger and Jangen (1997), Hoppenrath et al. (2009, 2014), Omura et al. (2012), and Lassus et al. (2016). The validity of names of the different taxa was checked on the World Register of Marine Species (Horton et al., 2018), the list of toxic species was checked on the Taxonomic Reference List of Harmful Micro Algae (Moestrup et al., 2009). Those species identified only at the genus level, but with clear morphological differences, were listed as sp.1, sp.2, etc.

Data Analysis

Exploratory data analysis (EDA) was conducted to both, environmental and biological databases previous to any statistical analysis. The EDA allowed us to confirm general assumptions of the 'family' distribution of the variables (i.e., binomial distribution for presence–absence), the presence of homoscedasticity, the independence of the values (i.e., lack of spatial autocorrelation) and collinearity (i.e., correlation among explanatory variables), as suggested by Zuur et al. (2010). Before fitting regression models all the oceanographic variables which showed a Pearson correlation coefficient (rho) higher than 0.75 under the collinearity analysis, were dropped from the model (**Supplementary Figure S1**).

Dinoflagellate community analysis considered the calculation of the species richness over the net samples. From an ecological perspective we looked for discrete groups (clusters) of species composition over the sampling area. To achieve this, the Sorensen dissimilarity matrix for presence–absence (binary) data (Gower and Legendre, 1986) was obtained using the *"dist.binary"* function from the ade4 package (Dray and Dufour, 2007). Then, a hierarchical clustering was performed using the Ward's minimum variance method by the use of the *"hclust"* function and *"method = ward.D2"* argument in the stats package (R Core Team, 2018; version 3.4.4).

In order to evaluate the role of environmental variables over the community structure of the most common species observed in net samples (frequency of occurrence higher than 75%), a canonical correspondence analysis (CCA) (ter Braak and Verdonschot, 1995) through the *"cca"* function of the vegan package (Oksanen et al., 2018) was fitted. This multivariate constrained ordination technique allows us to include unimodal relationships inside the correspondence analysis since the response of species to abiotic variables typically follows an unimodal response (Greenacre and Primicerio, 2013). To evaluate the significance of the CCA model the *"anova.cca"* function (from the vegan package, Oksanen, op.cit) which performs an ANOVA like permutation test using 999 permutations (Borcard et al., 2018) was used.

From a species-specific perspective, the effects of environmental variables over the presence probability were assessed for three toxic species present in net samples. *Dinophysis caudata* was selected considering the magnitude of the damage caused by their blooms (Reguera et al., 2014) and the high occurrence in water samples (see section "Harmful Species"). *Karlodinium* spp. was the potentially toxic genus observed in highest abundances in Van Dorn samples (see section "Harmful Species"). Finally, considering its benthic origin, *Ostreopsis* cf. *ovata* was studied as well.

Due to the binomial nature of the data in net samples (presence/absence) the Generalized Additive Logistic Models (GALoM) were fitted. The implementation of the "GALoM" models was done using the mgcv package through the *"gam"* function and the *"family"* argument defined as binomial (Wood, 2017). The model selection followed a backward selection method, based on the significance of each explanatory variable and using the Akaike's Information Criterion (AIC). This criterion negatively penalizes excess parameters, preventing from over-parameterization and allows for multimodel comparison where lower AIC values represent more parsimonious models.

All the statistical analysis and visualizations were conducted with the R software (R Core Team, 2018; version 3.4.4) and supervised by OneMind-DataScience[1].

[1]https://onemind-datascience.com

RESULTS

Hydrography and Environmental Variables

The spatial distribution of SST and sea surface salinity (SSS) (<5 m depth) was practically uniform in the area of study, with mean SST of 27.7°C, and with mean SSS of 34.5. The vertical profiles were generally characterized by the presence of an upper mixed layer with a varying thickness delimited by a sharp thermocline, halocline and oxycline (**Supplementary Figures S2–S4**).

Below the mixed layer, temperature and dissolved oxygen declined with depth and salinity tended to increase (**Supplementary Figures S2–S4**). In this study, a subsurface salinity maximum was revealed in several profiles at different depths, showing peaks of salinity maxima ($S > 35.5$) in Santa Fé (transect A, B, and D) and Santa Cruz Island (transect, B), while the higher subsurface salinity value ($S = 36.5$) was registered in Santa Fé Island, transect A, 5 nm, at about 13 m depth (**Supplementary Figure S2**).

A slight increase in dissolved oxygen concentrations was observed at depths where such subsurface maximum of salinity was present (**Supplementary Figure S4**). The lowest salinity value ($S < 34.0$) belongs to Santa Cruz (B, 10 nm from the coast) at surface (0 m depth). The mean surface pH value was homogeneous for the study area with values about 7.9. The pH profiles (**Supplementary Figure S5**) followed similar trends as those of temperature and dissolved oxygen, even though showing higher variability (**Supplementary Figure S5**).

Dinoflagellate Community

A total of 152 dinoflagellate taxa were identified in net samples, belonging to 7 orders, 22 families, and 38 genera. The most diverse genus was *Tripos* (25 species), followed by *Protoperidinium* (22 species), *Dinophysis* (13 species), *Oxytoxum* (9 species), and *Phalacroma* (7 species) (**Supplementary Table S1**). The taxa described as *Gonyaulax* spp., *Scrippsiella* spp. and *Heterocapsa* spp. were found in all stations, as well as *Gonyaulax spinifera*. Only 12 taxa were found in 75% of the samples, whereas 100 species appeared in 25% of them. Species with more occurrence were *Tripos furca* (88%), *Tripos muelleri* (79%), *Oxytoxum tesselatum* (85%), *Podolampas bipes* (85%), and *Protoperidinium steinii* (83%).

Regarding cell abundance estimation from Van Dorn bottles samples, no blooms were detected but higher abundances were registered in Seymour Island, especially in transect B (2 nm surface), with maximum values registered for *Heterocapsa* spp. (5,832 cell L^{-1}) and *Protoperidinium pellucidum* (4,000 cell L^{-1}) (**Supplementary Table S2**).

Dinoflagellate species richness varied among stations and ranged from a maximum of 53 to a minimum of 23 taxa per station (**Figure 2**). The lowest number of species was recorded at Santa Fé Island showing a maximum of 29 and a minimum of 23 taxa per station.

From the community structure analysis, a total of five discrete community composition groups were found. These assemblages showed a contrasting spatial distribution (**Figure 3**) with groups 4 and 5 being more representative of the coastal areas of Pinzón, Santa Cruz, and Seymour.

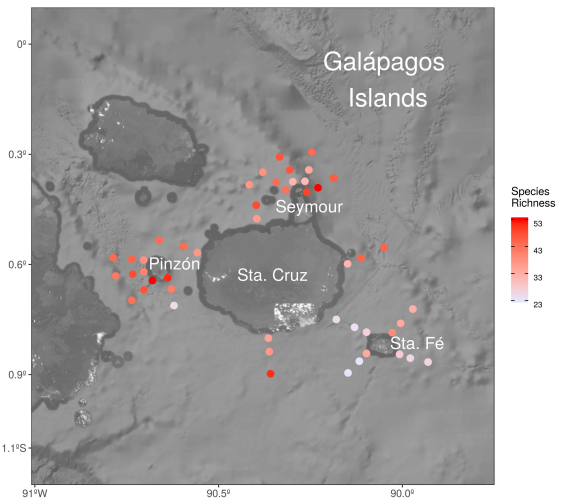


FIGURE 2 | Spatial (horizontal) distribution of the species richness in the area of study.

From the CCA analysis (based in the most frequent species), the total inertia (i.e., total variance in species distributions) was 0.49, representing the 100% of total inertia in the model. From this, the total variance explained by the environmental variables (i.e., constrained inertia) was low (8.4%) even though significant ($F = 1.346$, p-value < 0.05). The first two axes explained 7.2% of the constrained inertia with a total of 4.7% and 2.5% being explained by the first and second axes, respectively (**Figure 4**).

All the species represented in the CCA diagram, showed a homogenous distribution across the environmental variables, with some exceptions. A negative association with temperature was found for *Protoperidinium conicum*, *Dinophysis caudata*, and *Prorocentrum compressum*. In contrast, a positive relationship with temperature was found for *Protoperidinium pellucidum*, *Karlodinium* spp., and *Tripos fusus*; even though *Karlodinium* spp. and *T. fusus* are closer to a unimodal response with temperature (**Figure 4**). In relation with salinity, the species that showed a positive relationship were *Podolampas bipes*, *Ceratocorys horrida*, *Podolampas palmipes*, and *Histioneis costata*. However, a negative relationship was found for the following species: *Tripos lineatus*, *Tripos candelabrum*, *Cucumeridinium coeruleum*, and *Ostreopsis* spp. For *Ostreopsis* spp. this pattern is consistent with the GALoM model results (next section, **Figure 5**). No clear association between the species composition and pH were found.

Harmful Species

Nineteen potentially harmful taxa were identified in net samples (**Table 1**). The most frequent were *Gonyaulax spinifera*, that was present in all samples, *Phalacroma mitra*, which was present in 63% and followed by *Dinophysis caudata* that was found in 46% of the samples. Benthic species were also present in the water

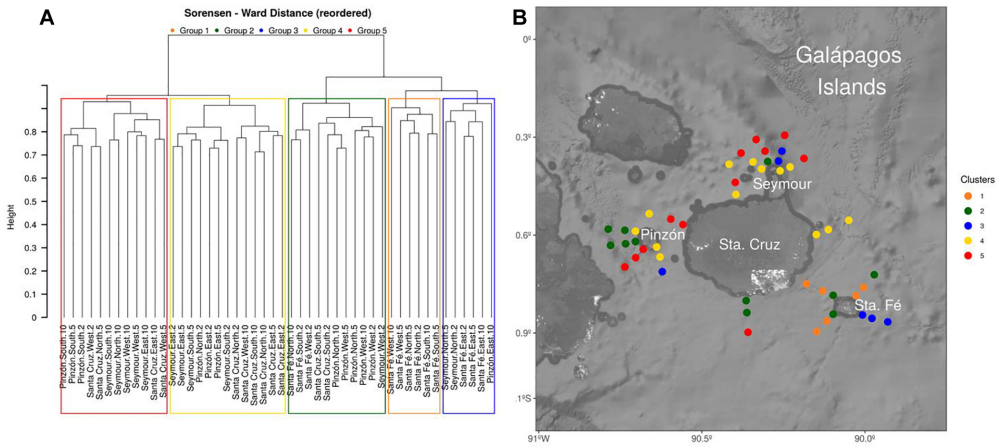


FIGURE 3 | (A) Hierarchical clustering of stations showing the final dendrogram with the five discrete groups and **(B)** spatial (horizontal) distribution of the five discrete dinoflagellates assemblages (clusters) found in the area of study.

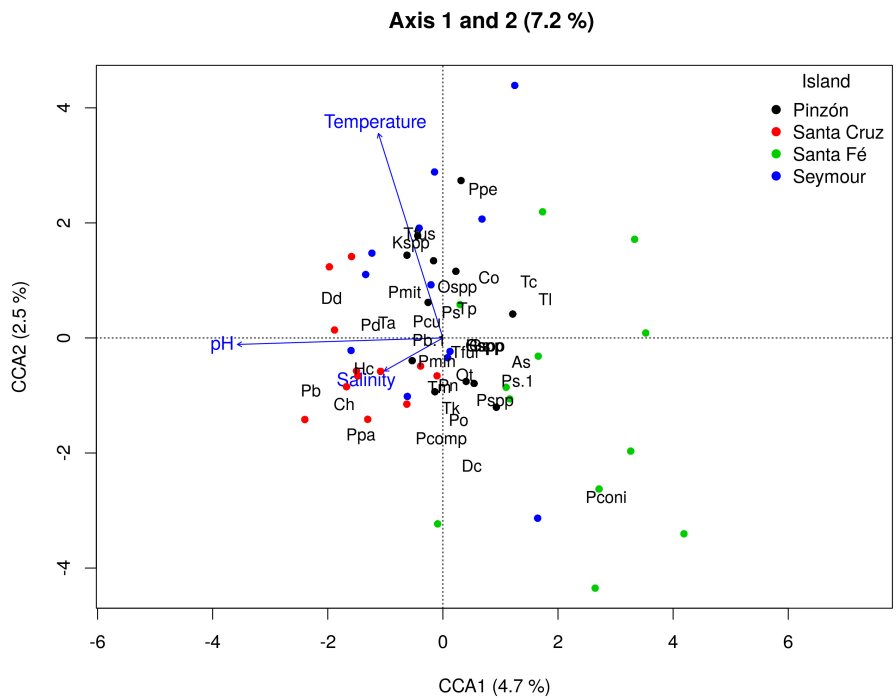


FIGURE 4 | Canonical correspondence analysis triplot. Sampling stations shown as colored dots according to each Island. The species present in the community follows: **Dd**, *Dinophysis doryphora*, **Kspp**, *Karlodinium* spp., **Pconi**, *Protoperidinium conicum*, **Ppe**, *Protoperidinium pellucidum*; **Dc**, *Dinophysis caudata*; **Ospp**, *Ostreopsis* spp.; **Pcu**, *Protoperidinium curtipes*; **Tfus**, *Tripos fusus*; **Pd**, *Protoperidinium diabolus*; **Ps**, *Podolampas spinifera*; **Po**, *Protoperidinium oviforme*; **Co**, *Cucumeridinium coeruleum*; **Hc**, *Histioneis costata*; **Pn**, *Ptychodiscus noctiluca*; **Pmit**, *Phalacroma mitra*; **As**, *Akashiwo sanguinea*; **Pb**, *Protoperidinium brochii*; **Pmin**, *Protoperidinium minutum*; **Tc**, *Tripos candelabrum*; **Ch**, *Ceratocorys horrida*; **Ppa**, *Podolampas palmipes*; **Ta**, *Tripos azaricum*; **Tl**, *Tripos lineatus*; **Tp**, *Tripos pentagonum*; **Tk**, *Tripos kofoidii*; **Pcomp**, *Prorocentrum compressum*; **Pspp**, *Pyrophacus* spp.; **Tm**, *Tripos muelleri*; **Ps**, *Protoperidinium steinii*; **Ot**, *Oxytoxum tesselatum*; **Pb**, *Podolampas bipes*; **Tfur**, *Tripos furca*; **Gs**, *Gonyaulax spinifera*; **Gspp**, *Gonyaulax* spp.; **Sspp**, *Scrippsiella* spp.; **Hspp**, *Heterocapsa* spp. Oceanographic (explanatory) variables are indicated in blue [temperature (°C), salinity, pH]. The percentage of variability (inertia) explained is indicated on each axis.

column: *Ostreopsis* cf. *ovata* (46%), *Ostreopsis* cf. *lenticularis* (4%), *Prorocentrum lima* (17%), and *Coolia* spp. (17%) (**Table 1**).

No HABs were detected in Van Dorn bottle samples. The highest abundance corresponded to an ichthyotoxic genus, *Karlodinium* spp. (3,940 cell L^{-1}) that was registered in transect A (2 nm, 10 m depth) at Seymour Island. Other harmful species were recorded in very low abundances (<280 cell L^{-1}), such as *Gonyaulax spinifera, Porocentrum micans, P. lima, P. cordatum, P. mexicanum, Karenia* spp., *Karenia papilionacea,* and *Alexandrium* spp. (**Supplementary Table S2**).

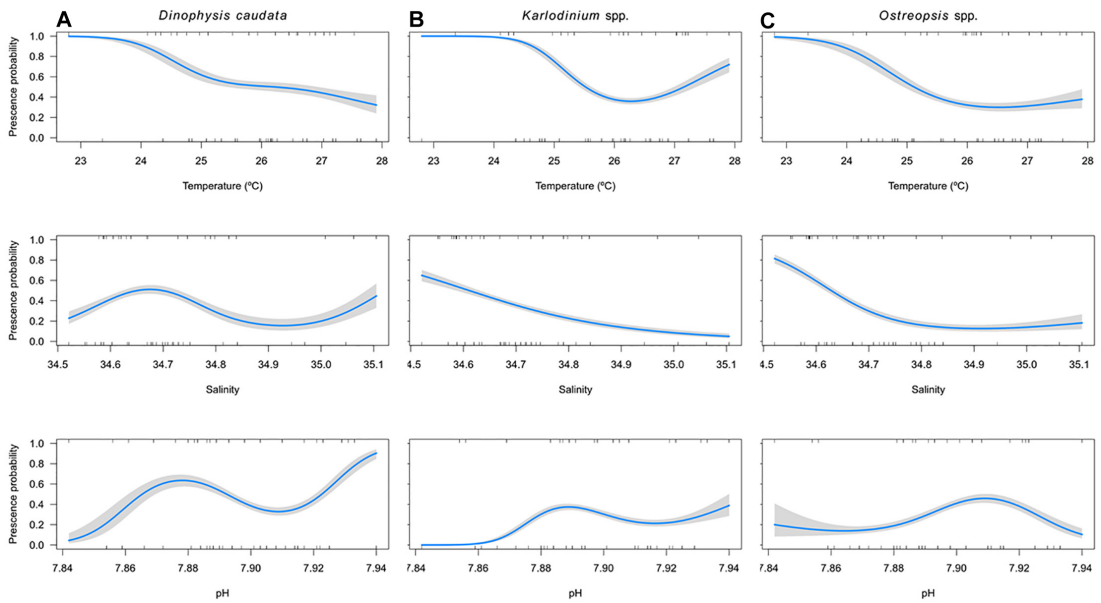


FIGURE 5 | Environmental association of **(A)** *Dinophysis caudata*, **(B)** *Karlodinium* spp., and **(C)** *Ostreopsis* spp. to temperature (°C), salinity and pH (upper, central, and lower panel, respectively). Central (blue) line represents the fitted GALoM model where the presence/absence probability is modulated for different environmental variable values, while keeping the rest variables at constant (average) values. Gray (shaded) areas represents the confidence intervals (i.e., uncertainity) associated to the fitted models.

TABLE 1 | Harmful species found in the water samples.

Harmful specie	Syndrome (toxins)	% occurrence
Alexandrium spp.	PSP (saxitoxin and analogs)	38
Dinophysis caudata Saville-Kent, 1881	DSP (okadaic acid and dinophysistoxins)	46
Dinophysis fortii Pavillard, 1923		6
Dinophysis infundibulum J.Schiller, 1928		6
Phalacroma mitra F.Schütt, 1895		63
Phalacroma rotundatum (Claparéde and Lachmann) Kofoid and Michener, 1911		25
Prorocentrum lima (Ehrenberg) F.Stein, 1878		17
Prorocentrum mexicanum Osorio-Tafall, 1942		17
Gonyaulax spinifera (Claparède and Lachmann) Diesing, 1866	Currently not associated with human illnesses (yessotoxin and analogs)	100
Lingulodinium polyedra (F.Stein) J.D.Dodge, 1989		8
Prorocentrum reticulatum M.A.Faust, 1997		4
Ostreopsis cf. *lenticularis* Y.Fukuyo, 1981	Palytoxin associated syndrome (palytoxin; ovatoxin and analogs; mascarenotoxins; ostreocins)	4
Ostreopsis cf. *ovata* Y.Fukuyo, 1981		46
Karenia papilionacea A.J. Haywood and K.A. Steidinger, 2004	NSP (brevetoxin and analogs)	2
Karenia spp.		23
Coolia spp.	Currently not associated with human illnesses (cooliatoxin)	17
Cochlodinium spp.	Ichthyotoxic	2
Karlodinium spp.		44
Prorocentrum cordatum (Ostenfeld) J.D.Dodge, 1975		19

PSP, Paralytic Shellfish Poisoning; DSP, Diarrhetic Shellfish Poisoning; NSP, Neurotoxic Shellfish Poisoning.

For *Dinophysis caudata* the GALoM model explained 7.94% of the deviance and the presence probability was significantly correlated with all the variables, showing a clear negative association with temperature (χ^2 = 106.2, *p*-value < 0.001) and a non-lineal positive response with salinity and pH (χ^2 = 60.9, *p*-value < 0.001 and χ^2 = 122.2, *p*-value < 0.001, respectively; **Figure 5A**).

For *Karlodinium* spp. the model explained 17.3% of the deviance and the presence probability was significantly correlated with all the variables. The effects of temperature over presence

probability showed a unimodal response, with the lower probability at about 26.5°C, thereafter the probability started to increase (χ^2 = 238.2, p-value < 0.001). In the case of salinity, the response showed a negative linear decrease with higher values of salinity (χ^2 = 157.6, p-value < 0.001). The effects of pH showed a positive non-lineal relationship (χ^2 = 192.1, p-value < 0.001; **Figure 5B**).

In the case of *Ostreopsis* cf. *ovata* the model explained 13% of the total deviance and the presence probability diminished significantly with temperature and salinity (χ^2 = 103.1, p-value < 0.001 and χ^2 = 183.2, p-value < 0.001, respectively). Additionally, the presence probability showed a unimodal response with pH (χ^2 = 104.7, p-value < 0.001), with the highest values found at pH about 7.9 (**Figure 5C**).

DISCUSSION

Dinoflagellates Assemblages

The number of taxa found in the present study is almost three times higher than the maximum observed in all the previous studies (Marshall, 1972; Torres and Tapia, 2000, 2002; Torres, 2002; Tapia and Naranjo, 2012; Torres and Andrade, 2014; Naranjo and Tapia, 2015), with 102 dinoflagellate taxa detected for the first time in the GMR. Some of the specimens, in this study, were only identified to genus level. Identification of armored dinoflagellates can be improved by the use of fluorescent stains such as calcofluor, which is an aid to examine the thecal plate pattern. Another method to improve identification consists in adding a drop of a diluted (5%) solution of sodium hypochlorite (bleach) applied to the edge of the coverslip which can be used for the separation of the dinoflagellate thecal plates (Tomas, 1997). These methodologies were not applied to the phytoplankton samples collected in the GMR in the present study. The use of these methods would probably have contributed to a deeper taxonomic identification of some of the specimens resulting in a longer list of species identified.

In addition, this study constitutes the first approach to the study of dinoflagellate assemblages in the GMR, since former studies have focused only on identification and estimation of cell abundance. The study was performed during a precise temporal sampling period corresponding to the wet season (March–April) during a weak La Niña event. Further discussion will be limited to the dinoflagellate community assemblages, its spatial variability and the response of specific species to the environmental conditions under this temporal span.

One of the first studies performed in the archipelago was done by Marshall (1972), covering 25 stations at seven depths in August 1968, during a moderate El Niño event. A total of 26 dinoflagellate taxa were reported at very low concentrations with a maximum of 326 cell L^{-1} in a station located north of Santa Cruz Island. Similarly, in August 2000, during the dry season and under the influence of a weak La Niña event, Torres and Tapia (2002) found 36 species of dinoflagellates during an expedition in the GMR and reported a high Chl a concentration north of Santa Cruz Island. This area has been previously defined as one of the most productive habitats in the GMR (Schaeffer et al., 2008), probably supported by topographic upwellings of the EUC and by the island-derived iron enrichment due to the IME (Palacios, 2002). This spatial abundance distribution coincides with the present study, where not only higher dinoflagellate abundances were observed in northern sites (North Seymour and Santa Cruz), but also the dinoflagellate richest stations (up to 53 taxa). Furthermore, Santa Cruz Island is considerably larger in size than the other islands. Biogeographically, there can be influenced differently either by the nutrient discharge or composition, depending on currents and winds. Those factors should be taken into account in future investigations in the area to study their contribution to the dynamics of dinoflagellate communities.

