Algal Indicators in Streams

Course No: H03-007

Credit: 3 PDH

Gilbert Gedeon, P.E.

Continuing Education and Development, Inc.
22 Stonewall Court
Woodcliff Lake, NJ 07677

P: (877) 322-5800
info@cedengineering.com

822B17002

ALGAL INDICATORS IN STREAMS: A REVIEW OF THEIR APPLICATION IN WATER QUALITY MANAGEMENT OF NUTRIENT POLLUTION

Michael J. Paul

Tetra Tech, Inc., Center for Ecological Sciences, Research Triangle Park, NC

Brannon Walsh, Jacques Oliver, and Dana Thomas

U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Washington, D.C.

PREFACE

This paper summarizes the application of algae as indicators of nutrient pollution in water quality management. It describes the use of algal indicators to develop water quality diagnostics for nutrient pollution in the United States (U.S.) and then reviews scientific developments in the use and application of algal indicators across the world. The paper is intended as a technical resource for the water quality manager/practitioner seeking to utilize algae to detect the presence of nutrient pollution and to estimate the risks of nutrient pollution in adversely affecting the condition of stream ecosystems.

INTRODUCTION

Algae are ubiquitous and essential components of all stream ecosystems (Stevenson 2014; Stevenson and Smol 2003). They are the primary energetic source for many stream food webs, fixing carbon from the atmosphere through photosynthesis, which is then transmitted through the web via consumer pathways. Algae are able to produce this energy for stream ecosystems across a wide range of physical and chemical conditions, from hot thermal spring fed stream ecosystems to cold, arctic stream ecosystems. They are represented by a vast range of different species, growth forms, and life histories.

Algae have a long history of use and possess many of the features valued in ecological indicators. They were part of the early saprobien indicator system development in Germany and were one of the first assemblages developed for use in biological assessment in the United States (Stevenson 2014; Stevenson et al. 2010; Stevenson and Smol 2003). Algae are relevant ecologically in streams and clearly impact the benefits that people obtain from these ecosystems. Also algae can be feasibly measured. They are easily sampled and processed using a wide variety of methods, and they can be identified for relatively low cost. Algal physiologies make them attractive for investigating biological responses across a range of stressors and stressor variability. Algae exhibit a wide variety of sensitivity/tolerance among their many naturally-occurring taxa. They respond quickly to disturbance and recover quickly after a stressor is removed, and while they vary naturally, as do most aquatic organisms, that natural variability can be quantified and factored into analysis. Lastly, algal measurements are readily interpreted and understood by scientists, policy makers, and the public (US Environmental Protection Agency (USEPA) 2000).

The ecological importance and distinguishing features of algae, particularly as indicators of nutrient pollution, make them conducive as assessment endpoints for numeric nutrient criteria development for water quality management purposes under the Clean Water Act (USEPA 2000, 2014). This value lies in both their sensitivity to nutrient pollution, as well as their linkage to aquatic life, drinking water source and recreational designated uses (Stevenson and Smol 2003; USEPA 2000). Indicators that have been developed using algae include measures of productivity, biomass and assemblage composition. Productivity measures include measures of photosynthesis and respiration using chamber and open system methods. Biomass indicators include cell abundance, cell biovolume, photopigments (e.g., chlorophyll *a*), and ash free dry mass (AFDM). Assemblage composition measures include taxonomic estimates of diversity, richness, and a suite of metrics characterizing an array of algal traits (e.g., pollutant sensitivity, motility).

Algal impacts on uses occur principally through the ecological phenomena of competition and productivity (Figure 1). Algae are aquatic life and therefore directly measure that use; their importance as primary resources in stream food webs means they are integrally linked to all other aquatic life, most directly higher trophic levels such as invertebrates and fish. Competition among algae for nutrients means that enrichment by nutrient pollution shifts the composition of the algal community, including edible forms, affecting food quality for higher trophic levels. In addition, nutrient pollution increases primary productivity, affecting the amount of food available to consumers whose composition is therefore altered in turn due to their competitiveness for food resources. In addition to these direct effects on aquatic life, algal productivity also affects aquatic life indirectly through dissolved oxygen and pH (through photosynthesis and decomposition of algal detritus) and physical habitat (through excess growth altering feeding and reproductive habitat).

Algae can also impact other important designated uses, such as drinking water and recreation, by the same pathways through which aquatic life uses are impacted. The competitive shift in species composition that occurs with nutrient pollution favors nuisance and harmful algal bloom taxa that produce toxins or compounds contributing to taste and odor issues that influence drinking water quality and treatment costs, as well as recreational uses of a water body. Similarly, increased productivity increases the concentration of dissolved organic compounds that contribute to disinfection by-product formation, and higher biomass can also increase operational costs associated with filtration. Many nuisance taxa have growth forms that are less desirable for recreation (e.g., long filaments and/or floating mats). High biomass also can affect water clarity making it aesthetically less desirable and also more difficult to see through.