In addition, in Santa Fé Island, which is located of south of Santa Cruz Island, the lowest dinoflagellate richness was observed. Moreover, a particular community assemblage (group 1 in **Figure 3B**), not observed in the rest of islands, was present in this area. This particular dinoflagellate composition found at Santa Fé Island could be related with special features of this area, characterized by the presence of high-salinity waters likely associated with the cooler, carbon-nutrient rich EUC (**Supplementary Figures S2**, **S3**). Regarding specific species, *Protoperidinium conicum* was linked with Santa Fé sites, being isolated from the rest of the species in the CCA analysis and related to cold waters (**Figure 4**). However, further investigation should be addressed to this area in order to identify potential species associations to different environmental conditions, representative of the entire niche occupied by the species, since the present study includes only one seasonal period under particular climatic perturbations like the presence of a weak la Niña event.

Reports performed by the INOCAR highlight consistent diatom dominance over other groups in phytoplankton communities in the GMR. Chávez et al. (1996) reported low phytoplankton biomass in the ETP, where more precisely, open ocean waters were dominated by small and solitary organisms such as dinoflagellates, while diatoms were associated with upwelling and the Equatorial Front. Moreover, during La Niña in May 2007, McCulloch (2011) reported a dominance of pelagophytes, haptophytes, euglenophytes, and chrysophytes relative to Chl a in the phytoplankton community of the archipelago. Thus, it is probable that these groups were dominant over dinoflagellates in the present study, which was conducted during a weak La Niña, explaining in part, the low dinoflagellates abundances.

There is scientific evidence that ENSO events explain, to a certain extent, Chl a concentration along the GMR (Kislik et al., 2017). During La Niña events, a shallower thermocline increases macronutrients supply (Chávez et al., 1996), however, it has not been linked with an increase in phytoplankton abundances in the ETP (Strutton et al., 2008). The study conducted by Kislik et al. (2017) pointed out that from 2003 to 2016, only one high Chl a concentration out of six, coincided with a La Niña event in the GMR. During this period of time, the variability in Chl a concentration found across the GMR occurred a few months later than the SST highest anomalies were registered. It is thus possible that Chl interannual variability could be partly explained by annual post-ENSO events. As the present study occurred

during a weak La Niña event, the low dinoflagellate abundances registered could be expected based on this statement. In addition, during the wet season, warmer waters from the Panama Current flow southward, southeast trade winds weaken, ITCZ migrates to the south and rainfall increases (Palacios, 2002; Edgar et al., 2004; Kessler, 2006; McCulloch, 2011; Kislik et al., 2017). Under those conditions, a low Chl a level has been observed, more pronounced in the far northern Islands, Darwin and Wolf (Kislik et al., 2017).

Moreover, changes in the composition and distribution of phytoplankton are not only due to seasonal sub-surface variations driven by large marine currents, but also due to the influence of local winds and local currents, different tidal inflows, mixing and sediment resuspension the long-distance larval transport capabilities of species, among other factors (Chávez et al., 1990; Lafabrie et al., 2013; Venrick, 2015; Anabalón et al., 2016; Jacox et al., 2016; Zhao et al., 2018). This makes the characterization and evaluation of the phytoplankton distribution a more complex process.

Harmful Algae

In this study, we registered the highest number of harmful dinoflagellates ever recorded in the GMR, with a total of 19 species (**Table 1**). The only other study focused on HABs in Ecuador reported proliferations of two species of diatoms, *Mesodinium rubrum* and *Bellerochea malleus* in the GMR (Torres, 2015). In addition, a bloom of *Prorocentrum gracile* (reported as *P. gracilis*) was associated with a fish mortality event in the archipelago in 1980, however, in the present study, this species was not observed.

Furthermore, Torres and Andrade (2014) in a study conducted during the dry season in September 2006, in Aeolián Bay (Baltra Island, south of Seymour Island), registered harmful species such as *Prorocentrum* cf. *mexicanum* and *Ostreopsis* cf. *siamensis*. The morphological identification of *Ostreopsis* species is very difficult since they overlap in size and have the same tear-drop shape (Penna et al., 2005). In the present study, identification of *O.* cf. *lenticularis* and *O.* cf. *ovata* is supported by molecular phylogeny and SEM images from epiphytic samples taken during the same period (Carnicer et al., in preparation). The presence of benthic species was also reported in a phylogenetic study on *Prorocentrum lima* (Nagahama et al., 2011) which included strains from Santa Cruz Island; unfortunately, the location was not specified. The present study also reported three other benthic taxa in the water column, *O.* cf. *lenticularis, P. lima,* and *Coolia* spp., with the dominant species being *O.* cf. *ovata* (46% occurrence). Their presence may be in response to blooms that occur on the substrate in the surrounding areas (Carnicer et al., 2015). However, the knowledge of epiphytic dinoflagellates assemblages and their blooming behavior along the GMR is not known.

Blooms of *O.* cf. *ovata* occur when a threshold temperature (25°C) is achieved during summer in the Mediterranean Sea, probably linked to cyst germination (Accoroni et al., 2015). In the Galápagos, the SST variability over the year (Schaeffer et al., 2008) is similar to temperate areas. During 2017, in Charles Darwin Research Station located in Academy Bay on Santa Cruz Island, a difference of 8.2°C was registered between April (27.3°C) and August (19.1°C). Thus, in the present study, this temperature variability may have contributed to *O.* cf. *ovata* bloom formations during the warm season. The environmental parameters used for the calibration of the GALoM model have to be taken with caution considering the benthic origin of this species. However, the statistical analysis showed that *Ostreopsis* spp. has a negative relationship with both, temperature and salinity. Other studies have found similar results, for instance those studies conducted in the Florida Keys and Hawaiian Islands (Parsons et al., 2012 and references therein). Few studies exist about the influence of pH over *Ostreopsis* spp. Di Cioccio et al. (2014) through their study about the possible effects of pH decrease on benthic HABs pointed out that *O.* cf. *ovata* seems to be tolerant to a wide range of pH values. In the present study, the pH values showed a narrow variability with the response of *Ostreopsis* spp. being unimodal with the highest occurrence probability at a pH about 7.9. Because the already cited work of Di Cioccio et al. (2014) was carried out in a much more dynamic scenario with respect to pH [stations located along marked pH gradients (6.8–8.1)], we can no longer compare and/or discuss their findings.

In the present study, the first record of *Karlodinium* spp. in the GMR is reported. There are at least 12 species described within the genus *Karlodinium* (Luo et al., 2018). Some species have been associated to fish kills: *K. armiger* (Garcés et al., 2006), *K. australe* (Lim et al., 2014), *K. conicum* (de Salas et al., 2008), *K. corsicum* (Paulmier et al., 1995), *K. gentienii* (Nézan et al., 2014), and *K. veneficum* (Place et al., 2012). The water column stratification has been suggested as the main factor that favors blooms of *Karlodinium* species such as *K. veneficum* (Place et al., 2012), as well as the abundance of preys (Lin et al., 2018). The stratification of the water column due to warm conditions and rising precipitation, a property of wet season, could benefit the presence of *Karlodinium* in the GMR area. Relative to the pH variable, the statistical analysis showed that the probability of presence of *Karlodinium* spp. rises with pH $>$ 7.8. A study of a bloom of *K. australe* conducted in west Johor Strait, Malaysia, by Lim et al. (2014) showed that the pH values found during the period of bloom in the strait was also $>$7.8. Those authors theorize the existence of a relation between the outbreak of *K. australe* blooms and the influx of nutrients mainly from anthropogenic activities from nearby areas. Under this scenario, further studies are required to shed light on the positive response of *Karlodinium* spp. to some pH values in order to known if this response corresponds to anthropogenic or natural forces. The abundance of *Karlodinium* spp. detected in the present study (5×10^4 cell L^{-1}) was low in comparison to the abundances that can be reached during bloom events in different areas of the world (Place et al., 2012; Lim et al., 2014). The fact that this study was conducted during a weak La Niña event could have contributed to a lower probability of blooms of *Karlodinium* spp. due to the stratification conditions being reduced and SST and precipitation being lower.

Dinophysis caudata is a neritic species associated to production of DSP toxins (Reguera et al., 2014). In this study, *D. caudata* presented a 46% of occurrence (**Table 1**), being one of the most common species in the GMR. The presence of this species was associated with temperate and tropical waters, as previously cited by Kofoid and Skogsberg (1928). A negative association with higher pH and temperature values, together with

the increase in the probability of presence of *D. caudata* to higher salinities (**Figure 5A**), could indicate local upwellings associated to the presence of *D. caudata*. The presence of *D. caudata* in the ETP is limited to Mexico, where a monitoring program has been running since the 1990s (Daguer et al., 2018). This species has been reported in the Gulf of California (Esqueda-Lara et al., 2013) and in Chiapas (Hernandez-Becerril et al., 2008; Maciel-Baltazar, 2015), but in very low abundances (Reguera et al., 2014). Thus, its limited distribution and its general low abundances could be the reason why it was not observed in the quantitative sampling (Van Dorn bottles).

The evaluation of the dinoflagellates-climate link provides baseline information to forecast biological responses under future environmental changes in the oceans (McCulloch, 2011). Hence, the study of dinoflagellate assemblages presented in this research constitute an advance in the knowledge about their diversity, abundance and spatial variability during the wet season in a weak La Niña event around Santa Cruz Island and nearby islands. This study provides original information about phytoplankton communities that will be useful for future work as a reference, not only to identify follow-up studies on potentially toxic species, but also to identify possible new species introduced by human activities and that may contribute to damage of the marine ecosystems of the GMR (Tian et al., 2017).

CONCLUSION

The dinoflagellate assemblage found in this study represents the highest diversity observed until now in the GMR, with 152 taxa identified. However, abundances were low and no blooms were detected, in agreement with similar abundance ranges previously reported. This is probably related with the environmental conditions occurring at the time of sampling (during the wet season and under a weak La Niña event). Although this work is from a restricted temporal scale, this study proposed and demonstrated a systematic data analysis that was able to reveal discrete dinoflagellate assemblages and among-island variability of environmental conditions. Moreover, a link between those assemblages and some particular dinoflagellate species was established with the environmental conditions prevailing during the sampling period.

Dinoflagellate species richness found in the north of Santa Cruz Island, together with higher abundances could be associated to the IME. A different dinoflagellate assemblage was distinguished in the surrounding waters of Santa Fé Island correlated with cooler and high-salinity waters possibly connected with the EUC.

A long-term monitoring program needs to be set up following an internationally validated protocol to assure accurate results considering the high ecological value of the GMR. Furthermore, the government must invest, both in sophisticated equipment and taxonomy training, in order to carry out a more extensive identification up to species level to avoid possible data misinterpretation.

Because this methodology is mainly focused in the response of dinoflagellate community (and particular species) to different environmental conditions, this approach can be easily extrapolable to different areas. However, this extrapolation should be taken carefully and must include the whole analytical procedure, including the spatio-temporal (when available) variability of environmental conditions and species presence (or abundance) in the new area, as well as the whole model calibration and variable selection procedure.

Efforts should also be addressed to the characterization of potentially toxic strains collected in the region considering the intra-specific variation among strains with different geographic origin. A parallel analysis of toxin content from water samples would further contribute to estimations of the potential HAB risk in the GMR.

AUTHOR CONTRIBUTIONS

OC and MF-T made substantial contributions to conception and design of the work. OC and IK conducted the sampling. ER-M and MF-T contributed to data collection. OC, MF-T, PD, and AC made substantial contributions in data analysis and interpretation, and participated in drafting the article. JD revised the article critically. OC, PD, AC, IK, ER-M, JD, and MF-T gave the final approval of the version to be submitted.

FUNDING

This work has been funded by the Pontifical Catholic University of Ecuador-Sede Esmeraldas through the Internal project "Characterization of the epi-benthic and phytoplanktonic microalgae community in the Galápagos Islands." IK thanks the Galápagos National Park Directorate (GNPD), the Galápagos Biosecurity Agency (ABG), the Ecuadorian Navy, and the Oceanographic Institute of the Ecuadorian Navy (INOCAR) for their support in this research and Galápagos Conservancy, Lindblad Expedition/National Geographic Fund, The Leona M. and Harry B. Helmsley Charitable Trust for the research funding that was provided for the Charles Darwin Foundation Marine Invasive Species Program that took part in this study. This publication is contribution number 2262 of the Charles Darwin Foundation for the Galapagos Islands.

ACKNOWLEDGMENTS

The authors would like to thank Josselyn Yépez Rendón for her help in field work and the reviewers for their valuable suggestions to improve the quality of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2019.00235/full#supplementary-material

FIGURE S1 | Collinearity analysis of the environmental variables measured in the study. The Pearson's correlation coefficients for each pair of variables are shown in

a color scale (red and blue represents positive and negative Pearson's correlation coefficients, respectively). Additionally, the value of each coefficient is given inside each comparison box.

FIGURE S2 | Vertical profiles for temperature (°C). The information for each island and transect is showed in columns and rows, respectively. Station is color code: blue color for 2 nm, green color for 5 nm, and yellow color for 10 nm.

FIGURE S3 | Vertical profiles for salinity. The information for each island and transect is showed in columns and rows, respectively. Station is color code: blue color for 2 nm, green color for 5 nm, and yellow color for 10 nm.

FIGURE S4 | Vertical profiles for dissolved oxygen (mg L^{-1}). The information for each island and transect is showed in columns and rows, respectively. Station is color code: blue color for 2 nm, green color for 5 nm and yellow color for 10 nm.

FIGURE S5 | Vertical profiles for pH. The information for each island and transect is showed in columns and rows, respectively. Station is color code: blue color for 2 nm, green color for 5 nm, and yellow color for 10 nm.

TABLE S1 | Presence of dinoflagellate species in net samples.

TABLE S2 | Cell abundance estimation from Van Dorn bottle samples.

REFERENCES

Accoroni, S., Glibert, P. M., Pichierri, S., Romagnoli, T., Marini, M., and Totti, C. (2015). A conceptual model of annual *Ostreopsis* cf. ovata blooms in the northern Adriatic Sea based on the synergic effects of hydrodynamics, temperature, and the N:P ratio of water column nutrients. *Harmful Algae* 45, 14–25. doi: 10.1016/j.hal.2015.04.002

Anabalón, V., Morales, C. E., González, H. E., Menschel, E., Schneider, W., Hormazabal, S., et al. (2016). Micro-phytoplankton community structure in the coastal upwelling zone off concepción (central chile): annual and inter-annual fluctuations in a highly dynamic environment. *Progr. Oceanogr.* 149, 174–188. doi: 10.1016/j.pocean.2016.10.011

Behrenfeld, M. J., O'Malley, R. T., Siegel, D. A., McClain, C. R., Sarmiento, J. L., Feldman, G. C., et al. (2006). Climate-driven trends in contemporary ocean productivity. *Nature* 444, 752–755. doi: 10.1038/nature05317

Berdalet, E., Fleming, L. E., Gowen, R., Davidson, K., Hess, P., Backer, L. C., et al. (2015). Marine harmful algal blooms, human health and wellbeing: challenges and opportunities in the 21st century. *J. Mar. Biol. Assoc. U.K.* 96, 61–91. doi: 10.1017/S0025315415001733

Borcard, D., Gillet, F., and Legendre, P. (2018). *Numerical Ecology With R*, 2nd Edn. New York, NY: Springer International Publishing, 441.

Boyce, D. G., Lewis, M. R., and Worm, B. (2010). Global phytoplankton decline over the past century. *Nature* 466, 591–596. doi: 10.1038/nature09268

Cai, W., Borlace, S., Lengaigne, M., van Rensch, P., Collins, M., Vecchi, G., et al. (2014). Increasing frequency of extreme El Niño events due to greenhouse warming. *Nat. Clim. Chang.* 4:111. doi: 10.1038/nclimate2100

Calvo-Vargas, E., Berrocal Artavia, K., and Boza Abarca, J. (2016). Floraciones algales nocivas durante el periodo 2008-2010 en el Golfo de Nicoya. Costa Rica. *Rev. Mar.* 8, 129–149. doi: 10.15359/revmar.8-1.9

Carnicer, O., Guallar, C., Andree, K. B., Diogène, J., and Fernández-Tejedor, M. (2015). *Ostreopsis* cf. ovata dynamics in the NW Mediterranean Sea in relation to biotic and abiotic factors. *Environ. Res.* 143, 89–99. doi: 10.1016/j.envres.2015.08.023

CEN (2006). *EN 15204:2006 Water Quality - Guidance Standard on the Enumeration of Phytoplankton Using Inverted Microscopy (Utermöhl Technique)*. London: BSI.

Chávez, F. P., Buck, K. R., and Barber, R. T. (1990). Phytoplankton taxa in relation to primary production in the equatorial Pacific. *Deep. Res. Part I Oceanogr. Res. Pap.* 37, 1733–1752. doi: 10.1016/0198-0149(90)90074-6

Chávez, F. P., Buck, K. R., Service, S. K., Newton, J., and Barber, R. T. (1996). Phytoplankton variability in the central and eastern tropical Pacific. *Deep Sea Res. Part. II Top Stud. Oceanogr.* 43, 835–870. doi: 10.1016/0967-0645(96)00028-8

Chávez, F. P., Strutton, P. G., Friederich, C. E., Feely, R. A., Feldman, G. C., Foley, D. C., et al. (1999). Biological and chemical response of the equatorial Pacific Ocean to the 1997-98 El Niño. *Science* 286, 2126–2131. doi: 10.1126/science.286.5447.2126

Daguer, H., Hoff, R. B., Molognoni, L., Kleemann, C. R., and Felizardo, L. V. (2018). Outbreaks, toxicology, and analytical methods of marine toxins in seafood. *Curr. Opin. Food. Sci.* 24, 43–55. doi: 10.1016/j.cofs.2018.10.006

Davidson, K., Gowen, R. J., Harrison, P. J., Fleming, L. E., Hoagland, P., and Moschonas, G. (2014). Anthropogenic nutrients and harmful algae in coastal waters. *J. Environ. Manage.* 146, 206–216. doi: 10.1016/j.jenvman.2014.07.002

de Salas, M. F., Laza-Martinez, A., and Hallegraeff, G. M. (2008). Novel unarmored dinoflagellates from the toxigenic family *Kareniaceae* (*Gymnodiniales*): five new species of *Karlodinium* and one new Takayama from the Australian sector of the Southern Ocean. *J. Phycol.* 44, 241–257. doi: 10.1111/j.1529-8817.2007.00458.x

Delwiche, C. F. (2007). *The Origin and Evolution of Dinoflagellates. Evolution of Primary Producers in the Sea*. Cambridge: Academic Press, 191–205.

Di Cioccio, D., Buia, M. C., Zingone, A., Kim, H. G., Reguera, B., Hallegraeff, G., et al. (eds) (2014). "Ocean acidification will not deliver us from Ostreopsis," in *Proceedings of the 15th International Conference on Harmful Algae*, (Changwon: International Society for the Study of Harmful Algae), 85–88.

Doney, S. C. (2006). Plankton in a warmer world. *Nature* 444:695. doi: 10.1038/444695a

Dray, S., and Dufour, A. B. (2007). The ade4 package: implementing the duality diagram for ecologists. *J. Stat. Softw.* 22, 1–20. doi: 10.18637/jss.v022.i04

Edgar, G. J., Banks, S., Fariña, J. M., Calvopiña, M., and Martínez, C. (2004). Regional biogeography of shallow reef fish and macro-invertebrate communities in the Galapagos archipelago. *J. Biogeogr.* 31, 1107–1124. doi: 10.1111/j.1365-2699.2004.01055.x

Esqueda-Lara, K., Parra-Toriz, D., and Hernández-Becerril, D. (2013). Morphology and taxonomy of *Dinophysis* species of the section *Hastata* (*Dinoflagellata*), including the description of *Dinophysis* conjuncta sp. nov., from the Mexican marine waters. *J. Mar. Biol. Assoc. U.K.* 93, 1187–1202. doi: 10.1017/S0025315412001750

Fiedler, P. C., and Talley, L. D. (2006). Hydrography of the eastern tropical Pacific: a review. *Prog. Oceanogr.* 69, 143–180. doi: 10.1016/j.pocean.2006.03.008

Field, C. B., Behrenfeld, M. J., Randerson, J. T., and Falkowski, P. (1998). Primary production of the biosphere: integrating terrestrial and Oceanic components. *Science* 281, 237–240. doi: 10.1126/science.281.5374.237

Garcés, E., Fernandez, M., Penna, A., Van Lenning, K., Gutierrez, A., Camp, J., et al. (2006). Characterization of new mediterranean *Karlodinium* spp. (*Dinophyceae*) strains using morphological, molecular, chemical, and physiological methodologies. *J. Phycol.* 42, 1096–1112. doi: 10.1111/j.1529-8817.2006.00270.x

Giraldo, A., Valencia, B., Acevedo, J. D., and Rivera, M. (2014). Fitoplancton y zooplancton en el área marina protegida de Isla Gorgona, Colombia, y su relación con variables oceanográficas en estaciones lluviosa y seca. *Rev. Biol. Trop.* 62, 117–132. doi: 10.15517/rbt.v62i0.15982

Glibert, P. M., Icarus Allen, J., Artioli, Y., Beusen, A., Bouwman, L., Harle, J., et al. (2014). Vulnerability of coastal ecosystems to changes in harmful algal bloom distribution in response to climate change: projections based on model analysis. *Glob. Chang. Biol.* 20, 3845–3858. doi: 10.1111/gcb.12662

Gower, J. C., and Legendre, P. (1986). Metric and Euclidean properties of dissimilarity coefficients. *J. Classif.* 3, 5–48.

Greenacre, M., and Primicerio, R. (2013). *Multivariate Analysis for Ecological Data*. Bilbao: Fundación BBVA.

Hallegraeff, G. M. (2003). "Harmful Algal Blooms: A Global Overview," in *Manual on Harmful Marine Microalgae. Monographs on Oceanographic Methodology*, 2nd Edn, eds M. Hallegraeff, D. M. Anderson, and A. D. Cembella (Paris: IOC-UNE-SCO), 25–49.

Hallegraeff, G. M. (2010). Ocean climate change, phytoplankton community responses, and harmful algal blooms: a formidable predictive challenge. *J. Phycol.* 46, 220–235. doi: 10.1111/j.1529-8817.2010.00815.x

Henson, S. A., Cole, H. S., Hopkins, J., Martin, A. P., and Yool, A. (2018). Detection of climate change-driven trends in phytoplankton phenology. *Glob. Chang. Biol.* 24, e101–e111. doi: 10.1111/gcb.13886

Hernandez-Becerril, D. U., Ceballos-Corona, G. A., Esqueda-Lara, K., Tovar-Salazar, M. A., and León-Alvarez, D. (2008). Marine planktonic dinoflagellates of the order Dinophysiales (Dinophyta) from coasts of the tropical Mexican Pacific, including two new species of the genus *Amphisolenia*. *J. Mar. Biol. Assoc. U.K.* 88, 1–15. doi: 10.1017/S0025315408000143

Hoppenrath, M., Elbrächter, M., and Debres, G. (2009). *Marine Phytoplankton*. Stuttgart: Schweizerbart Science Publishers.

Hoppenrath, M., Murray, S. A., Chomérat, N., and Horiguchi, T. (2014). *Marine Benthic Dinoflagellates - Unveiling Their Worldwide Biodiversity*. Stuttgart: Schweizerbart Science Publishers.

Horton, T., Kroh, A., Ahyong, S., Bailly, N., Boury-Esnault, N., Brandão, S. N., et al. (2018). *World Register of Marine Species*. Available at: http://www.marinespecies.org at VLIZ (accessed July 30, 2018).

Intergovernmental Oceanographic Commission [IOC] (2010). "Microscopic and molecular methods for quantitative phytoplankton analysis," in *IOC Manuals and Guides, No. 55*, eds B. Karlson, C. Cusack, and E. Bresnan (Paris: UNESCO).

Jacox, M. G., Hazen, E. L., and Bograd, S. J. (2016). Optimal environmental conditions and anomalous ecosystem responses: constraining bottom-up controls of phytoplankton biomass in the california current system. *Sci. Rep.* 6:27612. doi: 10.1038/srep27612

Kessler, W. (2006). The circulation of the eastern tropical Pacific: a review. *Prog. Oceanogr.* 69, 181–217. doi: 10.1016/j.pocean.2006.03.009

Kislik, E., Mantilla, G., Torres, G., and Borbor-Córdova, M. (2017). Biological hotspots in the galápagos islands: exploring seasonal trends of ocean climate drivers to monitor algal blooms. *Int. J. Bioeng. Life Sci.* 11, 784–794.

Kofoid, C. A., and Skogsberg, T. (1928). "Reports on the scientific results of the expedition to the Eastern Tropical Pacific, in charge of Alexander Agassiz, by the U.S. Fish Commission Steamer "Albatross" from October 1904 to March 1905, Lieut. Commanding. [No.] XXXV," in *The Dinoflagellata: the Dinophysoidae*, eds L. M. Commander and U. S. N. Garrett (Cambridge: Harvard College).

Lafabrie, C., Hlaili, A. S., Leboulanger, C., Tarhouni, I., Othman, H. B., Mzoughi, N., et al. (2013). Contaminated sediment resuspension induces shifts in phytoplankton structure and function in a eutrophic mediterranean lagoon. *Knowl. Managt. Aqua. Ecosyst.* 410:05. doi: 10.1051/kmae/2013060

Lassus, P., Chomérat, N., Hess, P., and Nézan, E. (2016). *Toxic and Harmful Microalgae of the World Ocean. Micro-Algues Toxiques et Nuisibles de l'Océan Mondial. IOC Manuals and guides, 68 (Bilingual English/French)*. Denmark: International Society for the Study of Harmful Algae/ Intergovernmental Oceanographic Commission of UNESCO.

Lim, H. C., Leaw, C. P., Tan, T. H., Kon, N. F., Yek, L. H., Hii, K. S., et al. (2014). A bloom of *Karlodinium australe* (*Gymnodiniales, Dinophyceae*) associated with mass mortality of cage-cultured fishes in West Johor Strait, Malaysia. *Harmful Algae* 40, 51–62. doi: 10.1016/j.hal.2014.10.005

Lin, C. H., Flynn, K. J., Mitra, A., and Glibert, P. M. (2018). Simulating effects of variable stoichiometry and temperature on mixotrophy in the harmful dinoflagellate *Karlodinium veneficum*. *Front. Mar. Sci.* 5:320. doi: 10.3389/fmars.2018.00320

Liu, Y., Xie, L., Morrison, J. M., Kamykowski, D., and Sweet, W. V. (2014). Ocean circulation and water mass characteristics around the galápagos archipelago simulated by a multiscale nested ocean circulation model. *Int. J. Oceanogr.* 2014, 1–16. doi: 10.1155/2014/198686

Luo, Z., Wang, L., Chan, L., Lu, S., and Gu, H. (2018). *Karlodinium zhouanum*, a new *dinoflagellate* species from China, and molecular phylogeny of *Karenia digitata* and *Karenia longicanalis* (*Gymnodiniales, Dinophyceae*). *Phycologia* 57, 401–412. doi: 10.2216/17-106.1

Maciel-Baltazar, E. (2015). Dinoflagelados (*Dinoflagellata*) tóxicos de la costa de Chiapas, México, Pacífico centro oriental. *Cuadernos de Invest.* 7, 39–48.

Marshall, H. G. (1972). Phytoplakton composition in the southern Pacific between Ecuador and Galápagos Islands (Archipelago de Colón). *Proc. Biol. Soc. Wash.* 85, 1–37.

McCulloch, A. (2011). *Spatio-Temporal Context for the Phytoplankton Community Patterns of the Galápagos Archipelago and the Northwest Florida Shelf*. Ph.D. thesis, North Carolina State University, Raleigh.

Moestrup, Ø, Akselmann, R., Fraga, S., Hoppenrath, M., Iwataki, M., Komárek, J., et al. (2009). *IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae*. Available at: http://www.marinespecies.org/hab/ (accessed December 1, 2018).

Nagahama, Y., Murray, S., Tomaru, A., and Fukuyo, Y. (2011). Species boundaries in the toxic dinoflagellate Prorocentrum lima (Dinophyceae, Prorocentrales), based on morphological and phylogenetic characters. *J. Phycol.* 47, 178–189. doi: 10.1111/j.1529-8817.2010.00939.x

Naranjo, D., and Tapia, M. E. (2015). Plancton en el canal Bolívar de la isla Isabela (Caleta Tagus), Islas Galápagos durante marzo de 2009. *Acta Oceanogr. del Pac.* 20, 71–87.

Nézan, E., Siano, R., Boulben, S., Six, C., Bilien, G., Chèze, K., et al. (2014). Genetic diversity of the harmful family Kareniaceae (Gymnodiniales, Dinophyceae) in France, with the description of *Karlodinium gentienii* sp. nov.: a new potentially toxic dinoflagellate. *Harmful Algae* 40, 75–91. doi: 10.1016/j.hal.2014.10.006

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2018). *Vegan: Community Ecology Package. R Package Version 2.5-2*. Available at: https://CRAN.R-project.org/package=vegan (accessed Febuary 4, 2019).

Omura, T., Iwataki, M., Borja, V. M., Takayama, H., and Fukuyo, Y. (2012). *Marine Phytoplankton of the Western Pacific*. Tokyo: Koseishakoseikaku.

Palacios, D. M. (2002). Factors influencing the island-mass effect of the Galápagos Archipelago. *Geophys. Res. Lett.* 29:2134. doi: 10.1029/2002GL016232

Palacios, D. M. (2004). Seasonal patterns of sea-surface temperature and ocean colour around the Galápagos: regional and local influences. *Deep Sea Res. Part. II Top Stud. Oceanogr.* 51, 43–57. doi: 10.1016/j.dsr2.2003.08.001

Parsons, M. L., Aligizaki, K., Bottein, M.-Y. D., Fraga, S., Morton, S. L., Penna, A., et al. (2012). *Gambierdiscus* and *Ostreopsis*: reassessment of the state of knowledge of their taxonomy, geography, ecophysiology, and toxicology. *Harmful Algae* 14, 107–129. doi: 10.1016/j.hal.2011.10.017

Paulmier, G., Berland, B., Billard, C., and Nezan, E. (1995). *Gyrodinium corsicum* nov. sp. (Gymnodiniales, Dinophycées), organisme responsable d'une "eau verte" dans l'etang marin de Diana (Corse), en avril 1994. *Cryptog. Algol.* 16, 77–94.

Penna, A., Vila, M., Fraga, S., Giacobbe, M. G., Andreoni, F., Riobó, P., et al. (2005). Characterization of *Ostreopsis* and *Coolia* (dinophyceae) isolates in the western Mediterranean Sea based on morphology, toxicity and internal transcribed spacer 5.8S rDNA sequences. *J. Phycol.* 41, 212–225. doi: 10.1111/j.1529-8817.2005.04011.x

Place, A. R., Bowers, H. A., Bachvaroff, T. R., Adolf, J. E., Deeds, J. R., and Sheng, J. (2012). Karlodinium veneficum-the little dinoflagellate with a big bite. *Harmful Algae* 14, 179–195. doi: 10.1016/j.hal.2011.10.021

Poloczanska, E. S., Burrows, M. T., Brown, C. J., García Molinos, J., Halpern, B. S., Hoegh-Guldberg, O., et al. (2016). Responses of marine organisms to climate change across Oceans. *Front. Mar. Sci.* 3:62. doi: 10.3389/fmars.2016.00062

R Core Team (2018). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. Available at: https://www.r-project.org/

Reguera, B., Alonso, R., Moreira, A., Méndez, S., and Dechraoui-Bottein, M.-Y. (eds) (2016). *Guide for Designing and Implementing a Plan to Monitor Toxin-Producing Microalgae IOC Manuals and Guides, No. 59*, 2nd Edn. Paris: Intergovernmental Oceanographic Commission (IOC) of UNESCO and International Atomic Energy Agency (IAEA).

Reguera, B., Riobó, P., Rodríguez, F., Díaz, P. A., Pizarro, G., Paz, B., et al. (2014). Dinophysis toxins: causative organisms. *Distrib. Fate Shellfish. Mar. Drugs* 12, 394–461. doi: 10.3390/md12010394

Ryan, J. P., Polito, P. S., Strutton, G. P., and Chávez, F. P. (2002). Unusual large-scale blooms in the equatorial Pacific. *Prog. Oceanogr.* 55, 263–285. doi: 10.1016/S0079-6611(02)00137-4

Ryan, J. P., Ueki, I., Chao, Y., Zhang, H., Polito, P. S., and Chávez, F. P. (2006). Western Pacific modulation of large phytoplankton blooms in the central and eastern equatorial Pacific. *J. Geophys. Res.* 111:G02013. doi: 10.1029/2005JG000084

Sachs, B. J. P., and Ladd, S. N. (2002). Climate and oceanography of the Galápagos in the 21st century: expected changes and research needs. *Galápagos Res.* 67, 50–54.

Schaeffer, B. A., Morrison, J. M., Kamykowski, D., Feldman, G. C., Xie, L., Liu, Y., et al. (2008). Phytoplankton biomass distribution and identification of productive habitats within the galápagos marine reserve by MODIS, a surface acquisition system, and in-situ measurements. *Remote Sens. Environ.* 112, 3044–3054. doi: 10.1016/j.rse.2008.03.005

Smayda, T. J., and Reynolds, C. S. (2003). Strategies of marine dinoflagellate survival and some rules of assembly. *J. Sea Res.* 49, 95–106. doi: 10.1016/S1385-1101(02)00219-218

Steidinger, K. A., and Jangen, K. (1997). "Chapter 3 - Dinoflagellates," in *Identifying Marine Phytoplankton*, ed. C. R. Tomas (San Diego: Academic Press), 387–584.

Stoecker, D. K. (1999). Mixotrophy among Dinoflagellates. *J. Eukaryot. Microbiol.* 46, 397–401. doi: 10.1111/j.1550-7408.1999.tb04619.x

Strutton, P. G., Evans, W., and Chávez, F. P. (2008). Equatorial Pacific chemical and biological variability, 1997–2003. *Glob. Biogeochem. Cycles* 22:GB2001. doi: 10.1029/2007GB003045

Sweet, W. V., Morrison, J. M., Kamykowski, D., Schaeffer, B. A., Banks, S., and McCulloch, A. (2007). Water mass seasonal variability in the Galápagos Archipelago. *Deep. Res. Part I Oceanogr. Res. Pap.* 54, 2023–2035. doi: 10.1016/j.dsr.2007.09.009

Tapia, M. E., and Naranjo, C. (2012). Aspectos Oceanográficos del Plancton y su relación con el frente ecuatorial, durante septiembre de 2011. *Acta Oceanogr. Del Pac.* 17, 67–91.

ter Braak, C. J. F., and Verdonschot, P. F. M. (1995). Canonical correspondence analysis and related multivariate methods in aquatic ecology. *Aqua. Sci.* 57:255. doi: 10.1007/BF00877430

Tian, W., Zhang, H., Zhao, L., Zhang, F., and Huang, H. (2017). Phytoplankton diversity effects on community biomass and stability along nutrient gradients in a Eutrophic Lake. *Int. J. Environ. Res. Public Health* 14:95. doi: 10.3390/ijerph14010095

Tomas, C. R. (ed.). (1997). *Identifying Marine Phytoplankton*. San Diego, CA: Academic Press, 858.

Torres, G. (2002). *Distribución Latitudinal Del Fitoplancton Entre Islas Cocos-Galápagos, Noviembre-Diciembre 2001*. Oostende: OceanDocs Repository of Ocean Publications.

Torres, G. (2015). Evaluación de Mareas Rojas durante 1968-2009 en Ecuador. *Acta Oceanogr. Del Pac.* 20, 89–98.

Torres, G., and Andrade, C. (2014). *Dinámica Del Plancton Marino Costero Con Relación Al Flujo De Mareas en Bahía Aeolián en Septiembre 2006 (Baltra-Galápagos-Ecuador)*. Oostende: OceanDocs Repository of Ocean Publications.

Torres, G., and Tapia, M. E. (2000). Distribución del fitoplancton y su comportamiento en el afloramiento en las islas Galápagos. *Acta Oceanogr. Del Pac.* 10, 137–150.

Torres, G., and Tapia, M. E. (2002). *Fitoplancton En El Afloramiento De Las Islas Galápagos, Durante Agosto 2000*. Oostende: OceanDocs Repository of Ocean Publications.

Utermöhl, H. (1958). *Zur Vervollkomnung der Quantitativen Phytoplankton-Methodik Mitteilungen. Internationale Vereiningung fuer Theoretische und Angewandte Limnologie*. Stuttgart: Schweizerbart.

Vargas-Montero, M., Bustamante, E. F., Guzman, J. C., and Vargas, J. C. (2008). Harmful blooms by noxious dinoflagellates in the Pacific coast of Costa Rica. *Hidrobiologica* 18, 15–23.

Venrick, E. L. (2015). Phytoplankton species in the California Current System off Southern California: the spatial dimensions. *Califor. Coopera. Ocean. Fish. Invest. Rep.* 56, 168–184.

Vila, M., Abós-Herràndiz, R., Isern-Fontanet, J., Àlvarez, J., and Berdalet, E. (2016). Establishing the link between *Ostreopsis* cf. ovata blooms and human health impacts using ecology and epidemiology. *Sci. Mar.* 80, 107–115. doi: 10.3989/scimar.04395.08A

Wasmund, N. (2017). The diatom/dinoflagellate index as an indicator of ecosystem changes in the baltic sea. 2. historical data for use in determination of good environmental status. *Front. Mar. Sci.* 4:153. doi: 10.3389/fmars.2017.00153

Wells, M. L., Trainer, V. L., Smayda, T. J., Karlson, B. S. O., Trick, C. G., Kudela, R. M., et al. (2015). Harmful algal blooms and climate change: learning from the past and present to forecast the future. *Harmful Algae* 49, 68–93. doi: 10.1016/j.hal.2015.07.009

Wood, S. N. (2017). *Generalized Additive Models: An Introduction with R*, 2nd Edn. London: Chapman and Hall/CRC.

Zhao, C., Daewel, U., and Schrum, C. (2018). Tidal impacts on primary production in the North Sea. *Earth Syst. Dynam.* 2018, 1–44. doi: 10.5194/esd-2018-74

Zuur, A. F., Ieno, E. N., and Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods Ecol. Evol.* 1, 3–14. doi: 10.1111/j.2041-210X.2009.00001.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Carnicer, De La Fuente, Canepa, Keith, Rebolledo-Monsalve, Diogène and Fernández-Tejedor. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Marine Science

ORIGINAL RESEARCH
published: 19 June 2019
doi: 10.3389/fmars.2019.00339

Effects of Salinity and Temperature on the Growth, Toxin Production, and Akinete Germination of the Cyanobacterium *Nodularia spumigena*

Savênia B. Silveira and Clarisse Odebrecht*

Phytoplankton and Microorganisms Laboratory, Institute of Oceanography, Federal University of Rio Grande, Rio Grande, Brazil

Harmful blooms of the cyanobacterium *Nodularia spumigena* occur in several parts of the world, and this species is notorious for its ability to produce cyanotoxins, such as nodularin. This species is also able to perform akinete differentiation, thus favoring the success of their populations under adverse conditions. In southern Brazil, *N. spumigena* has developed large blooms in experimental shrimp production farms. In this study, we implemented an experiment to evaluate the influence of salinity and temperature on several physiological attributes of a Brazilian strain of *N. spumigena*: cell growth, nodularin production, the ability to form akinetes and germination potential. A factorial experiment (3 × 4) was conducted to test the effects of temperature (15, 23, and 30°C) and salinity (1, 7, 15, and 30 ppm) on the growth, production of nodularin and the differentiation of akinetes. The germination potential of the akinetes was tested after incubation for 30, 60, 90, 120, 180, and 360 days at 4°C in the dark. *N. spumigena* grew under a wide range of salinity and temperatures, but salinity had a greater influence. The highest cell densities at the temperatures tested were observed at salinity 7 ppm and the lowest at salinity 1 ppm. The toxin nodularin was produced in all treatments, but there was an inverse relationship between the content of nodularin per cell and cell density. The akinetes were also differentiated in all the treatments, with the exception of S1T30. However, the largest proportion of akinetes (65% of the cells) was observed in the treatment with low cell growth at salinity 15 and 15°C, possibly indicating an effort of the population to survive over the long term. The akinetes germinated in all treatments, but those cultivated at salinity 7 and 15 ppm (except S15T30) showed a higher percentage of germination and, in contrast, the lowest germination rate was observed in the treatments with salinity 1 ppm. Thus, the low salinity (1) at all the temperatures tested has the potential to inhibit *N. spumigena* blooms, since it hampers cell growth and the formation of akinetes.

Keywords: cyanobacteria, nodularin, harmful, bloom, environmental factors

OPEN ACCESS

Edited by:
Jorge I. Mardones, Instituto de Fomento Pesquero (IFOP), Chile

Reviewed by:
Jonna Emilia Teikari, Universität Potsdam, Germany
Viviana Patricia Almanza, Universidad de Concepción, Chile

****Correspondence:***
Savênia B. Silveira
sbonoto@yahoo.com.br

Specialty section:
This article was submitted to Marine Ecosystem Ecology, a section of the journal Frontiers in Marine Science

Received: *21 May 2018*
Accepted: *03 June 2019*
Published: *19 June 2019*

Citation:
Silveira SB and Odebrecht C (2019) Effects of Salinity and Temperature on the Growth, Toxin Production, and Akinete Germination of the Cyanobacterium Nodularia spumigena. Front. Mar. Sci. 6:339. doi: 10.3389/fmars.2019.00339

INTRODUCTION

The planktonic cyanobacterium *Nodularia spumigena* Mertens ex Bornet and Flahault develops important blooms in several areas in the world (Blackburn et al., 1996; Van Buynder et al., 2001; Rakko and Seppälä, 2014). The first toxic bloom of *N. spumigena* was reported in southern Australia in the lakes that form the Murray estuary, in particular, Alexandrina Lake (Francis, 1878). In Brazil, blooms of this species were recently documented for the first time (Costa et al., 2013), reaching very high abundances in nursery ponds of the shrimp *Penaeus vannamei* Boone, 1931, in which they caused a reduction in the growth and survival rates. The identification of *N. spumigena* was confirmed using molecular methods and ultrastructural analysis (Popin et al., 2016; Silveira et al., 2017).

Among the common characteristics to the Order Nostocales, *N. spumigena* can differentiate its cells in heterocytes (molecular nitrogen fixation cells) and akinetes (resistance spores) (Komárek, 2013; Silveira et al., 2017). Both kinds of cells provide important advantages. Heterocytes favor the growth of *N. spumigena* under nitrogen low concentrations, while the akinetes remain viable for long periods in the sediment and can germinate in response to changes in environmental conditions (Hansson, 1993, 1996; Myers et al., 2010). The germinated akinetes may function as inoculum for the initiation of a harmful algal bloom and thus, represent an important mechanism of population propagation (Kim et al., 2005). In addition, this species can produce the toxin nodularin, a hepatotoxic cyclic pentapeptide (Rinehart et al., 1988), which accumulates in the animal liver and can act as a carcinogen in mammals (Carmichael, 1992; Ohta et al., 1994). Nodularin also causes the enzymatic inhibition of the eukaryotic proteins phosphatase 1 and 2A (Honkanen et al., 1991). *N. spumigena* can also produce other bioactive compounds, such as spumigins, aeruginosins, pseudoaeruginosins, and anabaenopeptins (Mazur-Marzec et al., 2016).

Important environmental factors that govern the distribution and bloom development of *N. spumigena* are salinity and temperature, light quality and intensity, and nutrient concentration. Salinity is considered to be a controlling factor of cyanobacteria in general (Kononen, 1992; Lehtimäki et al., 1994), affecting photosynthesis, the functioning of the plasma membrane (Singh et al., 2002) and therefore, cell growth (Moisander et al., 2002). In addition, the cyanobacterial morphology and life cycle vary in response to fluctuations in salinity (Iranshahi et al., 2014), as well as the turgor pressure on the cell (Ladas and Papageorgiou, 2000). It has been demonstrated that *N. spumigena* tolerates a large variation in water salinity (0–35 ppm), but its optimal growth differs between strains analyzed in the Baltic Sea and lakes and lagoons in Australia (Blackburn et al., 1996).

It is widely known that the temperature is directly related to the growth rate of cyanobacteria (Mackey et al., 2013) and that this relationship varies among species (Reynolds, 1984). The temperature also influences the toxin production of *N. spumigena*. It was shown that toxin production was favored at lower temperatures than cellular growth. Thus, a higher concentration of the intracellular toxin was observed in cells grown at 10°C, while the maximum biomass production was observed at 20–30°C (Hobson and Fallowfield, 2003). *Nodularia* strains isolated in Brazilian shrimp ponds were tested for their growth and toxin production under controlled conditions of salinity (7 ppm) and temperature (23°C) (Silveira et al., 2017). However, the optimal environmental conditions for the Brazilian strain and how they compare to strains from other sites in the world are not yet known. Phylogenetic analyses based on its 16S rRNA gene indicated that the Brazilian strain is closely related to the strains from Australia (Popin, 2017; Silveira et al., 2017). Alternatively, based on the analysis of genome, 31 proteins of this same Brazilian strain were grouped with the Baltic Sea strain (Popin, 2017). Currently, there is a lack of accessible data on the NCBI GenBank with only one Baltic Sea *N. spumigena* strain available for comparison, and thus, it is important to provide information on the strains from other geographical regions.

It is known that salinity and temperature influence the growth of *N. spumigena*. However, different strains respond differently to environmental variables. In this study, the effects of salinity and temperature on the growth, production of the nodularin toxin, and akinete differentiation were evaluated, as well as the germination potential of a strain of *N. spumigena* isolated from a shrimp production pond in southern Brazil. The results of this study will improve the understanding of the physiology and management of recurrent *N. spumigena* blooms in the environment and in aquaculture ponds, in which they cause great damage.

MATERIALS AND METHODS

Species Isolation and Stock Culture

Water samples containing *Nodularia* trichomes were collected on December 5, 2013 in production ponds of the marine shrimp *P. vannamei* at the Marine Aquaculture Station (EMA) of the Federal University of Rio Grande-FURG, Brazil. The isolated trichome gave rise to the NODSPU1 strain, which was identified as *N. spumigena* (Silveira et al., 2017). The strain was cultivated in f/2 medium (Guillard, 1975) at salinity 7 ppm in incubators with a light cycle control (12:12 h light-dark), at 80 μmol m^{-2} s^{-1} of light intensity supplied by white light fluorescent lamps (OSRAM, 20 W) and constant temperature (23 $\pm$ 1°C). The culture temperature and salinity values corresponded to those registered in the ponds on the sampling day.

Acclimation and Experimental Design

The conditions were gradually modified for the acclimation process every 7 days using the stock culture as the starting point. The cultures were acclimated at the test temperatures (15, 23, and 30°C) and salinities (1, 7, 15, and 30 ppm) through at least three transfers.

A factorial experiment (3 $\times$ 4) was conducted to concomitantly test the effect of temperature and salinity on the growth of *N. spumigena* using the f/2 medium and a light intensity of 80 μmol m^{-2} s^{-1}. Cultures for the experiments (three replicates) were maintained in incubators with a light cycle

control (12:12 h light: dark) provided by white light fluorescent lamps (OSRAM, 20 W). The light intensity in the incubators was measured using a LI-COR (LI-1400) spherical sensor. All the cultures were prepared in 500 ml of f/2 culture medium. To standardize the initial cell concentration, the volume of inoculum added corresponded to an optical density of 0.008 determined at absorbance 680 nm in a spectrophotometer (Varian Cary 1E) (Ma et al., 2006). The experiment lasted 40 days.