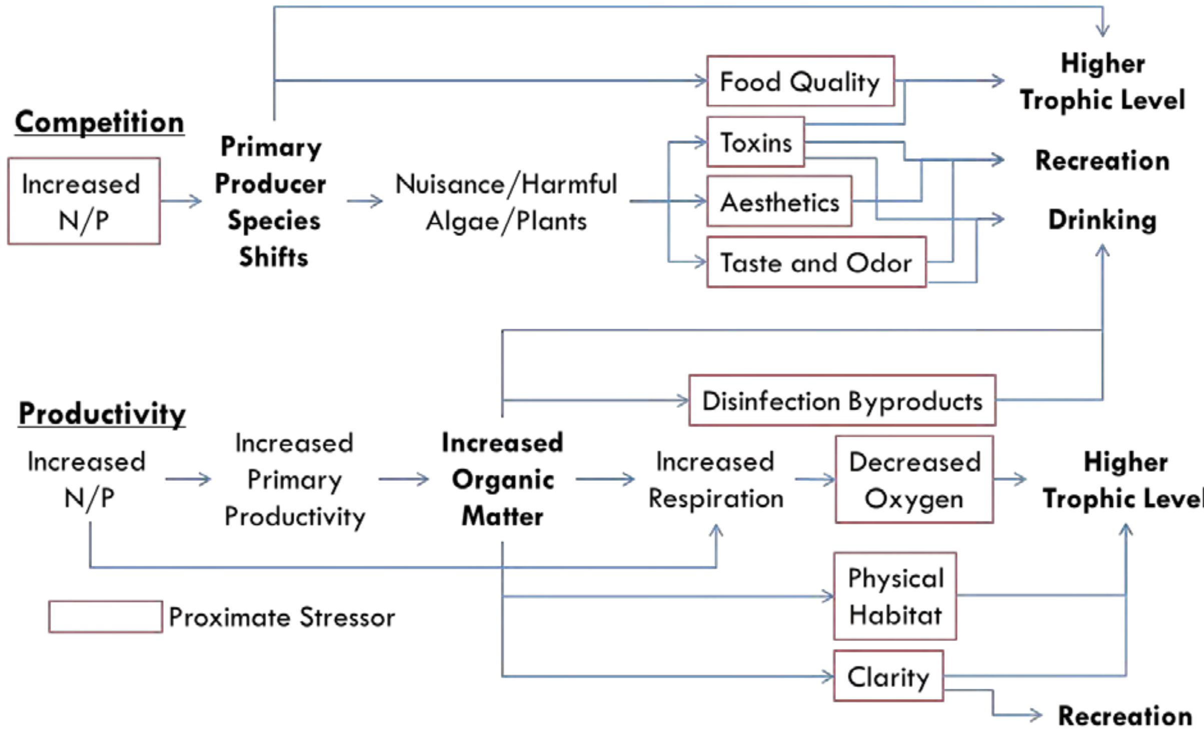

Figure 1. Conceptual model of effects of algae on designated uses.

Development of protective numeric nutrient criteria relies on the availability of assessment endpoints that are clearly responsive to nutrient pollution stress and linked to management goals. Algae are among the most important indicators of nutrient pollution stress and risk to designated use impairment in streams. As a result, EPA recommends that states and tribes consider the use of an algal biomass indicator, specifically chlorophyll *a*, because algae are not only a scientifically sound direct response to nutrient pollution, but algae in excess also stress aquatic ecosystems. At present, there is no comprehensive synthesis of the current state of diatoms and non-diatom algae application in streams as indicators of nutrient pollution that has been developed to assist water quality standards scientists and managers (See Stevenson (2014); Stevenson et al. (2010); Stevenson and Smol (2003) for excellent academic review of the subject). Therefore, the purpose of this review is to provide a reference document that details the current use of algae as indicators of nutrient pollution in streams to be helpful to states and tribes in the development of numeric nutrient criteria. This review includes descriptions and examples of the variety of methods and endpoints used, how algae are applied to assess biological condition and derive numeric pollutant endpoints, and the extent of their application in the U.S.

This review is comprised of two sections: the first section is a summary of U.S. applications by state and federal agencies; and the second section is a review of algal indicator research that has been pursued by scientists principally outside of state and federal agencies to advance the use of algae in nutrient pollution applications.

STATE AND FEDERAL AGENCY SUMMARY

EPA has encouraged states to use multiple assemblages, including algae, as part of the development of aquatic life use criteria (i.e., biocriteria, USEPA 2011, 2013a). EPA has also encouraged states to specifically use measures of primary productivity in the derivation of numeric nutrient criteria for streams and rivers (USEPA 2000). This encouragement for the use of algae in water quality standards and criteria development is reflected in EPA biological assessment programs. The Rapid Bioassessment Protocol (Barbour et al. 1999) has a chapter on algal methods, which are recommended for use because of algal sensitivity to stressors, especially nutrients, and importance to food webs. The chapter covers natural substrate and artificial substrate methods for diatom and non-diatom algae, lab processing, indicator development, and a rapid visual survey method. Similar algal methods and indicators were developed and included in the wadeable stream algal protocols for both the Environmental Monitoring and Assessment Program (EMAP) and the National Aquatic Resource Surveys (NARS) (Appendix I) (USEPA 2009).

In addition to EPA, other agencies also actively assess algae. For example, diatoms and non-diatom algae are a central component of the U.S. Geological Survey (USGS) National Water Quality Assessment (NAWQA) stream assessment protocol (Table 1) (Moulton et al. 2002), which is used by several state monitoring programs. The NARS and NAWQA protocols are both quantitative multiple habitat or transect methods that measure both benthic algal biomass as chlorophyll *a* and algal taxonomic composition. Quantitative multihabitat methods sample known areas of multiple habitats, thereby sampling a broad range of species, and transect methods are quantitative methods that also sample multiple habitats. Quantitative methods are important because they capture more taxa and increase precision, representativeness, and replicability, compared to qualitative methods. See Table 2 for a list of different types of quantitative methods frequently used by state and federal agencies. This information is translated using algal metrics or multimetrics. In the case of the recent NARS National Rivers and Streams Assessment, EPA calculated an algal multimetric index, or MMI (USEPA 2013b), which was used to interpret the biological condition of the nation's streams and rivers. In addition to quantitative methods, state and federal agencies use qualitative methods, which identify the taxa present, but not actual abundances, and