Cell Density, Growth, and Cyanotoxin Production

The growth was determined daily during the first four culture days based on the cell density, optical density and chlorophyll-*a* (Chl*a*) content and subsequently every 48 and 72 h until the end of the experiments. The Chl*a* content was determined in culture samples (5–10 mL) by retaining cells in glass fiber filters (Whatman GF/F, 25 mm) using a vacuum pump. The filters were stored in darkened flasks and kept in absolute methanol for 24 h in a freezer. The Chl*a* concentration ($\mu g\ L^{-1}$) was determined by measuring the absorbance at 665 and 750 nm as described by Mackinney (1941). Mean concentration of chlorophyll-*a* per cell (pg $cell^{-1}$) at the start, middle and end of growth curves was performed by dividing the value of chlorophyll-*a* by cell density. At the end of the logarithmic growth phase, the biomass was also determined using the dry weight (DW) and ash-free dry weight (AFDW) as described by Zhu and Lee (1997).

The correlation between cell density and optical density was higher (r = 0.93) compared to the correlation between cell density and Chl*a* concentration (r = 0.84) and between Chl*a* concentration and optical density (r = 0.87), but all correlations were significant (Pearson correlation, **Figure 1**). We presented the growth curves based solely on cell density for clarity. The growth rate (μ) was calculated as the slope of the linear regression between the time and cell density (Ln scale) during the exponential phase (Wood et al., 2005).

The density of the trichomes, vegetative cells and akinetes were estimated in samples fixed with acetic Lugol's solution in Sedgewick Rafter chambers using an inverted microscope (Olympus IX51; 200×). To estimate the number of vegetative cells per trichome, the total size of each trichome was measured, discounting the average size of the heterocysts and akinetes present, and divided by the average size of the vegetative cells. The counting error was maintained at a maximum of 20% and verified by estimating the coefficient of variation between the fields counted. The size of the vegetative cells (n = 50) and the akinetes (n = 30) were determined using an inverted microscope (Olympus IX71; 400×), and the cell volume was calculated assuming the geometric shape of a cylinder (Sun and Liu, 2003).

The toxin concentration was determined at each experimental treatment at the beginning of the stationary phase using an immunoassay ELISA plate kit for nodularin (Beacon Analytical Systems, Inc.). The concentration of nodularin ($\mu g\ L^{-1}$) was determined using the absorbance (450 nm) in a plate photometer (Quick ELISA Drake Instruments) as described by the manufacturer.

Akinete Isolation

Cultures of *N. spumigena* were maintained until the stationary/decline phase in salinity 15 ppm and temperature 15°C (S15T15). This condition was shown to favor the akinete differentiation as described above. The akinetes produced were separated from the vegetative cells as described by Sukenik et al. (2007). Cultures of *N. spumigena* with akinetes were harvested by suction, suspended and washed in Tris buffer (3×; 50 mM, pH 7.5, containing 10 mM EDTA and 1 mM Mg Cl_2). The biomass was washed in EDTA-free Tris buffer (50 mM pH 7.5, 1 mM $MgCl_2$) containing 5 mg/mL lysozyme, incubated (2 h, 37 ± 1°C) and centrifuged (3 min, 2000 rpm). The pellet was washed three times with EDTA-free Tris buffer to remove the cell debris, sonicated (3×, 10 s, 30 kHz), and the sample was examined under an optical microscope to confirm the separation of the akinetes. The material was centrifuged (10 min, 3000 rpm), and the pellet was suspended in ultrapure water and stored in a refrigerator (4°C) in the dark (Myers et al., 2010).

Germination tests were performed after incubation of the akinetes at 30, 60, 90, 120, 180, and 360 days maintained in the dark, salinity 15 and temperature 15°C.

Germination Tests

After incubation in the dark for different time periods, 100 akinetes for each treatment (in triplicate) were inoculated in 2.5 mL f/2 culture medium in cell culture plates under the microscope and maintained in incubators (light intensity 80 $\mu mol\ m^{-2}\ s^{-1}$). The effects of both temperature (15, 23 and 30°C) and salinity (1, 7, 15, and 30 ppm) were tested on the germination potential of the akinetes. The akinetes were exposed to the experimental conditions for 72 and 192 h, and the number

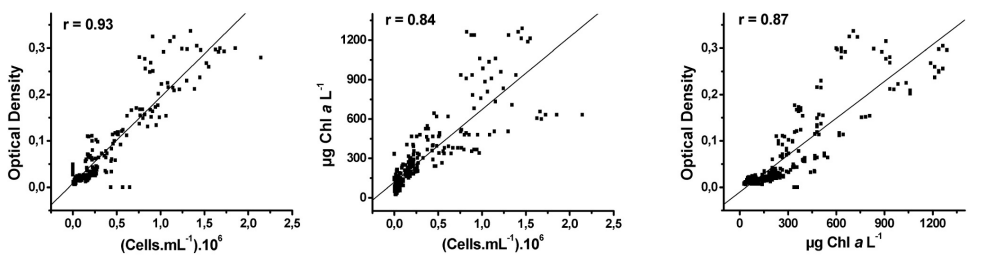


FIGURE 1 | Correlation of Pearson between cell density, optical density, and Chl*a* concentration.

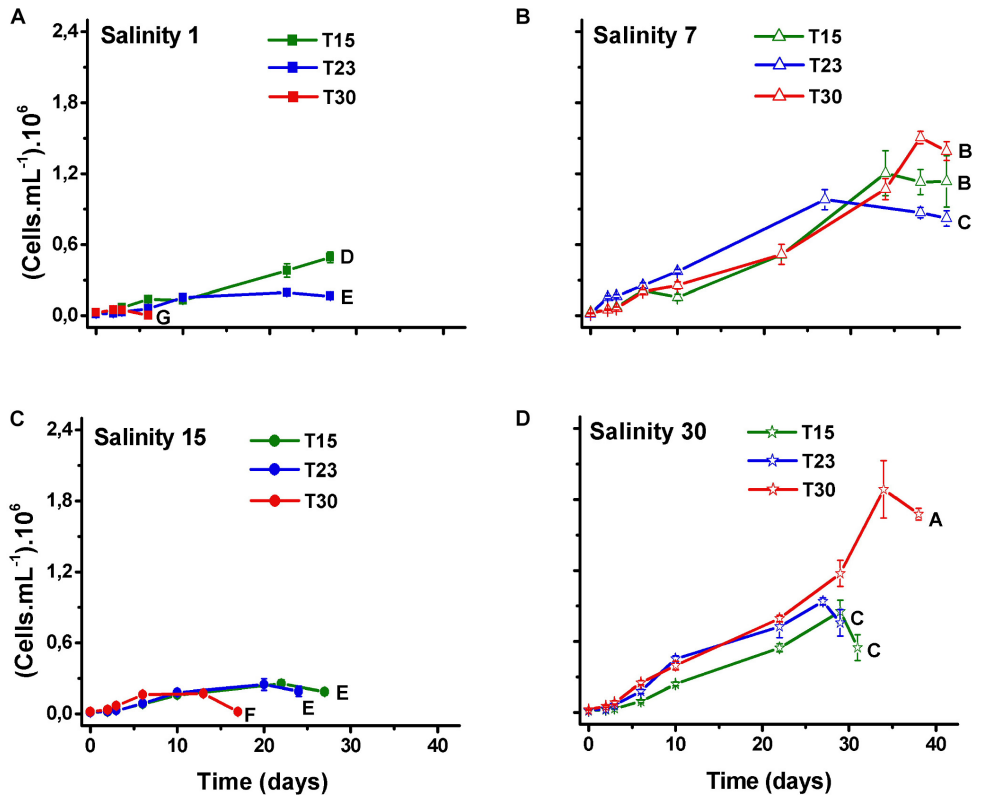


FIGURE 2 | Mean values of cell density (±SD; $n = 3$) of *N. spumigena* (NODSPU1) observed along the time in the factorial experiment, which analyzed the influence of temperature (T15, T23, T30°C) and salinity S1 **(A)**, S7 **(B)**, S15 **(C)**, and S30 **(D)**. Significant difference between treatments is indicated by letters (ANOVA, Tukey's $p < 0.05$).

of germinated akinetes was determined in the cell culture plates using an optical microscope (200×).

Statistical Analysis

To determine the cell density differences between treatments, a two-way ANOVA was applied and the Tukey's *a posteriori* test to specify the differences at 95% significance level (Zar, 2010). The data of the percentage of akinetes, cell volume, DW and AFDW, Chl*a* L^{-1}, Chl*a* $cell^{-1}$, toxin concentration and akinete germination were analyzed using a one-way ANOVA and Tukey's *a posteriori* test to specify the differences at 95% significance level. All data were tested for their normality and homoscedasticity (Cochran C., Hartley e Bartlett). The cell density, akinete percentage, cell volume of the vegetative cells and Chl*a* $cell^{-1}$ were transformed using logarithmic transformations and the square root for the cell volume of the akinetes to ensure normal distributions.

RESULTS

Cell Density and Growth

The comparison between the *N. spumigena* growth curves shows that both the time to reach the maximum cell density and the maximum values differed among the treatments (**Figure 2**; ANOVA $p < 0.001$, F 435.3; **Supplementary Table 1**). The highest value (1.9×10^6 cells mL^{-1}) was recorded at the highest values of salinity and temperature (S30T30) (**Figure 2D**), followed by treatments with salinity 7 ppm at the three temperature values (S7T30, S7T15, S7T23) (**Figure 2B**). At the lowest salinity level (S1) (**Figure 2A**), the cultures showed practically no growth and either collapsed within a week (S1T30) or remained at low density for approximately 1 month (S1T23, S1T15). At salinity 15 ppm (**Figure 2C**), the cultures persisted for approximately 1 month at low density (S15T15, S15T23) or died within 2 weeks (S15T30). The growth rates (μ) varied between 0.2 (S1T23) and 0.4 d^{-1} (S7T30, S30T30).

As expected, the number of trichomes also increased over time in all the treatments, except in those treatments that rapidly died (S1T30; S15T30; **Figure 3**). The number of cells per trichome remained relatively constant or decreased, indicating that the trichome length was reduced over time with some variation among treatments and with time. The treatment with high salinity and temperature (S30T30), which presented the largest cell number (**Figure 2**) also presented the longest trichomes at the end of the experiment with 140 cells per trichome (**Figure 4**).

The percentage of akinetes in relation to the number of cells (0.01–65.2%) increased over time, particularly in the S15T15 treatment but also in S7T15, S30T15 and S30T23. Other

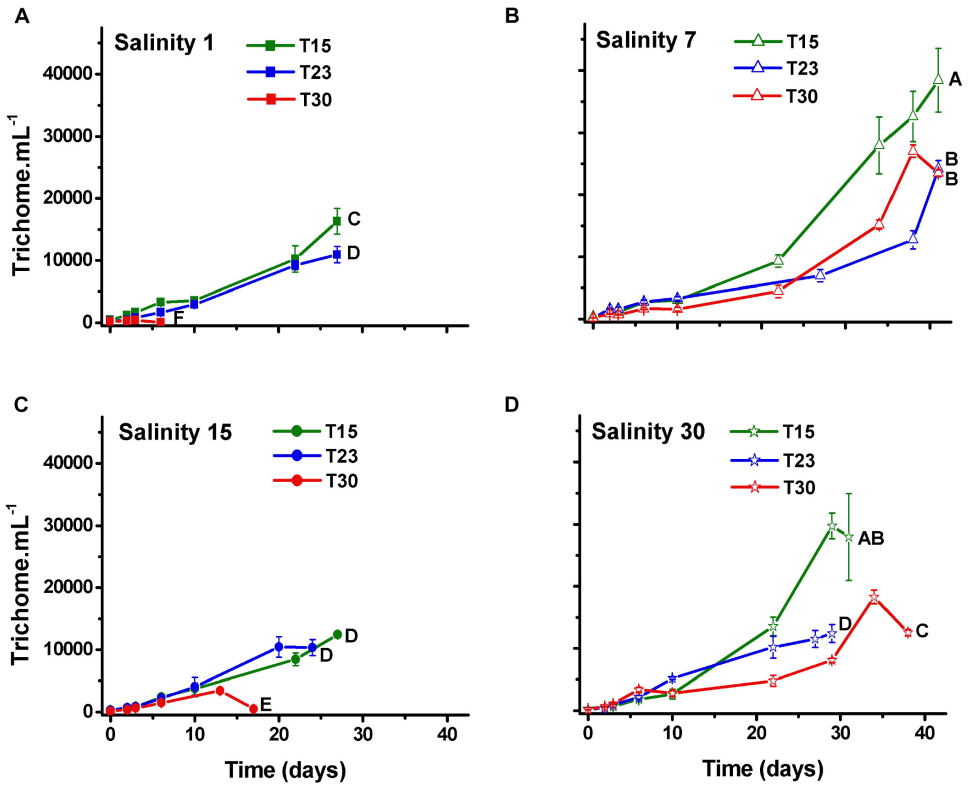


FIGURE 3 | Trichome density (±SD; $n = 3$) of *N. spumigena* (NODSPU1) observed along the time in the factorial experiment, which analyzed the influence of temperature (T15, T23, T30°C) and salinity S1 **(A)**, S7 **(B)**, S15 **(C)**, and S30 **(D)**. Significant difference between treatments is indicated by letters (ANOVA, Tukey's $p < 0.05$).

treatments showed low percentages of akinetes (<5%), except for S1T30, in which no akinetes were observed (**Figure 5**).

Biomass

The biomass values determined by both the DW and AFDW (**Figure 6A**) and the Chl*a* content (**Figure 6B**) were generally consistent with the cell density results. All were higher in the treatments with salinity 7 and lower in salinity 1. However, the S30T30 treatment, which demonstrated the highest cell density and largest trichomes, did not correspond to the largest DW, AFDW and Chl*a*. The lowest values of DW, AFDW and Chl*a* were observed in high temperature treatments at salinity 1 and 15 ppm (S1T30; S15T30) in treatments in which no growth was observed. In these treatments, the Chl*a* cell^{-1} (**Figure 7A**) was higher at the end of the experiment, as opposed to the general decreasing trend from the beginning to the end of the experiment.

The mean volume of the vegetative cells (116–342 μm^3) and akinetes (520–1054 μm^3) varied without a clear pattern (**Figure 7B**). However, it is noteworthy that the size of vegetative cells in the salinity 30 ppm treatment decreased with increasing temperature, being largest at 15°C. This trend was inverse to the cell density, as smallest vegetative cells at temperature 30°C coincided with highest cell density (**Figure 2**). The mean volume of the akinetes was approximately three times larger than that of the vegetative cells and, unlike these, the largest akinetes were observed in treatments with low cell numbers (S1T23, S15T15). The smallest akinetes were observed in the high salinity and temperature treatment (S30T30) (**Figure 7B**).

Toxin Concentration

All the cultures of *N. spumigena* produced the nodularin toxin at the beginning of the stationary phase, ranging between 1.3 $\mu g\ L^{-1}$ (S1T30) and 6.0 $\mu g\ L^{-1}$ (S7T23) (**Figure 8A**). High values were observed in salinity 7 ppm (S7T15, S7T23, S7T30) associated with large cell density but also in treatments with low cell density and low salinity (S1T15, S1T23) and intermediate (S15T15). The toxin concentration per cell (2.8×10^{-9} to 3.4×10^{-7} pg cell^{-1}) (**Figure 8B**) was generally lower in the treatments of salinity 7 and 30 ppm, i.e., in the treatments with higher cell density. The highest values of toxin per cell were observed at salinity 1 and 15 ppm, particularly in S1T30 (3.4×10^{-7} pg cell^{-1}) and S15T30 (2.0×10^{-7} pg cell^{-1}), i.e., in the treatments with low cell density.

Akinete Germination

After incubation in the dark for 30, 60, 90, 120, 180, and 360 days, akinetes exposed to light at the respective treatments germinated in a relatively high percentage (8–53%) in all the treatments (**Figure 9**). The same germination pattern was observed among the treatments in all the tests. The lowest percentage of germination was always observed in the treatments of salinity

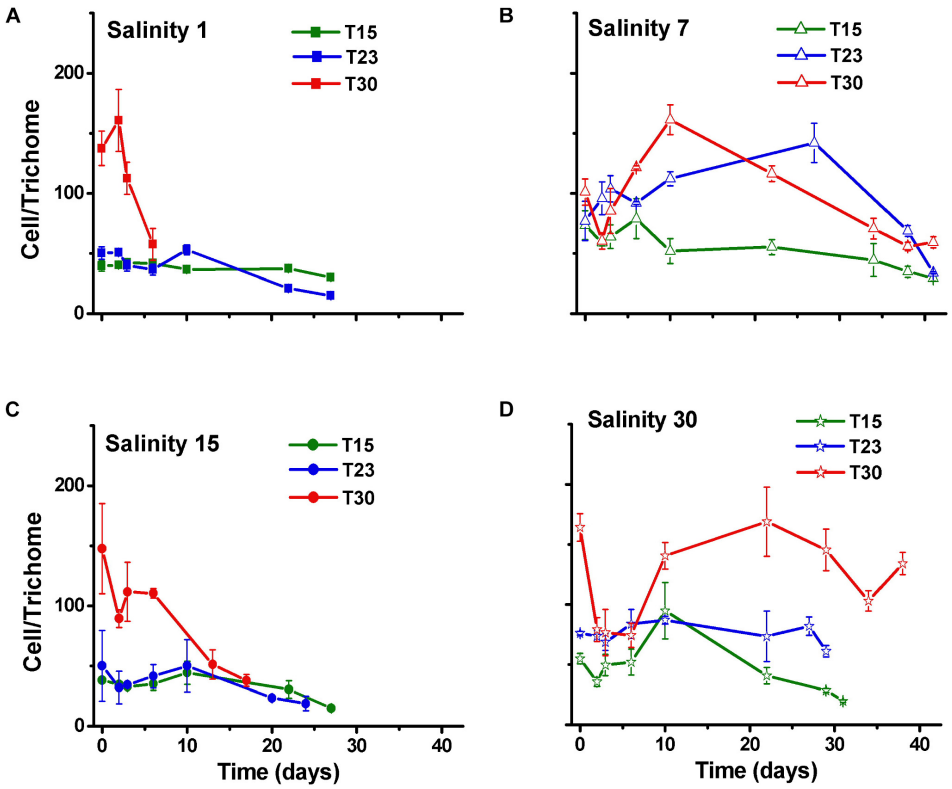


FIGURE 4 | Cell number per trichome (±SD; *n* = 3) of *N. spumigena* (NODSPU1) observed along the time in the factorial experiment, which analyzed the influence of temperature (T15, T23, T30°C) and salinity S1 **(A)**, S7 **(B)**, S15 **(C)**, and S30 **(D)**.

1 ppm, particularly in S1T30 with the lowest germination rate. The highest germination rate was observed in treatments with salinity 7 and 15 ppm (S7T15; S7T23; S7T30; S15T15; S15T23), with the exception of S15T30. These differences between the treatments became more significant after 120 days of akinete incubation in the dark when the germination rate was the highest. The time of exposure to light (72 or 192 h) after the dark incubation did not appear to influence the germination rate.

DISCUSSION

Cell Density, Growth, and Biomass

In our study, the highest cell densities of *N. spumigena* were observed in the treatments with salinity 7 and 30 ppm that demonstrated a relatively high (μ = 0.3–0.4 d^{-1}) growth rate. In the former treatment (S7), the growth persisted for a longer period in the three test temperatures. Thus, it can be considered that this condition was more favorable for the development of *N. spumigena*. The cell growth in salinity 1 ppm was negligible and was different than the results obtained with two strains from the Baltic Sea that demonstrated a similar growth rate over a wide range of salinity (0–20, Moisander et al., 2002). Alternatively, studies conducted with other *N. spumigena* strains isolated from the Baltic Sea did not tolerate salinity values lower than 2 ppm, and the highest growth rate (μ = 0.15–0.20 d^{-1}) was observed in salinity 8–10 ppm under conditions similar to those in our experiment (Mohlin and Wulff, 2009; Rakko and Seppälä, 2014). A strain (001E) from Australia showed a maximum rate of photosynthesis in salinity 6.6 ppm (Hobson and Fallowfield, 2001). Mazur-Marzec et al. (2005) observed a substantial difference when comparing strains from the Baltic Sea and Australia: while the Australian strain (NSPI-05) showed similar growth in treatments exposed to a wide range of salinity (3–35 ppm) with a slight inhibition in salinity 0 ppm, the Baltic Sea strain (NSGG-1), showed higher growth (μ = 0.11 d^{-1}) in salinity 7 ppm, and negligible growth (μ = 0.05 d^{-1}) in zero salinity. Thus, the physiology of the different *Nodularia* strains is complex, since strains isolated in different regions in the same country present different physiological responses. In our study, the 15 ppm salinity treatments, independently of temperature (S15T15; S15T23; S15T30), demonstrated very low growth unlike the salinity 7 and 30 ppm treatments (see **Figure 2**). We repeated the test with the S15 treatment (data not shown), and the same pattern was obtained. To understand this question it would be important to study the biochemical mechanisms that affect growth under the influence of differing salinities. Salinity affects the osmotic equilibrium and concentration of inorganic ions responsible for optimal cell function and growth (Teikari et al., 2018). Photosynthetic activity and cell division can be stopped with salinity changes (Moisander et al., 2002), but it is clear that the strains obtained in different environments, and even

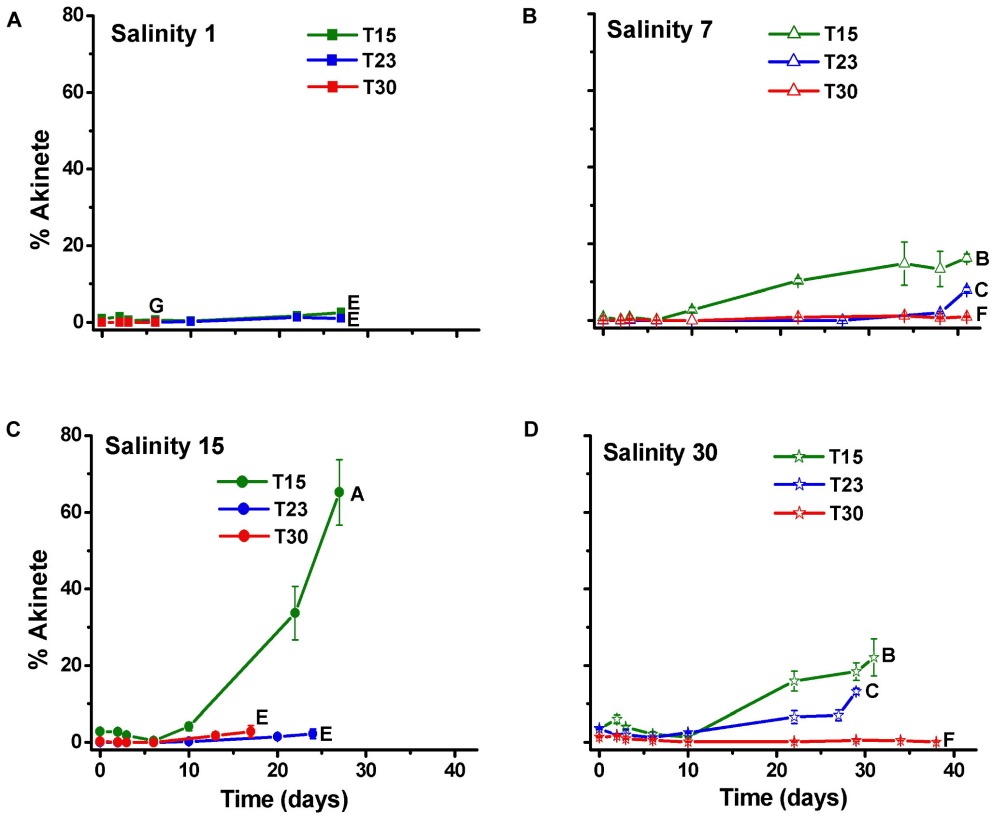


FIGURE 5 | Percentage of akinete per cell (±SD; $n = 3$) of *N. spumigena* (NODSPU1) observed along the time in the factorial experiment, which analyzed the influence of temperature (T15, T23, T30°C) and salinity S1 **(A)**, S7 **(B)**, S15 **(C)**, and S30 **(D)**. Significant difference between treatments is indicated by letters (ANOVA, Tukey's $p < 0.05$).

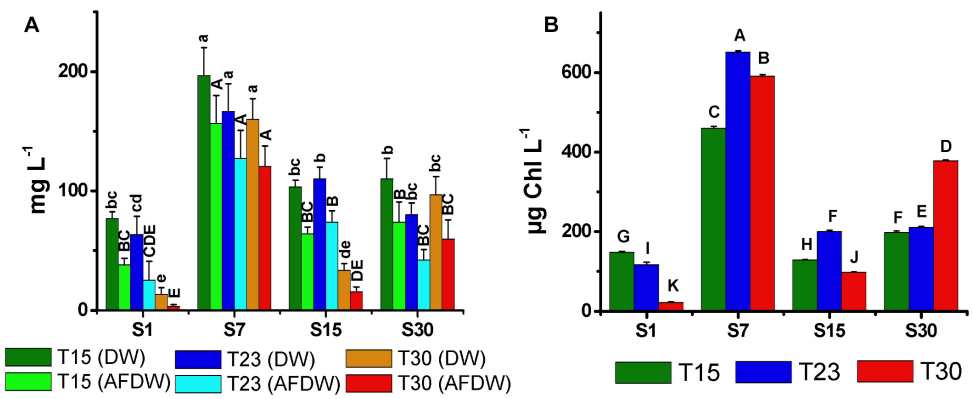


FIGURE 6 | Total biomass dry weight (DW), ash free dry weight (AFDW) **(A)**, and mean concentration of chlorophyll-*a* **(B)** of *N. spumigena* (NODSPU1) (±SD; $n = 3$). Significant difference between treatments is indicated by letters (ANOVA, Tukey's $p < 0.05$). Lower case for DW and upper case for AFDW.

from the same one, do not always show a similar response to variations in salinity.

Our experiment on the effect of temperature showed that the Brazilian *N. spumigena* strain isolated grows equally well in the range tested (15–30°C) with differences related to its interactions with salinity. Studies conducted in the Baltic Sea (Pliński et al., 2007) and Lake Alexandrina, Australia (Hobson and Fallowfield, 2003) showed that temperature was more important and that values above 20°C favored bloom development, which did not happen in our study. Our experiments on growth indicated that the number of cells per trichome of *N. spumigena* generally decreased, particularly in the treatments that reached higher cell density. The shorter trichomes may have resulted from reproduction by hormogonia,

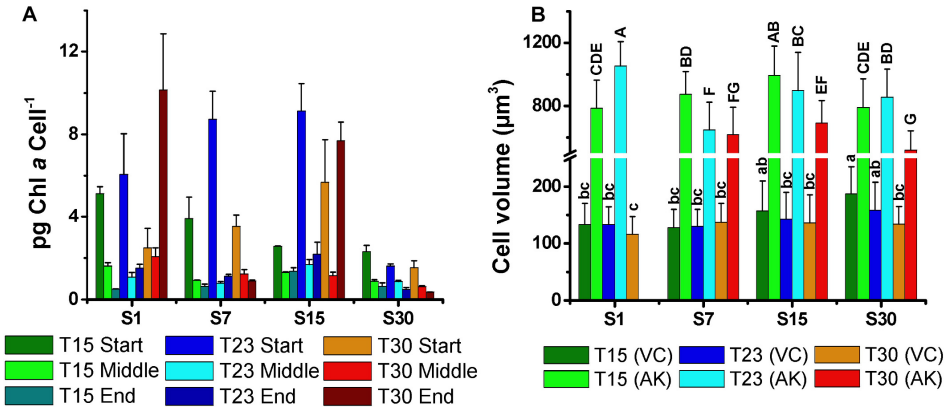


FIGURE 7 | Mean concentration of chlorophyll-*a* per cell (pg cell^{-1}) at the start, middle, and end of growth curves **(A)**, and **(B)** mean cell volume of the vegetative cells (VC) and akinetes (AK) of *N. spumigena* (NODSPU1) (±SD; $n = 3$). Significant difference is indicated by letter (ANOVA, Tukey's $p < 0.05$). Lower case for VC and upper case for AK.

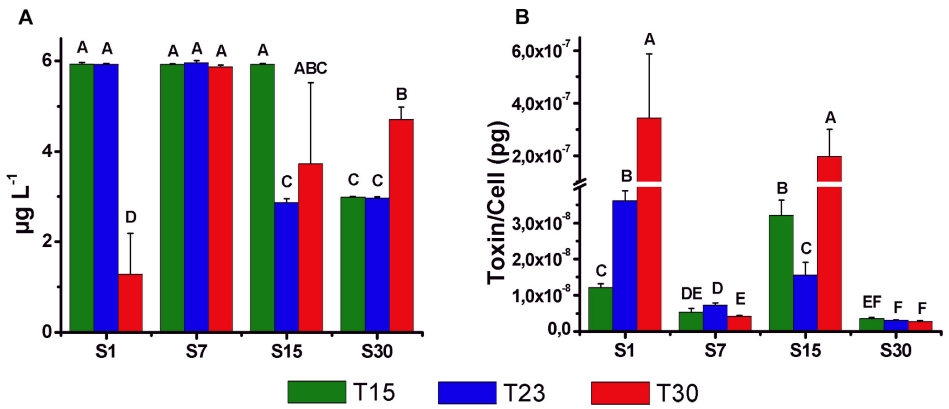


FIGURE 8 | Mean concentration (±SD; $n = 3$) of intracellular nodularin (µg l^{-1}) **(A)** and toxin content per cell (pg cell^{-1}) **(B)** at the beginning of the stationary phase of the growth curves for different treatments. Significant differences are indicated by letters (ANOVA, Tukey's $p < 0.05$).

or even by stress, as reported for *Raphidiopsis brookii* (Yunes et al., 2009). On exception was the high salinity and temperature treatment (S30T30) with the highest cell density of low density of longer trichomes.

Under adverse conditions in the environment, akinetes are responsible for the viability of the populations in the long term. In our experiment, the differentiation of the akinetes markedly increased in the treatments with a lower temperature (15°C), particularly at S15T15 in which 65% of the cells were akinetes, followed by S30T15 and S7T15. In S1T30, the culture did not grow well, and in the other low salinity (S1) treatments, only a few akinetes were observed (<3%). In fact, Li et al. (1997) reported that the differentiation of the akinetes in *Anabaena* spp. is also triggered at low temperatures. It is interesting to note that the volume of the akinetes tended to be smaller at a higher temperature (30°C) (see **Figure 7B**), indicating that low temperatures favor the formation not only of a large number of akinetes but also of a larger size. Salinity apparently did not influence the akinete differentiation or size, as was also found by Myers et al. (2011). However, Jones et al. (1994) reported more akinetes with increased salinity, and Mazur-Marzec et al. (2005) observed a large number of akinetes in high salinity and a reduction at zero salinity. It is likely that the interaction between temperature and salinity, and possibly other factors, are important in the control of akinete differentiation.

The distinct biomass measurements presented significant differences. For example, the S30T30 treatment with the highest cell density, presented low DW and AFDW, possibly due to the observed relatively low number of small akinetes (<5%). The high density of vegetative cells in this treatment was also not associated with Chl*a*, which was highest in the salinity 7 ppm treatments. In the latter, highest Chl*a* in the S7T23 did not correspond with density, which was highest in S7T30 and lowest in S7T23. These differences may be associated with the amount of Chl*a* per cell, indicating a strong reduction compatible with cell growth over time, except for two treatments with minimal growth in high temperature (S1T30; S15T30). Given the constraints described above, and the best correlation obtained between optical density and cell density (**Figure 1**), we suggest using the optical density measurements for estimating the biomass of

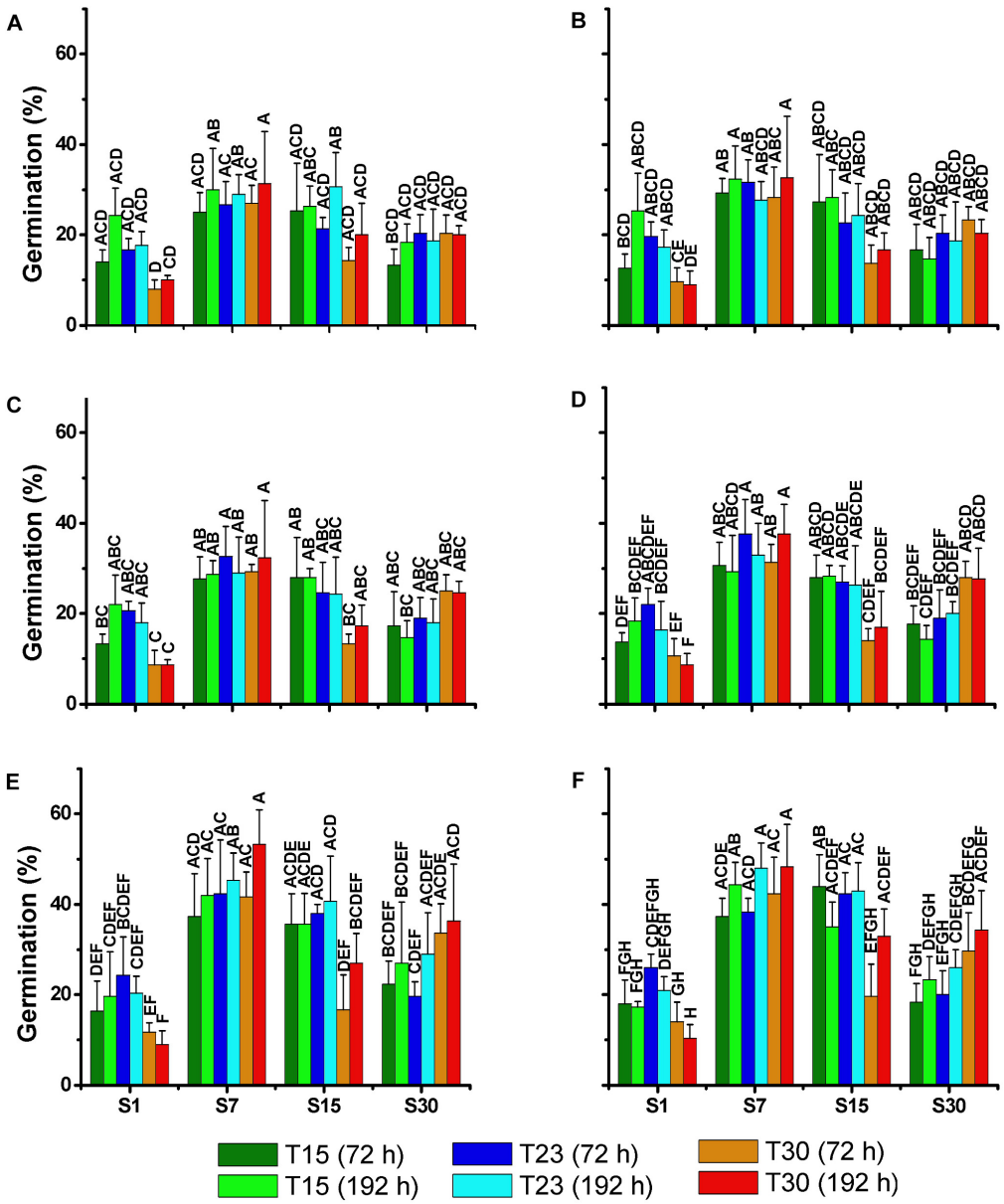


FIGURE 9 | Mean percentage (±SD; $n = 3$) of akinetes germination of *N. spumigena* (NODSPU1) after 72 and 192 h of exposure to different treatments. Significant differences are indicated by letters. Previous to the germination experiment, dark incubation lasted for 30 **(A)**, 60 **(B)**, 90 **(C)**, 120 **(D)**, 180 **(E)**, and 360 **(F)** days. Significant differences for each germination test are indicated by letters (ANOVA, Tukey's $p < 0.05$).

N. spumigena, instead of DW or AFDW. Chl*a* measurements should be analyzed in conjunction with the cell density and cell size, as the latter presents a general pattern of reduction over time (Mohlin and Wulff, 2009).

Nodularin Production

Three *N. spumigena* strains isolated from the same shrimp ponds in southern Brazil were shown to produce nodularin (Silveira et al., 2017). It was also shown that one of the strains contains the genes to produce other bioactive compounds, such as spumigin and anabaenopeptin (Popin et al., 2016). The concentration of nodularin recorded in the stationary phase of our experiment (1.3–6.0 μg L^{-1}) was relatively low, considering that values of 0.8–220 μg L^{-1} (Heresztyn and Nicholson, 1997; McGregor et al., 2012) and greater than 15,000 μg L^{-1} (Mazur and Pliński, 2003) had previously been found in the environment. It must be noted that the ELISA method used in our study does not detect the nodularin variants, such as NOD-R and [D-Asp'] NOD, which can be produced by our strain (Popin, 2017). Thus, the nodularin concentration found in this study is possibly underestimated. However, even low concentrations of nodularin potentially cause environmental problems, such as

animal mortality, contamination of seafood, degradation of water quality (Van Buynder et al., 2001), a reduction in the production rate of copepod eggs (Karjalainen, 2005) and lower fish growth (Pääkkönen et al., 2008). In Southern Brazil, shrimp survival and growth were negatively affected in ponds with *N. spumigena* blooms (Costa et al., 2013).

In the present study, toxin production was not related to temperature or salinity but to cell density (see **Figure 8**), and the highest (underestimated) values ($2.0–3.4 \times 10^{-7}$ pg cell^{-1}) were observed in the salinity treatments 15 and 1 ppm, in which cell growth was reduced. In contrast, low values of nodularin ($3.5–7.3 \times 10^{-9}$ pg cell^{-1}) were recorded in salinity treatments 30 and 7 ppm with higher cell density. This type of relationship could be associated with cellular metabolism, which during the exponential phase is directed to cell growth, while there would be a deviation to toxin production during low growth conditions (Sivonen, 1996; Orr and Jones, 1998). However, Repka et al. (2001) suggested that toxin production would be positively related to cell division, and Hobson and Fallowfield (2003) observed higher production of nodularin at low temperature, indicating that the results cannot be easily generalized to other strains or conditions.

Akinete Germination

The recruitment of resistant cells from the sediment could be a key factor for the success of a species in the plankton community (Hansson, 1996). Knowledge of the conditions that favor akinete germination helps to predict cyanobacterial blooming events and represents an important step in management processes. In our study, the akinetes of *N. spumigena* germinated in all the treatments tested. However, the highest percentage of germination was observed in the treatments with salinity 7 and 15 ppm, in contrast to the lowest in low salinity, especially at a higher temperature (S1T30). A higher germination at salinity values between 5 and 15 ppm was also found in a strain isolated from Australia (Myers et al., 2010). In general, the effect of temperature influenced the akinete germination less than salinity in spite of the slight decrease in germination at high temperature (30°C). Huber (1985) observed that the influence of temperature on akinete germination varies depending on the species with *N. spumigena* demonstrating a high germination rate between 15 and 25°C. The population with the greatest potential recruitment success that was observed from the akinetes in salinity 7 matches our results of cellular growth. However, in salinity 15, high akinete germination coinciding with low cell density indicates that even without significant growth, the akinetes could potentially initiate the development of a population.

An examination of the storage time of the akinetes indicated that germination tended to increase with a longer storage period (see **Figure 9**), as has been observed for both *N. spumigena* (Myers et al., 2010) and *Anabaena circinalis* (Baker and Bellifemine, 2000). The longer storage period resulting in higher germination may be related to the maturation period of the akinetes (Karlsson-Elfgren et al., 2004). Yamamoto (1975) showed that the akinetes of a strain of *Anabaena cylindrica* survived for 5 years in a dark and dry environment.

CONCLUSION

The cyanobacterium *N. spumigena* isolated from shrimp ponds in southern Brazil grows under a wide range of salinity and temperature values, but responded more strongly to salinity. The highest cell densities at the temperatures tested were observed at salinity 7 ppm and the lowest at salinity 1 ppm. The toxin nodularin was produced in all the treatments, demonstrating an inverse relationship between the content of nodularin per cell and cell density. The akinetes were also differentiated in all the treatments, with the exception of S1T30. The largest proportion (65% of cells) occurred in the treatment S15T15 with low cell growth, possibly indicating an effort of the population to survive in the long term. The akinetes also germinated in all the treatments, particularly those cultivated at salinity 7 and 15 ppm with a higher germination percentage (except S15T30). In contrast, the lowest germination was observed in the treatments with salinity 1 ppm. Thus, the low salinity (1 ppm) at all temperatures tested has the potential to inhibit *N. spumigena* blooms, since it hampers cell growth and the differentiation and germination of the akinetes. These results could be useful to manage aquaculture nurseries and production ponds, in which the presence and persistence of *N. spumigena* has been a serious problem.

AUTHOR CONTRIBUTIONS

SS performed all the activities, including the sampling, laboratory experiments, and the final drafting of the results. CO provided support to carry out the work from the planning and execution of the experiments to writing the final draft.

FUNDING

Financial support for this investigation was provided by the Brazilian Long-Term Ecological Research Program (PELD) of the Ministry of Sciences/National Research Council (CNPq, Proc. 403805/2012-0) and FAPERGS (Proc. 003122-2551/12-7) to CO and the Ministry of Education (CAPES) grant to SS.

ACKNOWLEDGMENTS

We would like to thank Dr. Wilson Wasielesky for providing the experimental shrimp ponds of the Marine Station of Aquaculture (EMA), Federal University of Rio Grande-FURG.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2019.00339/full#supplementary-material

REFERENCES

Baker, P. D., and Bellifemine, D. (2000). Environmental influences on akinete germination of *Anabaena circinalis* and implications for management of cyanobacterial blooms. *Hydrobiologia* 427, 65–73.

Blackburn, S., McCausland, M. A., Bolch, C. J. S., Newman, S. J., and Jonas, G. J. (1996). Effect of salinity on growth and toxin production in cultures of the bloom-forming cyanobacterium *Nodularia spumigena* from Australian waters. *Phycologia* 35, 511–522. doi: 10.2216/i0031-8884-35-6-511-1

Carmichael, W. W. (1992). Cyanobacteria secondary metabolites – the cyanotoxins. *J. Appl. Bacteriol.* 72, 445–459. doi: 10.1111/j.1365-2672.1992.tb01858.x

Costa, C. M., Fóes, G. K., Odebrecht, C., Poersh, L., and Wasielesky, W. (2013). "Impacto da cianobactéria Nodularia spumigena em cultivo de camarões marinhos em sistema de bioflocos," in *Proceedings of the Reunião Latino Americana Sobre Algas Nocivas*. Florianópolis

Francis, G. (1878). Poisonous Australian lake. *Nature* 18, 11–12. doi: 10.1038/018011d0

Guillard, R. R. L. (1975). "Culture of phytoplankton for feeding marine invertebrates," in *Culture of Marine Invertebrate Animals*, eds W. L. Smith and M. H. Chanley (New York, NY: Plenum Press).

Hansson, L. A. (1993). Factors initiating algal life-form shift from sediment to water. *Oecologia.* 94, 286–294. doi: 10.1007/BF00341329

Hansson, L. A. (1996). Algal recruitment from lake sediments in relation to grazing sinking, and dominance patterns in the phytoplankton community. *Limnol. Oceanogr.* 41, 1312–1323. doi: 10.4319/lo.1996.41.6.1312

Heresztyn, T., and Nicholson, B. C. (1997). Nodularin concentrations in lakes Alexandrina and Albert, South Australia, during a bloom of the cyanobacterium (Blue-Green Alga) *Nodularia spumigena* and degradation of the toxin. *Environ. Toxicol.* 12, 273–282. doi: 10.1002/(sici)1098-2256(1997)12:4<273::aid-tox1>3.3.co;2-j

Hobson, P., and Fallowfield, H. (2001). Effect of salinity on photosynthetic activity of *Nodularia spumigena*. *J. Appl. Phycol.* 13, 493–499.

Hobson, P., and Fallowfield, H. J. (2003). Effect of irradiance, temperature and salinity on growth and toxin production by *Nodularia spumigena*. *Hydrobiologia* 493, 7–15.

Honkanen, R. E., Dukelow, M., Zwiller, J., Moore, R. R., Khatra, B. S., and Boynton, A. L. (1991). Cyanobacterial nodularin is a potent inhibitor of type 1 and type 2A protein phosphatases. *Mol. Pharmacol.* 40, 577–583.

Huber, A. L. (1985). Factors affecting the germination of akinetes of *Nodularia spumigena* (*Cyanobacteriaceae*). *Appl. Environ. Microb.* 49, 73–78.

Iranshahi, S., Nejadsattari, T., Soltani, N., Shokravi, S., and Dezfulian, M. (2014). The effect of salinity on morphological and molecular characters and physiological responses of Nostoc sp. ISC 101. *Iran. J. Fish. Sci.* 13, 907–917.

Jones, G. J., Blackburn, S. I., and Parker, N. S. (1994). A toxic bloom of *Nodularia spumigena* Mertens in Orielton Lagoon, Tasmania. *Aust. J. Mar. Fresh. Res.* 45, 787–800.

Karjalainen, M. (2005). *Fate and Effects of Nodularia Spumigena and its Toxin, Nodularin, in Baltic Sea planktonic food webs*. Dissertação de Mestrado. Finland: University of Helsinki.

Karlsson-Elfgren, I., Rengefors, K., and Gustafsson, S. (2004). Factors regulating recruitment from the sediment to the water column in the bloom-forming cyanobacterium *Gloeotrichia echinulata*. *Fresh. Biol.* 49, 265–273. doi: 10.1111/j.1365-2427.2004.01182.x

Kim, B. H., Lee, W. S., Kim, Y.-O., Lee, H.-O., and Han, M.-S. (2005). Relationship between akinete germination and vegetative population of *Anabaena flos-aquae* (*Nostocales, Cyanobacteria*) in Seokchon reservoir (Seoul, Korea). *Arch. Hydrobiol.* 163, 49–64. doi: 10.1127/0003-9136/2005/0163-0049

Komárek, J. (2013). "Cyanoprokaryota -3. Teil/Part 3: Heterocytous genera," in *Süßwasserflora von Mitteleuropa Freshwater Flora of Central Europe*, eds B. Büdel, G. Gärtner, L. Krienitz, and M. Schagerl (Heidelberg: Springer).

Kononen, K. (1992). Dynamics of the toxic cyanobacterial blooms in the Baltic Sea. *Finn. Mar. Res.* 261, 3–36.

Ladas, N. P., and Papageorgiou, G. C. (2000). Cell turgor: a critical fator for the proliferation of cyanobacteria at unfavourable salinity. *Photosysth. Res.* 65, 155–164.

Lehtimäki, J., Sivonen, K., Luukkainen, R., and Niemelä, S. I. (1994). The effect of incubation time, temperature, light, salinity and phosphorus on growth and hepatotoxin production by *Nodularia spumigena* strains. *Arch. Hydrobiol.* 130, 269–282.

Li, R., Watanabe, M., and Watanabe, M. M. (1997). Akinete formation in planktonic *Anabaena* spp. (*cyanobacteria*) by treatment with low temperature. *J. Phycol.* 33, 576–584. doi: 10.1111/j.0022-3646.1997.00576.x

Ma, J., Lu, N., Qin, W., Xu, R., Wang, Y., and Chen, X. (2006). Differential responses of eight cyanobacterial and green algal species, to carbamate insecticides. *Ecotox. Environ. Safe.* 63, 268–274. doi: 10.1016/j.ecoenv.2004.12.002

Mackey, K. R. M., Payton, A., Caldeira, K., Grossman, A. R., Moran Mallvin, D., et al. (2013). Effect of temperature on photosynthesis and growth in marine *Synechococcus* spp. *Plant Physiol.* 163, 815–829. doi: 10.1104/pp.113.221937

Mackinney, G. (1941). Absorption of light by chlorophyll solutions. *J. Biol. Chem.* 140, 315–322.

Mazur, H., and Pliński, M. (2003). *Nodularia spumigena* blooms and the occurrence of hepatotoxin in the Gulf of Gdánsk. *Oceanologia* 45, 305–316.

Mazur-Marzec, H., Bertos-Fortis, M., Toruńska-Sitorg, A., Fidor, A., and Legrand, C. (2016). Chemical and genetic diversity of *Nodularia spumigena* from the Baltic Sea. *Mar. Drugs* 14, 209. doi: 10.3390/md14110209

Mazur-Marzec, H., Żegliñska, L., and Pliński, M. (2005). The effect of salinity on the growth, toxin production, and morphology of *Nodularia spumigena* isolated from the Gulf of Gdañsk, southern Baltic Sea. *J. Appl. Phycol.* 17, 171–179. doi: 10.1007/s10811-005-5767-1

McGregor, G. B., Stewart, I., Sendall, B. C., Sadler, R., Reardon, K., Carter, S., et al. (2012). First report of a toxic *Nodularia spumigena* (*Nostocales/Cyanobacteria*) bloom in sub-tropical Australia. I. phycological and public health investigations. *Int. J. Environ. Res. Public Health* 9, 2396–2411. doi: 10.3390/ijerph9072396

Mohlin, M., and Wulff, A. (2009). Interaction effects of ambient UV radiation and nutrient limitation on the toxic cyanobacterium. *Microbial Ecol.* 57, 675–686. doi: 10.1007/s00248-008-9427-2

Moisander, P. H., McClinton, E., III., and Paerl, H. W. (2002). Salinity effects on growth, photosynthetic parameters, and nitrogenase activity in estuarine planktonic cyanobacteria. *Microb. Ecol.* 43, 432–442. doi: 10.1007/soo248-001-1044-2

Myers, J. H., Beardall, J., Allinson, G., Salzman, S., and Gunthorpe, L. (2010). Environmental influences on akinete germination and development in *Nodularia spumigena* (*Cyanobacteriaceae*), isolated from the Gippsland Lakes, Victoria, Australia. *Hydrobiologia* 649, 239–247. doi: 10.1007/s10750-010-0252-5

Myers, J. H., Beardall, J., Allinson, G., Salzman, S., Robertson, S., and Gunthorpe, L. (2011). Potential triggers of akinete differentiation in *Nodularia spumigena* (Cyanobacteriaceae) isolated from Australia. *Hydrobiologia* 671, 165–180. doi: 10.1007/s10750-011-0714-4

Ohta, T., Sueoka, E., Iida, N., Komori, A., Suganuma, M., Nishiwaki, R., et al. (1994). Nodularin, a Potent inhibitor of protein phosphstases 1 and 2A, is a new environmental carcinogen in male F344 rat liver. *Cancer Res.* 54, 6402–6406.

Orr, P. T., and Jones, G. J. (1998). Relationship between microcystin production and cell division rates in nitrogen-limited *Microcystis aeruginosa* cultures. *Limnol. Oceanogr.* 43, 1604–1614. doi: 10.4319/lo.1998.43.7.1604

Pääkkönen, J.-P., Rönkkönen, S., Karjalainen, M., and Viitasalo, M. (2008). Physiological effects in juvenile three-spined sticklebacks feeding on toxic cyanobacterium *Nodularia spumigena*-exposed zooplankton. *J. Fish. Biol.* 72, 485–499. doi: 10.1111/j.1095-8649.2007.01707.x

Pliński, M., Mazur-Marzec, H., Jóźwiak, T., and Kobos, J. (2007). The potential causes of cyanobacterial bloom in Baltic Sea estuaries. *Int. J. Oceanogr. Hydrobiol.* 36, 125–137. doi: 10.2478/v10009-007-0001-x

Popin, R. V. (2017). *Análise genômica e funcional da Nodularia spumigena CENA596 formadora de florações em tanques de produção de camarões*. (Dissertação de Mestrado). Piracicaba: Universidade de São Paulo.

Popin, R. V., Rigonato, J., Abreu, V. A. C., Andreote, A. P. D., Silveira, S. B., Odebrecht, C., et al. (2016). Draft genome assembly of the bloom-forming cyanobacterium *Nodularia spumigena* strain CENA596 in shrimp production ponds. *Genome Announc.* 4, e466–e416. doi: 10.1128/genomeA.00466-416

Rakko, A., and Seppälä, J. (2014). Effect of salinity on the growth rate and nutrient stoichiometry of two Baltic Sea filamentous cyanobacterial species. *Estonian J. Ecol.* 63, 55–70. doi: 10.3176/eco.2014.2.01

Repka, S., Mehtonen, J., Vaitomaa, J., Saari, L., and Sivonen, K. (2001). Effects of nutrients on growth and nodularin production of Nodularia strain GR8b. *Microb. Ecol.* 42, 606–613. doi: 10.1007/s00248-001-0026-8

Reynolds, C. S. (1984). *The Ecology of Freshwater Phytoplankton*. Cambridge: Cambridge University Press.

Rinehart, K. L., Harada, K., Namikoshi, M., Chen, C., Harvis, C. A., Munro, M. H. G., et al. (1988). Nodularin, microcystin, and the configuration of adda. *J. Am. Chem. Soc.* 110, 8557–8558. doi: 10.1021/ja00233a049

Silveira, S. B., Wasielesky, W., Andreote, A. P. D., Fiore, M. F., and Odebrecht, C. (2017). Morphology phylogeny, growth rate and nodularin production of *Nodularia spumigena* from Brazil. *Mar. Biol. Res.* 13, 1095–1107. doi: 10.1080/17451000.2017.1336587

Singh, S. C., Sinha, R. P., and Häder, D. P. (2002). Role of lipids and fatty acids in stress tolerance in cyanobacteria. *Acta Protozool.* 41, 296–308.

Sivonen, K. (1996). Cyanobacteria toxins and toxin production. *Phycologia* 35, 12–24. doi: 10.2216/i0031-8884-35-6S-12.1

Sukenik, A., Beardall, J., and Hadas, O. (2007). Photosynthetic characterization of developing and mature akinetes of *Aphanizomenon ovalisporum* (*Cyanoprokaryota*). *J. Phycol.* 43, 780–788. doi: 10.1111/j.1529-8817.2007.00374.x

Sun, J., and Liu, D. (2003). Geometric models for calculating cell biovolume and surface area for phytoplankton. *J. Plankton Res.* 25, 1331–1346. doi: 10.1093/plankt/fbg096

Teikari, J. E., Hou, S., Wahlsten, M., Hess, W. R., and Sivonen, K. (2018). Comparative genomics of the Baltic Sea toxic cyanobacteria *Nodularia spumigena* UHCC0039 and its response to varying salinity. *Front. Microbiol.* 9:356. doi: 10.3389/fmicb.2018.00356

Van Buynder, P. G., Oughtred, T., Kirkby, B., Phillips, S., Eaglesham, G., Thomas, K., et al. (2001). Nodularin uptake by seafood during a cyanobacterial bloom. *Environ. Toxicol.* 16, 468–471. doi: 10.1002/tox.10004

Wood, A. M., Everroad, R. C., and Wingard, L. M. (2005). "Measuring growth rates in microalgal cultures," in *Algal Culturing Techniques*, ed. R. Andersen (Cambridge: Academic Press).

Yamamoto, Y. (1975). Effect of desiccation on the germination of akinetes of *Anabaena cylindrical*. *Plant Cell. Physiol.* 16, 749–752. doi: 10.1093/oxfordjournals.pcp.a075195

Yunes, J. S., De La Rocha, S., Giroldo, D., Silveira, S. B., Comin, R., Bicho, M. S., et al. (2009). Release of carbohydrates and proteins by a subtropical strain of *Raphidiopsis brookii* (*Cyanobacteria*) able to produce saxitoxin at three nitrate concentrations. *J. Phycol.* 45, 585–591. doi: 10.1111/j.1529-8817.2009.00673.x

Zar, J. H. (2010). *Biostatistical Analysis*, 5th Edn. New Jersey: Pearson.

Zhu, C. J., and Lee, Y. K. (1997). Determination of biomass dry weight of marine microalgae. *J. Appl. Phycol.* 9, 189–194.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Silveira and Odebrecht. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Marine Science

MINI REVIEW
published: 20 August 2019
doi: 10.3389/fmars.2019.00463

The State of Knowledge of Harmful Algal Blooms of *Margalefidinium polykrikoides* (a.k.a. *Cochlodinium polykrikoides*) in Latin America

David J. López-Cortés[1†], Erick J. Núñez-Vázquez[1,2], Juan J. Dorantes-Aranda[3], Christine J. Band-Schmidt[4], Francisco E. Hernández-Sandoval[1], José J. Bustillos-Guzmán[1], Ignacio Leyva-Valencia[5] and Leyberth J. Fernández-Herrera[4]*

[1] Centro de Investigaciones Biológicas del Noroeste, La Paz, Mexico, [2] Investigación para la Conservación y el Desarrollo, La Paz, Mexico, [3] Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, TAS, Australia, [4] Instituto Politécnico Nacional-Centro Interdisciplinario de Ciencias Marinas, La Paz, Mexico, [5] CONACyT, Instituto Politécnico Nacional-Centro Interdisciplinario de Ciencias Marinas, La Paz, Mexico

OPEN ACCESS

Edited by:
Angel Borja,
Centro Tecnológico Experto en Innovación Marina y Alimentaria (AZTI), Spain

Reviewed by:
Maximo Jorge Frangopulos,
University of Magallanes, Chile
Kyoko Yarimizu,
National Research Institute of Fisheries Science, Japan Fisheries Research and Education Agency, Japan

***Correspondence:**
Erick J. Núñez-Vázquez
enunez04@cibnor.mx

[†]Deceased

Specialty section:
This article was submitted to Marine Ecosystem Ecology, a section of the journal Frontiers in Marine Science

Received: 05 July 2018
Accepted: 10 July 2019
Published: 20 August 2019

Citation:
López-Cortés DJ, Núñez-Vázquez EJ, Dorantes-Aranda JJ, Band-Schmidt CJ, Hernández-Sandoval FE, Bustillos-Guzmán JJ, Leyva-Valencia I and Fernández-Herrera LJ (2019) The State of Knowledge of Harmful Algal Blooms of Margalefidinium polykrikoides (a.k.a. Cochlodinium polykrikoides) in Latin America. Front. Mar. Sci. 6:463. doi: 10.3389/fmars.2019.00463

The marine dinoflagellate *Margalefidinium polykrikoides* is a harmful species that has affected aquaculture, fisheries and tourism activities. It produces reactive oxygen species (ROS) as well as hemolytic and neurotoxic-like substances that have been associated with mass mortalities of marine organisms. It has a tropical and subtropical distribution that has mainly affected Asia and North America. The economic impacts for aquaculture industries have been estimated to be up to US$140M. In Latin America, no economic estimates have been performed. Harmful algal blooms by *M. polykrikoides* are more frequent in Mexico and Central America. Proliferations of this dinoflagellate are associated with winds, upwelling, convergence areas, local convection of seawater, and eutrophication of coastal areas by nitrogen and phosphorus compounds from rainwater runoff, as well as agricultural and aquaculture activities, into coastal waters. Eco-physiological and toxicological studies have provided detailed descriptions of the growth of algal strains from these regions and the harmful effects on fish and shrimp, as well as the role the production of ROS and polyunsaturated fatty acids in their toxicity. It is also possible that *M. polykrikoides* has an ecological role in the regulation of blooms of other harmful algae. In this contribution, we review the records of harmful algal blooms of *M. polykrikoides* in Latin America and the research that has been conducted with this species.

Keywords: dinoflagellates, *Margalefidinium polykrikoides*, *Cochlodinium polykrikoides*, HAB, Latin America

INTRODUCTION

Global Harmful Algal Blooms of *M. polykrikoides*

Margalefidinium polykrikoides (=*Cochlodinium polykrikoides*) is a photosynthetic and mixotrophic marine dinoflagellate that forms cysts. It forms harmful algal blooms (HABs), causing economic losses in fish farming areas (US$140M in world wide; Dorantes-Aranda, 2012) mainly in Asia (Kim, 1998; Kim et al., 1999; Zhong and Gobler, 2009). *M. polykrikoides* was first identified in

Phosphorescent Bay, Puerto Rico by Margalef (1961), and since then *Margalefidinium* blooms have been reported across Asia, Europe and North America. In the last three decades, *Margalefidinium* HABs have impacted coastal regions of Australia, Canada, China, Croatia, India, Iran, Italy, Japan, Korea, Malaysia, Oman, Philippines, Russia, Saudi Arabia, United Arab Emirates, and the United States (Kudela and Gobler, 2012). The largest bloom recorded occurred in the Arabian Gulf from August to October 2008 affecting 1,200 km of coastline, killing wild, and farmed fish as well as damaging coral reefs (Richlen et al., 2010). A wide variety of harmful substances have been reported for this species (**Figure 1**), including reactive oxygen species (ROS) (superoxide anion, hydrogen peroxide, hydroxyl radical), hemolytic and neurotoxic-like substances, hemagglutinins, and free polyunsaturated fatty acids (Onoue and Nozawa, 1989; Hallegraeff, 1992; Lee, 1996; Landsberg, 2002; Jeong et al., 2004; Dorantes-Aranda et al., 2009, 2010). The genus *Cochlodinium* was established in the late nineteenth century with the identification of *C. strangulatum* (Schütt, 1895), and the first record of this genus in the Gulf of California occurred in 1942 (Osorio-Tafall, 1943). Recently, *C. polykrikoides* was re-assigned to the new genus *Margalefidinium* and renamed as *M. polykrikoides* (Gómez et al., 2017). In North America, HABs of *M. polykrikoides* have been observed in Canada and the US with impacts on fish and shellfish industries (Whyte et al., 2001; Gobler et al., 2008, 2012; Tomas and Smayda, 2008; Mulholland et al., 2009; Zhong and Gobler, 2009; Griffith et al., 2019). The damage by a *M. polykrikoides* bloom in Canada was estimated in a loss of US$2M in 1999 (Whyte et al., 2001). In Latin America (LA) there are no estimates of the economic losses caused by this dinoflagellate. The purpose of this work is to review the HABs of *M. polykrikoides* and the impacts they have caused in LA by country.

MEXICO

Gulf of California

M. polykrikoides was first observed in Bahía de Mazatlán, Sinaloa, in spring of 1979 proliferating together with *Gymnodinium catenatum*. It was suggested that *M. polykrikoides* HABs are commonly formed in Bahía de Mazatlán during winter and early spring coinciding with the upwelling of cold waters in the region (Morey-Gaines, 1982; Cortés-Altamirano, 1987). From September to October 2000, *M. polykrikoides* was present during 22 days causing a notable odor and fish mortalities (Cortés-Altamirano and Gómez-Aguirre, 2001; Cortés-Altamirano et al., 2002; **Table 1**). Fish kills were also reported in 2003 and 2012 (Cortés-Altamirano et al., 2019). A record of HABs in Bahía de Mazatlán between 1979 and 2014 by Cortés-Altamirano et al. (2019) revealed that this species forms blooms during summer and fall when El Niño exerts its inhibitory effect. This dinoflagellate has also been reported forming blooms in shrimp farming ponds and coastal lagoons of Sinaloa with adverse effects such has fish, oysters and octopus mortality, as well as skin hyperpigmentation in swimmers (Alonso-Rodríguez et al., 2004, 2008). Despite the occurrence of mortalities, the economic impacts of these blooms have not been determined.

An outbreak of this dinoflagellate was reported for the first time in Bahía de La Paz, in the southern part of the Gulf of California, in September 2000 (Gárate-Lizárraga et al., 2000). Chlorophyll *a* concentrations ranged from 2.7 to 56.8 mg L^{-1}. Gárate-Lizárraga et al. (2004) described another bloom in this bay in November 2000. The bloom emerged after 2 days of heavy rain and wind coinciding with an increase in nutrient concentrations (**Table 1**). Another bloom occurred in September-November 2001, which extended outside the bay probably a result of Hurricane Juliette; mortalities of 166 fish (76 adults and 90 sub-adults fish) were observed in ponds, including the species *Lutjanus peru* (n = 102), *Pomadasys macracanthus* (n = 60), and *L. argentiventris* (n = 4), with abundant cells of *M. polykrikoides* observed in the gills (Núñez-Vázquez et al., 2003; Gárate-Lizárraga et al., 2004; **Figure 2**). Muciño-Márquez (2010) also reported *M. polykrikoides* near tuna pens in Bahía de la Paz in September 2006, however, no adverse effects were reported.

López-Cortés et al. (2014) followed the development of a bloom of *M. polykrikoides* in Bahía de La Paz from the beginning (September) to its decay (October) in 2012. This event reached a maximum cell density of 8.6×10^6 cell L^{-1}, with a chlorophyll *a* content of 121.2 mg m^{-3} and peridinin of 40.2 mg m^{-3}, coinciding with a N:P (nitrogen:phosphorus) ratio of 2:3. This event was associated with NNE winds and rain, which may have contributed to the enrichment of nutrients in the water column; the direction of the wind changed to the SE, and its intensity decreased to <1.3 m s^{-1}, which was when the bloom appeared. No mortality of marine organisms were observed during this period. According to Gárate-Lizárraga (2013) and López-Cortés et al. (2014), recurrent blooms of *M. polykrikoides* in this bay are associated with the mixing of the water column and upwelling of deep waters.

Mexican Pacific

The first record of *M. polykrikoides* was recorded in Bahía de Manzanillo, Colima in 2000 (López, 2000; Morales-Blake et al., 2001). Another extensive bloom by *Margalefidinium* sp. was reported during late winter and early spring of 1999, where the paralytic toxin producing dinoflagellate *G. catenatum* was also found. However, no impacts in wildlife were reported (Morales-Blake et al., 2000). Another event was recorded in this bay between March and May in 2007, involving again several HAB species such as *Akashiwo sanguinea*, *Karenia mikimotoi*, *G. catenatum* as well as *M. polykrikoides*. This event was associated with upwelling of deep waters (González-Chan et al., 2007).

The most extensive bloom by *M. polykrikoides* (1.1×10^6–3.0×10^6 cell L^{-1}) reported in Mexico lasted 12 weeks (September to November 2000), affecting 63 km of coastline in Bahía de Banderas. This event impacted tourism activities in Puerto Vallarta and Nuevo Vallarta in the states of Jalisco and Nayarit, respectively (Cortés-Lara et al., 2004). Another *M. polykrikoides* bloom was reported in the area the following year (Cortés-Lara, 2002). Cortés-Lara (2002) mentioned that the phenomenon remained for 5 months in Puerto Vallarta causing

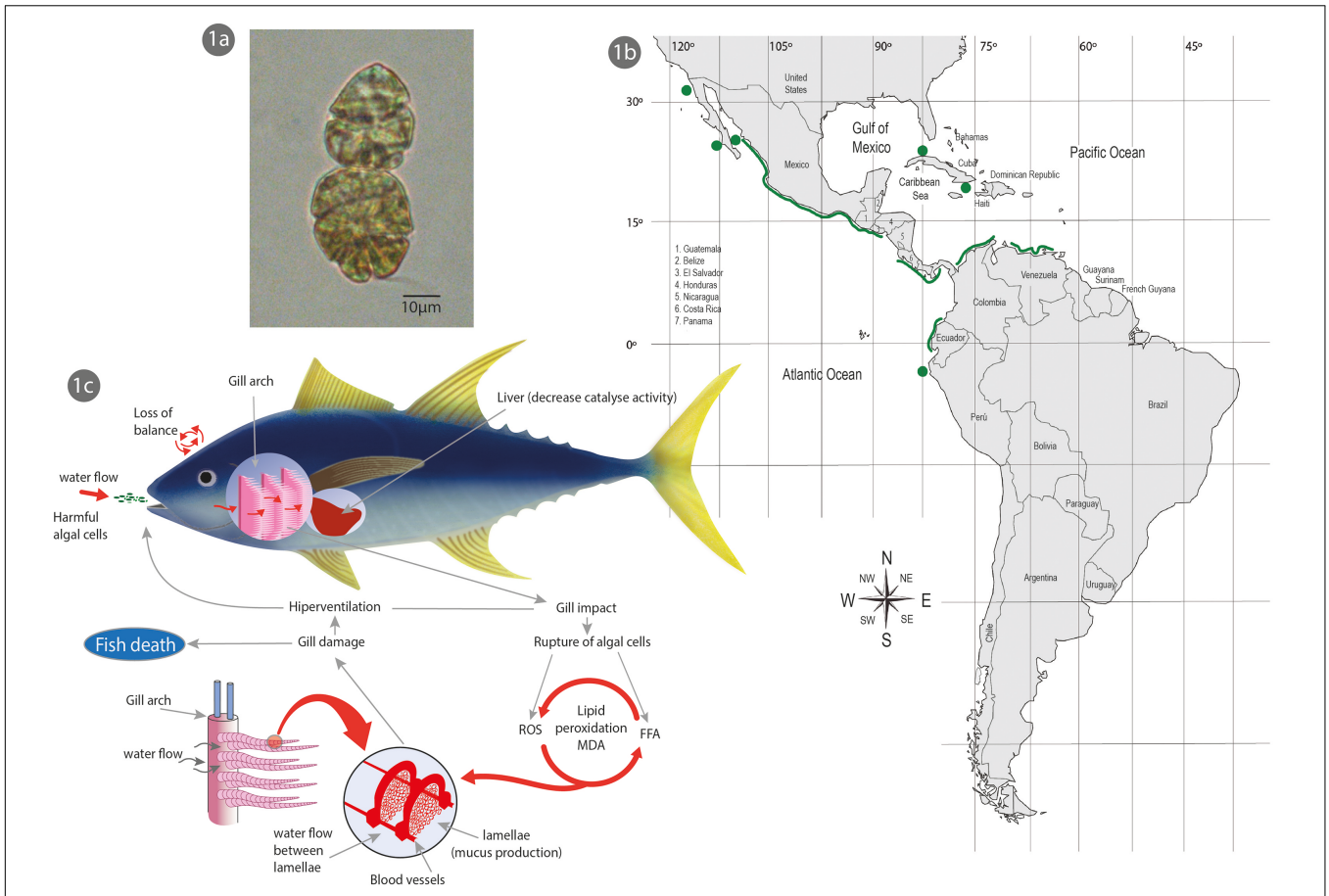


FIGURE 1 | (a) Microscopic image (63×) of *Margalefidinium polykrikoides* in culture (chain of two cells), isolated from Bahía de La Paz, Gulf of California, Mexico. Scale bar = 10 µm. **(b)** HABs records of *M. polykrikoides* in coastal waters of Latin America. **(c)** Suggested route of ichthyotoxicity caused by *M. polykrikoides*: fish gills are impacted when algal cells in the water lyse, releasing reactive oxygen species (ROS) and free polyunsaturated fatty acids that cause lipid peroxidation and physiological disturbances leading to fish mortality (modified after Hallegraeff et al., 2017).

mortality of eels, octopuses, and 13 species of fish, including flounders, sardines, snappers and puffers. Gómez-Villarreal et al. (2008) monitored HABs in Bahía Banderas using satellite images during 2000 and 2001 (**Figure 2**). High chlorophyll *a* levels were associated with blooms of *M. polykrikoides* during the summer-fall season, which were more intense in 2000 than in 2001, with a range in cell abundances from 7×10^3 to 1.2×10^6 cell L^{-1} in 2000, and an average cell abundance of 1×10^6 cell L^{-1} in 2001.

Gárate-Lizárraga and Muñetón-Gómez (2008) reported mortalities of farmed tuna in Bahía Magdalena, B.C.S associated with *M. polykrikoides* and reported that this dinoflagellate is distributed from Ensenada, B.C. to the coasts of Oaxaca. Gárate-Lizárraga et al. (2009) also reported HAB events of *M. polykrikoides* and *G. catenatum* in Bahía de Acapulco. Both species were recorded from December 2005 to December 2007. The abundance of *M. polykrikoides* ranged from 39×10^3 cell L^{-1} in January 2008 to $8{,}228 \times 10^3$ cell L^{-1} in December 2005, within a thermal range of 25–29°C; however, no mortalities of marine organisms were observed. Maciel-Baltazar and Hernández-Becerril (2013) reported this species in 2009 for the first time in the Gulf of Tehuantepec.

Recently, Fimbres-Martínez et al. (2018), Fimbres-Martínez (2019) described the presence of *Margalefidinium* sp. and raphidophytes of the genera *Chattonella*, *Heterosigma* and *Fibrocapsa* in Bahía de Todos Santos, in the northern Pacific of Mexico, which were the suspected cause of mortalities of farmed tuna (*Thunnus thynnus*) in this region (García-Mendoza et al., 2018).

Experimental Studies

During the last decade, efforts have been directed toward the isolation of strains from Bahía de La Paz to study their ecology and toxicology. Growth rates calculated for these strains varied between 0.11 and 0.39 div day^{-1} with maximum biomasses of 7.1 ± 0.5 and 9.4–11.0 × 10^6 cell L^{-1} using modified GSe and GSe media (Dorantes-Aranda et al., 2009, 2010; Zumaya-Higuera, 2017). Some authors have suggested that *M. polykrikoides* is an invasive species that has been transported by seawater currents and ship ballast water (Sierra-Beltrán et al., 2001; Cortés-Altamirano et al., 2006; Meave del Castillo, 2014; Páez-Osuna et al., 2017). However, the sequences of strains isolated from Bahía de La

TABLE 1 | Events of harmful algal blooms by the ichthyotoxic dinoflagellate *Margalefidinium polykrikoides* in Latin America.

Country and region	Date	Number of cells (cell L^{-1})	Temperature (°C)	Impact	References
Mexico					
Bahía de Mazatlán	April 1985	5.0×10^5	∼20.0	Mortality of fish, crustaceans and annelids	Cortés-Altamirano, 1987
	September–October 2000	8.9×10^5 to 3.4×10^6	No data	Fish mortality	Cortés-Altamirano and Gómez-Aguirre, 2001
	2003 and 2012	No data	No data	Fish kill	Cortés-Altamirano et al., 2019
Bahía de Manzanillo	March, April, and May 1999	No data	23.0–25.0	Foam accumulation	Morales-Blake et al., 2000
	March–April 2000	3.2×10^6	21.2–25.5/34.5–34.6	No data	Morales-Blake et al., 2001
Puerto de Manzanillo	March–May 2007	4.0×10^3	24.8–32.6	No data	González-Chan et al., 2007
Bahía de Banderas	October–November 2001	3.0×10^6 to 1.1×10^6	No data	Fish mortality. Skin irritation, excess of nasal mucosa, eyes, and dermatitis in humans	Cortés-Lara, 2002
	July–December 2000	10.8×10^6	25.0–32.0/33–35	Fish mortality. Impact in tourist activities	Cortés-Lara et al., 2004
	2000–2001	7.0×10^3–1.2×10^6 1.0×10^6	No data	No data	Gómez-Villarreal et al., 2008
Ensenada de La Paz, Bahía de La Paz	15–28 September 2000	3.6×10^5 to 7.0×10^6	29.0–321.50	Fish mortality	Gárate-Lizárraga et al., 2000; Núñez-Vázquez et al., 2003; Gárate-Lizárraga et al., 2004
Ensenada de La Paz, Bahía de La Paz	September 2000	3.6×10^5 to 7.0×10^6	20.0–32.5	Fish mortality	Gárate-Lizárraga et al., 2004
Bahía de La Paz	April–June 2006, April 2007	3.6×10^5 to 8.0×10^9	20.0–26.0 and 29.0–31.0	Fish mortality	Gárate-Lizárraga and Muñetón-Gómez, 2008
Bahía de La Paz	August 16–17, 2012	0.073–1.4×10^6	29.0–30.0	No impact	Gárate-Lizárraga, 2013
Ensenada de La Paz, Bahía de La Paz	September–November 2012	6.2×10^5–8.6×10^6	30.3–31.3/35.2–36.8	No impact	López-Cortés et al., 2014
Bahía de Acapulco	March 1999–October 2008	0.7–7.8×10^4 to 8.2×10^6	25.0–29.0	No impact	Gárate-Lizárraga et al., 2009
Cuba					
Bahía de Santiago	April–May 2005	No data	No data	Mortality of juvenile wild fishes	Gómez et al., 2007
Channels of Marine Hemingway in Havana	September 2015	1.8×10^6 to 7.5×10^5		No Impact	
Guatemala					
Guatemala Coast	May 28, 2004	9.6×10^5	30.3–32.7	Fish mortality	Carrillo-Ovalle et al., 2007
	January 8–29 2007	6.9×10^6	28.0	Fish mortality	
El Salvador					
Punta Roca and Puerto de Punta Libertad	March 2012	2.1×10^8	28.5–34.8	Fish mortality, putrid odors, headache to tourists and local people	Espinoza et al., 2013
La Libertad	October 2013	No data	No data	Massive sea turtle mortality	Amaya et al., 2014
Costa Rica					
Gulf of Nicoya	February–March 1979	80.0×10^6	28.0/32.2	Co-occurring with other toxic species, damage to human health, such as. . .	Hargraves and Víquez, 1981

(Continued)

TABLE 1 | Continued

Country and region	Date	Number of cells (cell L^{-1})	Temperature (°C)	Impact	References
Gulf of Nicoya	June–November 1986	2.2×10^6	26.0–31.0	Mortality of fish and coral	Guzmán et al., 1990; Víquez and Hargraves, 1995
Isla Caño	July 12, 1985	8.3×10^5			
Pacific Coast	November 2000–December 2001	14.4×10^6	No data	Co-occurring with others toxic species, impact fisherman's economy	Valverde, 2002
Pacific Coast	May 2002	1.2×10^5	No data	Fish mortality	Vargas-Montero and Freer, 2004
Pacific coast	January 2003–June 2004	1.7×10^8	No data	Fish mortality	Vargas-Montero et al., 2006
Pacific coast	April 2004	3.8×10^8	No data	Fish mortality, foam formation on the beach	Vargas-Montero et al., 2008
Gulf of Nicoya	January 2008–December 2010	11.7×10^6	26.6–28.7/27.9–31.4	Unpleasant odor, sickness and vomiting in children	Calvo et al., 2016
Colombia					
Caribe, Bahía de Santa María	October 2010 and November 2011	5.0×10^6	29.0–31.0/~20.0–33.0	No impact	Malagón and Perdomo, 2013
	2010–2017	No data	No data	No impact	Cuellar-Martínez et al., 2018
Ecuador					
Puerto bolívar	February 1992	5.2×10^6	No data	No data	Torres-Zambrano, 2000
Estero salado	1993				
Guayas river	June 1999				
Peru					
Bahía de Sechura	February 2010, May 2014	9.6×10^5	17.1–23.3/32.8	No data	Orozco et al., 2017

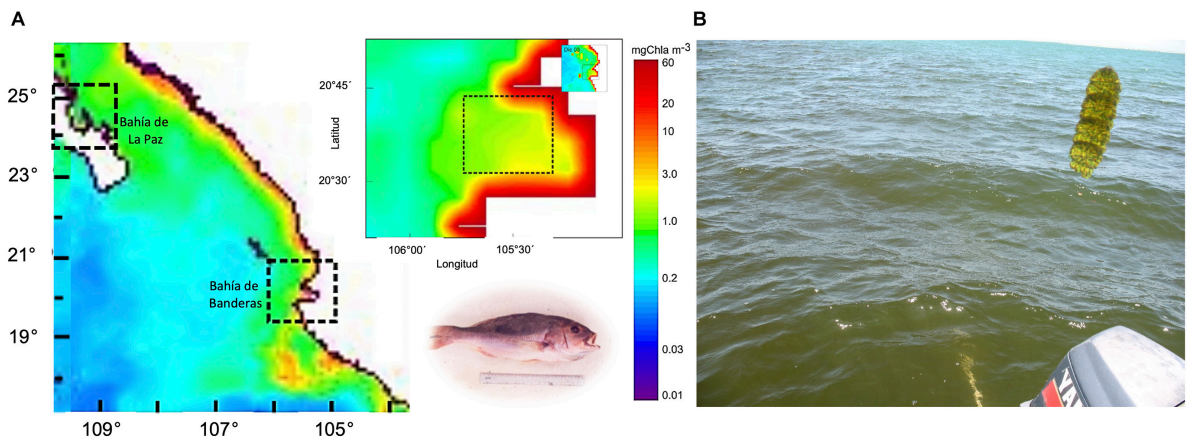


FIGURE 2 | (A) Satellite images showing chlorophyll *a* concentration in mgChla m^{-3} in December 2001. Dotted box corresponds to Bahía de La Paz and Bahía de Banderas, some of these blooms could have been formed by *M. polykrikoides*. Maps from SeaWiFS level 3 images. The bottom left image shows fish kills of Pacific red snappers (*Lutjanus peru*) in culture ponds caused by *M. polykrikoides* in Bahía de La Paz in 2001–2002. **(B)** Brown coloration of the sea surface created by *M. polykrikoides* in Bahía de la Paz (2016).

Paz were identical to that of American and Malaysian strains (Zumaya-Higuera, 2017).

Núñez-Vázquez et al. (2003) observed that fish juveniles of *Mugil* sp. died when exposed to *M. polykrikoides* at a cell abundance of 4.1×10^6 cell L^{-1}. However, cell extracts showed no toxicity by mouse bioassay and did not have adverse effects on juvenile shrimp *Litopenaeus vannamei*. However, Pérez-Morales et al. (2017) reported 100% mortality of *L. vannamei* zoea larvae after exposing them for 120 h to cells of *M. polykrikoides* (3.0×10^6 cell L^{-1}). Dorantes-Aranda et al. (2010), also observed

100% mortality of the spotted rose snapper *Lutjanus guttatus* after exposure to $\geq 3.0 \times 10^6$ cell L^{-1} of *M. polykrikoides*. Fish showed loss of balance, breathing difficulty, oxidative stress in gill lamellae and liver, abnormal production of mucus and asphyxiation, suggesting that the production of ROS by the dinoflagellate caused oxidative damage that lead to their death. In a complimentary study, the same authors observed that extracts from 5.2×10^6 *M. polykrikoides* cell L^{-1} and 27.0×10^6 cell L^{-1} caused 50% hemolysis in *L. guttatus* and human erythrocytes, respectively. The authors also reported hexadecaenoic (16:0), docosahexaenoic (22:6n3), and octadecapentanoic (18:5n3) as the most abundant fatty acids (Dorantes-Aranda et al., 2009). The latter fatty acid has also been found in other nocive microalgae showing fish cell toxicity, suggesting that it plays a key role in the ichthyotoxicity caused by microalgae (Dorantes-Aranda et al., 2009; Mooney et al., 2011). The possible ichthyotoxic pathway of *M. polykrikoides* is shown in **Figure 1c**, as suggested by Hallegraeff et al. (2017).

Allelopathy of *M. polykrikoides* has been demonstrated by exposing cells and filtrates of *M. polykrikoides* to live cells of *G. catenatum*. *M. polykrikoides* caused cell damage to *G. catenatum*, such as detachment of the membrane, deformation, prominent nuclei, loss of flagella, and lysis, suggesting that *M. polykrikoides* could inhibit or regulate the growth of *G. catenatum* in the natural environment (Zumaya-Higuera, 2017).

Caribbean and Central America

Cuba

M. polykrikoides proliferated in Bahía de Santiago in April and May 2005 covering an area of 0.8 km^2. The HAB was short (4 days), however, it caused mortality of wild juvenile fish (*Mujil curema*, *Opisthonema oglinum*, *Acanthurus chirurgus*, *Haemulon* spp.) and crabs (*Callinectes sapidus*). During the bloom, cysts and high cell concentrations were recorded (**Table 1**; Gómez et al., 2007). Another bloom occurred in the channels of the Marina Hemingway in Havana in September 2015. A cell concentration of 1.8×10^6 cell L^{-1} was reported, and the bloom declined after 2 days of torrential rain, decreasing to 7.5×10^5 cell L^{-1}. No impact on wildlife was reported (Delgado et al., 2016).

Guatemala to Nicaragua

A HAB of *M. catenatum* was recorded for the first time in Guatemala in 2004, which lasted 32 days and occurred from May to June. Toward the end of the bloom, on the 23rd of June, samples for species identification and cell density were obtained that contained 9.6×10^5 cell L^{-1} which explained the abnormal values of chlorophyll *a* (>30 mg m^{-3}) observed in satellite images (Carrillo-Ovalle et al., 2007). High chlorophyll *a* levels (10–39 mg m^{-3}) were also found off the coast of Honduras, El Salvador and the Gulf of Fonseca (gulf shared between El Salvador, Honduras and Nicaragua). Rain contributed to high nutrient concentrations (**Table 1**), and the water temperature was 30.3°C. A second event was recorded in January 2007 with a higher cell concentration (6.9×10^6 cell L^{-1}). This bloom occurred at a lower temperature (28°C) during the influence of coastal water upwelling. Wild fish mortalities were observed during both events (Carrillo-Ovalle et al., 2007).

El Salvador

Espinoza et al. (2013), documented a bloom of *M. polykrikoides* off the coast of El Salvador in March 2012, with a maximum cell density of 2.1×10^8 cell L^{-1}. The bloom was 3 km wide and 13 km long, and mortality of benthic fish, bad odor and severe headaches in tourists and local residents were reported. The major tourist season in El Salvador is from March to April, however, no economic losses were estimated for this event. Mass sea turtle mortalities occurred in La Libertad in October 2013 (Amaya et al., 2014). PSP toxins were detected in turtle tissues as well as in oysters. Although the most abundant species during this event was *G. catenatum*, other species such as *P. bahamense*, *A. monilatum*, and *M. polykrikoides* were also reported. In July 2017 a mixed bloom caused by *M. polykrikoides* and *Scripsiella trochoidea* affected several coastal areas of El Salvador (Ochoa-Arguello, 2017). During this event, a sanitary closure was applied.

Costa Rica and Panama

A bloom of *M. polykrikoides* reported as *M. catenatum* occurred in the Gulf of Nicoya, Costa Rica in February-March 1979 with a width of 200 m and length of 2–3 km. A strain was isolated from this event and growth rate of 0.3 div day^{-1} was estimated. The maximum abundance of cells during the event was 80.0×10^6 cell L^{-1} (Hargraves and Víquez, 1981; **Table 1**).

An annual monitoring program of harmful dinoflagellates was conducted in the middle and upper Gulf of Nicoya between January 1985 and March of 1986. HABs of *M. polykrikoides* were commonly observed in the lower gulf between June and October, mainly during the rainy season. The highest abundance of *M. polykrikoides* was 5.0×10^6 cell L^{-1} during this period (Víquez and Hargraves, 1995). Ramírez et al. (1989) suggested that the decline of anchovy eggs in the estuary of Punta Morales recorded was caused by a red tide near the estuary in August 1985 (**Table 1**).

In the second half of 1985, in Panama (Caño Island, Costa Rica, and Uva Island), mass mortalities of reef fish, invertebrates and corals were associated with a bloom of *M. catenatum* and *Gonyaulax monilata*. In Caño Island, a coral mortality up to 100% was observed between the surface and 3 m of depth. The species most affected were *Pocillopora elegans* and the zooxantella *Tubastrea coccinea*. Additionally, hundreds of fish of the family Scarideae, Balistidae, Acanthuridae, Pomancentridae, and Tetraodontidae; hermit crabs, brachyuran crabs and gastropods. In Uva Island, 13% mortality of *Pocillopora* spp. occurred. Mucus adhesion to polyps and interference with the expansion of polyps appeared to be the cause of coral mortality (Guzmán et al., 1990). Valverde (2002) reported a toxic bloom of *Pyrodinium bahamense* var. *compressum*, *G. catenatum*, and *M. polykrikoides,* on the Pacific Coast of Costa Rica from late November 2000 to December 2001 (**Table 1**). No impact was observed when the abundance of cells of *M. polykrikoides* reached 14.4×10^6 cell L^{-1}. Vargas-Montero and Freer (2004) reported a bloom in Puntarenas and Caldera beach in the Gulf of Nicoya in May 2002 (1.2×10^5 cell L^{-1}). This event

was dominated by *M. polykrikoides* and the cyanobacterium *Trichodesmium erythraeum*. Large fish died, and deformity of fish larvae was also observed.

M. polykrikoides bloomed again on the Pacific region of Costa Rica between January 2003 and June 2004. Cell abundance was 1.7×10^8 cell L^{-1} (**Table 1**), and cysts were also observed in October 2003 (Vargas-Montero et al., 2006). The following bloom, in January 2004, covered an area of 50 km^2 and in April of the same year a high number of cells were also found (3.8×10^8 cell L^{-1}). During these events, corals died and fish mortalities of the families Carangidae, Lutjanidae, Muraenidae, Engraulidae, and the Solidae occurred (Vargas-Montero et al., 2006). *M. polykrikoides* commonly bloomed in this region with low abundance of other dinoflagellate species such as *Ceratium dens*, *Gonyaulax spinifera*, *Heterocapsa* sp., *Mesodinium rubrum*, *P. bahamense* var. *compressum* and the cyanobacterium *T. erytraeum*. Vargas-Montero et al. (2006, 2008) concluded that northeaster winds may be the most influential factor in the formation of HABs of *M. polykrikoides* in Costa Rica.

A total of 11 HABs of different species of dinoflagellates were reported in the Gulf of Nicoya from 2008 to 2010. Of these, eight were observed during the rainy season (May to November) and three during the dry season (December to April) (Calvo et al., 2016). Authors emphasize that the most impacting HAB occurred in September 2010, when 16 phytoplankton species were observed, with *M. polykrikoides* as the dominant species, occurring during a La Niña event. Heavy rain contributed to the enrichment of nutrients in the Gulf of Nicoya, as a result of runoff discharges of the rivers Tempisque and Grande de Tárcoles.

South America

Venezuela

Only one report exists on a bloom of *Margalefidinium* sp. on the coast of Sucre, Venezuela, which occurred in August 1977. Cells of *Margalefidinium* were found in the digestive tract of the mussel *Perna perna*. During this bloom, *Gonyaulax tamarensis* var. *excavate* (=*Alexandrium catenella*) and *Noctiluca miliaris* were also observed, and the presence of paralytic shellfish toxins were detected in mussels, which were associated with human intoxications (Reyes-Vásquez et al., 1979).

Colombia

A bloom of *M. polykrikoides* was recorded for the first time in Bahía de Santa María, in the Colombian Caribbean in October and November 2010. The maximum density was 5.0×10^6 cell L^{-1}, and the event covered an area of 6 km^2, however, no impact on fauna was observed (Malagón and Perdomo, 2013). The key factor for the proliferation was the contribution of terrestrial nutrients during the rainy season. This bloom coincided with SSE and SE winds during the day, and NNW and NW during the night. Cuellar-Martínez et al. (2018) reported two more blooms in the same bay between 2010 and 2017 with no harmful effects.

Ecuador

HABs are frequent in Ecuador, more commonly in the Gulf of Guayaquil. There have also been reports of blooms in the southern part of the Santa Elena peninsula, Manglar alto, Manta, Cojimies and in the Galápagos Islands. One of the most frequent and abundant species is *Margalefidinium* sp., which bloomed in Puerto Bolívar (March 1992), Estero Salado (March 1993), and Guayas river in June 1999, reaching a maximum cell abundance in Guayas River of 5.2×10^6 cell L^{-1} (Torres-Zambrano, 2000). The high frequency of HABs in the Gulf of Guayaquil is associated with poor water quality due to mangrove removal, ship traffic, shrimp farming, population increase, combined with El Niño events.

Peru

Individual blooms of *A. sanguinea*, *M. rubrum*, *Protoceratium minimum*, and *M. polykrikoides* occurred in Bahía de Pisco in February 2010 and May 2014. These events were associated with upwelling of deep waters, with a temperature range from 17.1 to 23.3°C. The bacteria *Vibrio alginolyticus*, *V. metschmicovich*, *V. vulfenicus*, and *V. parahaemolyticus* were isolated during the bloom. A strain of *V. parahaemolyticus* was virulent and a public health event with diarrhetic symptoms was associated with the bacteria (Orozco et al., 2017).

CONCLUSION

The toxic dinoflagellate *M. polykrikoides* is wide spread in Latin American coastal waters. It has formed recurrent blooms that have been documented mainly in Mexico and Central American countries. *M. polykrikoides* has been able to proliferate in a wide temperature range (17–32°C), and has affected several marine organisms, including fish, crabs, corals, shrimp, eels, octopuses, and gastropods. Blooms of *M. polykrikoides* in Latin America have been associated with periods of heavy rain that cause an increase in nutrients in coastal waters due to runoff from rivers. Moreover, coastal upwellings associated with wind patterns, mixing of the water column and rain, all of which can create nutrient enrichment of surface waters, seem to favor proliferation and recurrence of this dinoflagellate. Although significant economic losses have been reported in other countries due to the negative impacts of *M. polykrikoides*, this information is still lacking in Latin America, possibly due to scarce socioeconomic studies. A continuous monitoring program is required to obtain information, during and after bloom occurrence. Also, data of cyst beds should be included to generate ecological information that can provide the opportunity to evaluate the potential impacts on fisheries and aquaculture. Especially to address the economic and social repercussions that HABs may have, given the recent experience of two continuous blooms created by *Pseudochattonella verruculosa* and *Alexandrium catenella* in Chile in 2016 that caused economic losses of US$800M, creating unemployment and social riots triggered by protests (Mascareño et al., 2018). Mitigation approaches for HABs of this dinoflagellate are also a subject not yet investigated in Latin America. The higher amount of reports from Mexico might be because this country possesses a longer coastal area, as well as having a high number of experts on harmful algae, or simply because

M. polykrikoides has formed more blooms in the Mexican coastline than in other Latin America countries. The presence of *Margalefidinium* as well as other species of marine fish-killing microalgae in Latin America will require constant monitoring mainly in fish-growing areas to avoid severe economic impacts, such as the recent cases in Chile and Mexico (Clement et al., 2016; García-Mendoza et al., 2018; León-Muñoz et al., 2018; Mardones et al., 2019).

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

ACKNOWLEDGMENTS

We thank the institutional projects PAC (Planeación Ambiental y Conservación) of the CIBNOR, to G. Hernández-García end edition of figures (CIBNOR), Dr. J. L. Peña-Manjarrez (CETMAR-DGECyTM, SEP, Ensenada) for the satellite image HAB of the *M. polykrikoides*, Instituto Politécnico Nacional (IPN Grant SIP 2019–5649), and Don Johnson, Ph.D. for improving the language part of the manuscript. We are grateful to the Consejo Nacional de Ciencia y Tecnología (FORDECyT Grant 260040), and RedFAN-CONACyT. CB-S is a COFFA-IPN and EDI fellow. We also thank the reviewers for the suggestions provided during editing of this manuscript.

REFERENCES

Alonso-Rodríguez, R., Hernández-Becerril, D. U., and Gárate-Lizárraga, I. (2008). "Catálogo de microalgas de las lagunas costeras de Sinaloa," in *Serie Lagunas Costeras de Sinaloa 4*, ed. F. P. áez-Osuna (Sinaloa, ME: Universidad Nacional Autónoma de México).

Alonso-Rodríguez, R., Páez-Osuna, F., and Gárate-Lizárraga, I. (2004). *El fitoplancton en la camaronicultura y larvicultura: Importancia de un buen manejo*. México, ME: Comité Estatal de Sanidad Acuícola de Sinaloa.

Amaya, O., Ruiz, G., Espinoza, J., and Rivera, W. (2014). Saxitoxin analyses with a receptor binding assay (RBA) suggest PSP intoxication of sea turtles in El Salvador. *Harmful Algae News* 48, 6–7. doi: 10.3389/fmars.2018.00409

Calvo, V. E., Berrocal, A. K., and Boza, A. J. (2016). Floraciones algales nocivas durante el periodo 2008-2010 en el Golfo de Nicoya. *Costa Rica. Rev. Mar. Coast.* 8, 129–149. doi: 10.15359/revmar.8-1.9

Carrillo-Ovalle, L., Cortés-Altamirano, R., Leiva-Cerezo, V., and Ramírez, V. (2007). *Cochlodinium catenatum* on Guatemala coast (2004 & 2007). *Harmful Algae News* 33, 15–17.

Clement, A., Lincoqueo, L., Saldivia, M., Brito, C. G., Muñoz, F., Fernández, C., et al. (2016). Exceptional summer conditions and HABs *Pseudochattonella* in southern Chile create record impacts on salmon farms. *Harmful Algae News* 53, 1–3.

Cortés-Altamirano, R. (1987). Observaciones de mareas rojas en la Bahía de Mazatlán. Sinaloa, México. *Cienc. Mar.*. 13, 1–19. doi: 10.7773/cm.v13i4.557

Cortés-Altamirano, R., Alonso-Rodríguez, A., and Salas-de León, D. A. (2019). Historical observations of algal blooms in Mazatlán bay, Sinaloa, Mexico (1979-2014). *PLoS One* 14:e0210631. doi: 10.1371/journal.pone.0210631

Cortés-Altamirano, R., and Gómez-Aguirre, S. (2001). "Florecimientos algales nocivos en la Bahía de Mazatlán, México (2000)," in *Proceedings of the Memoria in Extenso del Congreso Internacional COLACMAR* 16-20 de septiembre, San Andrés.

Cortés-Altamirano, R., Cifuentes, J. L., and Gaxiola López, J. (2002). "Mareas rojas: biodiversidad de microbios que pintan el mar," in *Atlas de Biodiversidad de Sinaloa*, (México, ME: Colegio de Sinaloa), 29–41.

Cortés-Altamirano, R., Lavín, M. F., Sierra-Beltrán, A., and Cortés-Lara, M. C. (2006). Una hipótesis sobre el transporte de microalgas invasoras desde el Pacífico oeste tropical hasta el Golfo de California por las corrientes marinas. *Cienc. Del. Mar. U. A. S.* 18, 19–26. doi: 10.1371/journal.pone.0210631

Cortés-Lara, M. C. (2002). Informe del fenómeno de marea roja en Bahía de Banderas Jalisco-Nayarit, octubre-noviembre de 2001. *Rev. Biomed.* 13, 73–75.

Cortés-Lara, M. C., Cortés-Altamirano, R., and Sierra-Beltrán, A. P. (2004). Presencia de *Cochlodinium catenatum* (*Gymnodiniales: Gymnodiniaceae*) en mareas rojas de Bahía de Banderas, Pacífico Mexicano. *Rev. Biol. Trop.* 52(Suppl. 1), 35–49.

Cuellar-Martínez, T., Ruiz-Fernández, A. C., Alonso-Hernández, C., Amaya-Monterrosa, O., Quintanilla, R., Carrillo-Ovalle, H. L., et al. (2018). Addressing the problem of harmful algal blooms in Latin America and the Caribbean-a regional network for early warning and response. *Front. Mar. Sci.* 5:409. doi: 10.3389/fmars.2018.00409

Delgado, G., Arencibia, G., Lucas, F., and Rosquete, C. M. A. (2016). *Cochlodinium polykrikoides* Margalef bloom in the channels of marine Hemingway, La Habana, Cuba. *Harmful Algae News* 53:8.

Dorantes-Aranda, J. J. (2012). *Development and novel application of an in vitro fish gill cell assay to elucidate the ichthyotoxic mechanism of the microalga Chattonella marina (Raphidophyceae)*. Ph.D. Thesis, University of Tasmania: Burnie, TAS

Dorantes-Aranda, J. J., García-de la Parra, L. M., Alonso-Rodríguez, R., and Morquecho, L. (2009). Hemolytic activity and fatty acids composition in the ichthyotoxic dinoflagellate *Cochlodinium polykrikoides* isolated from Bahía de la Paz, Gulf of California. *Mar. Poll. Bull.* 58, 1401–1405. doi: 10.1016/j.marpolbul.2009.06.007

Dorantes-Aranda, J. J., García-de la Parra, L. M., Alonso-Rodríguez, R., Morquecho, L., and Voltolina, D. (2010). Toxic effect of the harmful dinoflagellate *Cochlodinium polykrikoides* on the spotted rose snapper *Lutjanus guttatus*. *Environm. Toxicol.* 25, 319–326. doi: 10.1002/tox.20507

Espinoza, J., Amaya, O., Reguera, B., Alvarado, Y., Ruiz, G., and Escobar, D. (2013). A *Cochlodinium polykrikoides* bloom in El Salvador. *Harmful Algae News* 47:13.

Fimbres-Martínez, M. (2019). Distribución y abundancia de microalgas ictiotóxicas en la costa Noroeste de Baja California, México. MSc. Thesis, Centro de Investigación Científica y de Educación Superior de Ensenada: Mexico, ME.

Fimbres-Martínez, M., García-Mendoza, E., Olivos-Ortiz, A., Rivas-Camargo, D., and Helenes-Escamilla, J. (2018). "Spatial and temporal abundance of ichthyotoxic microalgae in Todos Santos bay, Baja California, Mexico. abstract book ICHA 2018," in *Proceedings of the 18th International Conference on Harmful Algae*, (Nantes).

Gárate-Lizárraga, I. (2013). Bloom of *Cochlodinium polykrikoides* (dinophyceae): gymnodiniales) in Bahía de La Paz. Gulf of California. *Mar. Poll. Bull.* 67, 217–222.

Gárate-Lizárraga, I., and Muñetón-Gómez, M. C. (2008). Los riesgos de las mareas rojas en el Pacífico Mexicano. *Conversus* 3, 20–23.

Gárate-Lizárraga, I., Bustillos-Guzmán, J. J., Morquecho, L. M., and Lechuga-Devéze, C. H. (2000). First outbreak of *Cochlodinium polykrikoides* in the Gulf of California. *Harmful Algae News* 21:7.

Gárate-Lizárraga, I., Díaz-Ortiz, J., Pérez-Cruz, B., Alarcón-Tacuba, M., Torres-Jaramillo, A., Alarcón-Romero, M. A., et al. (2009). *Cochlodinium polykrikoides* and *Gymnodinium catenatum* in Bahía de Acapulco, Mexico (2005-2008). *Harmful Algae News* 40, 8–9.

Gárate-Lizárraga, I., López-Cortés, D. J., Bustillos-Guzmán, J. J., and Hernández-Sandoval, F. E. (2004). Blooms of *Cochlodinium polykrikoides* (*Gymnodiniaceae*) in the Gulf of California, Mexico. *Rev. Biol. Trop.* 52(Suppl. 1), 51–58.

García-Mendoza, E., Cáceres-Martínez, J., Rivas, D., Fimbres-Martínez, M., Sánchez-Bravo, Y., Vásquez-Yeomans, R., et al. (2018). Mass mortality of cultivated northern bluefin tuna *Thunnus thynnus orientalis* associated with *Chattonella* species in Baja California, Mexico. *Front. Mar. Sci.* 5:454. doi: 10.3389/fmars.2018.00454

Gobler, C., Berry, J., Anderson, D., Burson, O. R., Koch, A., Rodgers, F., et al. (2008). Characterization, dynamics, and ecological impacts of harmful *Cochlodinium polykrikoides* blooms on eastern long island NY, USA. *Harmful Algae* 17, 293–307. doi: 10.1016/j.hal.2007.12.006

Gobler, C., Burson, J., Koch, A., Tang, F., and Mulholland, M. R. (2012). The role of nitrogenous nutrients in the occurrence of harmful algal blooms caused by *Cochlodinium polykrikoides* in New York estuaries (USA). *Harmful Algae* 17, 64–74. doi: 10.1016/j.hal.2012.03.001

Gómez, F., Richlen, M. L., and Anderson, S. M. (2017). Molecular characterization and morphology of *Cochlodinium strangulatum*, the type species of *Cochlodinium*, and *Margalefidinium* gen. nov. for *C. polykrikoides* and allied species (Gymnodiniales, Dinophyceae). *Harmful Algae* 63, 32–44. doi: 10.1016/j.hal.2017.01.008

Gómez, L., Licea, Y., Díaz, U., and Álvarez, I. (2007). Primer reporte de *Cochlodinium polykrikoides* (Margalef, 1961) en aguas Cubanas. *Rev. Invest. Mar.* 28, 87–89.

Gómez-Villarreal, M. C., Martínez-Gaxiola, M. D., and Peña-Manjarrez, J. L. (2008). Proliferaciones algales 2000-2001 en Bahía de Banderas, México según el sensor SeaWiFS. *Rev. Biol. Trop.* 56, 1653–1664.

González-Chan, R. B., Hernández-Silva, L., Navarro-Ornelas, J. G., and Blanco-Padilla, M. A. (2007). *Proliferación Algal Nociva en Las Bahías del Puerto de Manzanillo, Colima (Marzo-Mayo 2007)*. Nuevo Vallarta: Memorias del XIV congreso de Ciencia y Tecnología del Mar.

Griffith, A. W., Shumway, S. E., and Gobler, C. J. (2019). Differential mortality of north atlantic bivalve molluscs during harmful algal blooms caused by the dinoflagellate, *Cochlodinium* (a.k.a. *Margalefidinium*) *polykrikoides*. *Estuaries Coasts* 42, 190–203. doi: 10.1007/s12237-018-0445-0

Guzmán, M. H., Cortés, J., Glynn, W. P., and Richmond, H. R. (1990). Coral mortality associated with dinoflagellate bloom in the eastern Pacific (Costa Rica and Panama). *Mar. Ecol. Prog. Ser.* 60, 299–303.

Hallegraeff, G. M. (1992). Harmful algal blooms in the Australian region. *Mar. Pollut. Bull.* 25, 186–190. doi: 10.1016/0025-326X(92)90223-S

Hallegraeff, G., Dorantes-Aranda, J. J., Mardones, J., and Seger, A. (2017). "Review of progress in our understanding of fish-killing microalgae: implications for management and mitigation," in *Proceedings of the 17th International Conference on Harmful Algae. International Society for the Study of Harmful Algae and Intergovernmental Oceanographic Commission of UNESCO 2017*, eds L. A. O. Proença and G. Hallegraeff (Brasil).

Hargraves, E. P., and Víquez, R. (1981). The dinoflagellate red tide in Golfo de Nicoya. *Costa Rica. Rev. Biol. Trop.* 29, 31–38.

Jeong, H. J., Yoo, Y. D., Kim, J. S., Kim, T. H., Kim, J. H., Kang, N. S., et al. (2004). Mixotrophy in the phototrophic harmful alga *Cochlodinium polykrikoides* (Dinophycean): prey species, the effects of prey concentration, and grazing impact. *J. Eukaryot. Microbiol.* 51, 563–569.

Kim, C. S., Lee, S. G., Kim, H. G., and Jung, J. (1999). Reactive oxygen species as causative agents in the ichthyotoxicity of the red tide dinoflagellate *Cochlodinium polykrikoides*. *J. Plankton Res.* 21, 2105–2115.

Kim, H. G. (1998). "*Cochlodinium polykrikoides* blooms in Korean coastal waters and their mitigation," in *Harmful Algae*, eds B. Reguera, J. Blanco, T. Fernández, and T. Wyatt (Vigo: Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO), 227–228.

Kudela, R. M., and Gobler, C. J. (2012). Harmful dinoflagellate blooms caused by *Cochodinium* sp.: global expansion and ecological strategies facilitating blooms formation. *Harmful Algae* 14, 71–86. doi: 10.1016/j.hal.2011.10.015

Landsberg, J. H. (2002). The effects of harmful algal blooms on aquatic organisms. *Rev. Fish. Sci.* 10, 113–390. doi: 10.1080/20026491051695

Lee, J. S. (1996). Bioactive components from the red tide plankton *Cochlodinium polykrikoides*. *J. Korean Fish. Soc.* 29, 165–173.

León-Muñoz, J., Urbina, M. A., Garreaud, R., and Iriarte, J. L. (2018). Hydroclimatic conditions trigger record harmful algal bloom in western Patagonia (summer 2016). *Sci. Rep.* 8:1330. doi: 10.1038/s41598-018-19461-4

López, L. (2000). Marea roja en bahía de banderas. *Gac. Univ.* 179, 6.

López-Cortés, D. J., Band-Schmidt, C. J., Bustillos-Guzmán, J. J., Hernández-Sandoval, F. E., Mendoza-Flores, A., and Núñez-Vázquez, E. J. (2014). Condiciones ambientales durante un florecimiento de *Cochlodinium polykrikoides* (*Gymnodiniales, Dinophyceae*) en la Ensenada de La Paz, Golfo de California. *Rev. Biol. Mar. Oceanog.* 40, 97–110. doi: 10.4067/S0718-19572014000100011

Maciel-Baltazar, E., and Hernández-Becerril, D. U. (2013). Especies de dinoflagelados atecados (Dinophyta) de la costa de Chiapas, sur del Pacífico Mexicano. *Rev. Bio. Mar. Oceanog.* 48, 245–259. doi: 10.4067/S0718-19572013000200005

Malagón, L. A., and Perdomo, L. V. (2013). *Cochlodinium polykrikoides* bloom in the Colombian caribbean. *Harmful Algae News* 47, 14–15.

Mardones, J. I., Fuenzalida, G., Zenteno, K., Alves-de-Souza, C., Astuya, A., and Dorantes-Aranda, J. J. (2019). Salinity-growth response and ichthyotoxic potency of the Chilean *Pseudochattonella verruculosa*. *Front. Mar. Sci.* 6:24. doi: 10.3389/fmars.2019.00024

Margalef, R. (1961). Hidrografía y fitoplancton de un área marina de la costa meridional de Puerto Rico. *Invest. Pesq.* 18, 33–96.

Mascareño, A., Cordero, R., Azócar, G., Billi, M., Henríquez, P. A., and Ruz, G. A. (2018). Controversies in social-ecological systems: lessons from a major red tide crisis on Chiloe Island, Chile. *Ecol. Soc.* 23:art15. doi: 10.5751/ES-10300-230415

Meave del Castillo, M. E. (2014). "Plancton marino introducido por agua de lastre," in *Especies Acuáticas Invasoras En México*, eds R. Mendoza and P. Kolef (México, ME: Comisión Nacional para el conocimiento y Uso de la Biodiversidad), 289–308.

Mooney, B. D., Dorantes-Aranda, J. J., Place, A. R., and Hallegraeff, G. M. (2011). Ichthyotoxicity of gymnodinioid dinoflagellates: PUFA and superoxide effects in sheepshead minnow larvae and rainbow trout gill cells. *Mar. Ecol. Prog. Ser.* 426, 213–224.

Morales-Blake, A., Cavazos-Guerra, C., and Hernández-Becerril, D. U. (2001). Unusual HABs in Manzanillo bay. Colima, Mexico. *Harmful Algae News* 22:6.

Morales-Blake, A., Hernández-Becerril, D. U., and Cavazos-Guerra, C. (2000). "Registros de mareas rojas en la Bahía de Manzanillo, Colima, México," in *Estudios sobre plancton en México y el Caribe*, eds E. Ríos-Jara, E. Juárez-Carrillo, M. Pérez-Peña, E. López-Uriarte, E. G. Robles-Jarero, D. U. Hernández-Becerril, et al. (México, ME: Universidad de Guadalajara), 81–82.

Morey-Gaines, G. (1982). *Gymnodinium catenatum* Graham (*Dinophyceae*): morphology and affinities with armoured forms. *Phycologia* 21, 151–163.

Muciño-Márquez, R.E. (2010). Variación estacional de la comunidad fitoplanctónica en las granjas atuneras en la Bahía de La Paz, Baja California Sur. Master Thesis, Centro Interdisciplinario de Ciencias Marinas-IPN: La Paz.

Mulholland, M. R., Morse, R. E., Boneillo, G. E., Bernhardt, P. W., Filipinno, K. C., Procise, L. A., et al. (2009). Understanding causes and impacts of the dinoflagellate *Cochlodinium polykrikoides* blooms in the Chesapeake. *Estuaries Coasts* 32, 734–747.

Núñez-Vázquez, E. J., Heredia-Tapia, A., Pérez-Urbiola, J. C., Alonso-Rodríguez, R., Arellano-Blanco, J., Cordera-Tapia, A., et al. (2003). "Evaluation of dinoflagellates toxicity in recent HAB event in the Gulf of California, Mexico," in *Proceedings of the HABTECH, Workshop*, eds P. Holland, L. Rhodes, and L. Brown, Nelson.

Ochoa-Arguello, I. (2017). *Marea roja en Centroamérica avanza hacia costas de Chiapas. El Orbe*. Available at: https://elorbe.com/seccion-politica/local/2017/07/10/marea-roja-en-centroamerica-avanza-hacia-costas-de-chiapas.html (accessed July 10, 2017).

Onoue, Y., and Nozawa, K. (1989). "Separation of toxins from harmful red tides occurring along the coast of Kagoshima prefecture," in *Red tides: Biology, Environmental, Science, and Toxicology*, eds T. Okaichi, D. M. T. Anderson, and T. Nemoto (New York, NY: Elsevier), 371–374.

Orozco, R., Quispe, Y., Lorenzo, A., and Zamudio, M. L. (2017). Asociación de floraciones de algas nocivas y *Vibrio* spp., en áreas de pesca y acuicultura de bivalvos de moluscos en las bahías de Sechura y Pisco. *Perú. Rev. Per. Biol.* 24, 111–116. doi: 10.15381/rpb.v24i1.13111

Osorio-Tafall, B. F. (1943). El mar de Cortés y la productividad fitoplanctónica de sus aguas. *An. Esc. Nal. Cienc. Biolog. Mex.* 3, 73–118.

Páez-Osuna, F., Álvares-Borrego, S., Ruiz-Fernández, A. C., and García-Hernández, J. (2017). Environmental status of Gulf of California: a pollution review. *Earth Sci. Rev.* 166, 181–2005. doi: 10.1016/j.earscirev.2017.01.014

Pérez-Morales, A., Band-Schmidt, C. J., and Martínez-Díaz, S. F. (2017). Mortality on zoea stage of the pacific white shrimp *Litopenaeus vannamei* caused by *M. polykrikoides (Dinophyceae)* and *Chattonella* spp. *(Raphidophyceae)*. *Mar. Biol.* 164:57. doi: 10.1007/s00227-017-3083-3

Ramírez, A., Szelistowski, W., and Lopez, M. (1989). Spawning pattern and larval recruitment in Gulf of Nicoya anchovies (Pisces: Engraulidae). *Rev. Biol. Trop.* 37, 55–62.

Reyes-Vásquez, G., Ferraz-Reyes, E., and Vásquez, E. (1979). "Toxic dinoflagellate blooms in northeastern Venezuela during 1977," in *Toxic Dinoflagellate Blooms*, eds D. L. Taylor and H. H. Seliger (Amsterdam, NE: Elsevier-North Holland, Inc:).

Richlen, M. L., Morton, S. L., Jamali, E. A., Rajan, A., and Anderson, D. M. (2010). The catastrophic 2008-2009 red tide in the Arabian Gulf region, with observations on the identification and phylogeny of the fish-killing dinoflagellate *Cochlodinium polykrikoides*. *Harmful Algae* 9, 163–172. doi: 10.1016/j.hal.2009.08.013

Schütt, F. (1895). *Die Peridineen der Plankton-Expedition. I. Theil Studien Über die Zellen der Peridineen. Ergebnisse der Plankton-Expedition der Humboldt-Stiftung, IV. M.a.A.* Kiel, Leipzig: Lipsius und Tischler.

Sierra-Beltrán, A., Lluch-Cota, D. B., Cortés-Altamirano, R., Castillo-Chávez, M., Carrillo, L., Pacas, L., et al. (2001). "Possibilities for environmental and biological monitoring in the Pacific coast of central and north america as a strategy for prevention and mitigation of harmful algal blooms (HABs). an example of small budget cooperative efforts leading to a mayor benefit," in *Proceeding of the Second International Conference on Harmful Algae Management and Mitigation*, eds Z. Mingyuan, Z. Yinglin, L. Cheong, and S. Hall,Brasil.

Tomas, C. R., and Smayda, T. J. (2008). Red tides blooms of *Cochlodinium polykrikoides* in a coastal cove. *Harmful Algae* 7, 308–317. doi: 10.1016/j.hal.2007.12.005

Torres-Zambrano, G. (2000). Mareas rojas durante 1989-1999, en aguas ecuatorianas. *Acta Oceanog. Pacific, INOCAR, Ecuador.* 10, 127–136.

Valverde, J. O. (2002). *The Longest and Recent Red Tide Event in Costa Rica. Book of Abstracts, Molluscan Shellfish Safety*. Galicia: Santiago de Compostela.

Vargas-Montero, M., and Freer, E. (2004). Proliferaciones algales nocivas de cianobacterias (*Oscillatoriaceae*) y dinoflagelados (*Gymnodiniaceae*) en el Golfo de Nicoya, Costa Rica. *Rev. Biol. Trop.* 52(Suppl. 1), 121–125.

Vargas-Montero, M., Freer, B. E., Guzmán, J. C., and Vargas, J. C. (2008). Florecimientos de dinoflagelados nocivos en la costa Pacífico de Costa Rica. *Hidrobiológica* 18(Suppl. 1), 15–23.

Vargas-Montero, M., Freer, E., Jiménez-Montealegre, R., and Guzmán, J. C. (2006). Occurrence and predominance of the fish killer *Cochlodinium polykrikoides* on the Pacific coast of Costa Rica. *Afr. J. Mar. Sci.* 28, 215–217. doi: 10.2989/18142320609504150

Víquez, R., and Hargraves, E. P. (1995). Annual cycle of potentially harmful dinoflagellate in the Gulf of Nicoya. *Costa Rica. Bull. Mar. Sci.* 57,0 467–475.

Whyte, J. N. C., Haigh, N., Ghinter, N. G., and Keddy, L. (2001). First record of blooms of *Cochlodinium* sp. *(Gymnodiniales, Dinophyceae*) causing mortality to aquacultured salmon on the west coast of Canada. *Phycologia* 40, 298–304. doi: 10.2216/i0031-8884-40-3-298.1

Zhong, T. Y., and Gobler, C. J. (2009). Characterization of the toxicity of *Cochlodinium polykrikoides* isolates from Northeast US estuaries to finfish and shellfish. *Harmful Algae* 8, 454–462. doi: 10.1016/j.hal.2008.10.001

Zumaya-Higuera, M. G. (2017). *Interacciones Bióticas entre los Dinoflagelados Cochlodinium polykrikoides y Gymnodinium catenatum (Dinophyceae) en condiciones de Laboratorio*. MSc. Thesis, Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas, La Paz.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 López-Cortés, Núñez-Vázquez, Dorantes-Aranda, Band-Schmidt, Hernández-Sandoval, Bustillos-Guzmán, Leyva-Valencia and Fernández-Herrera. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership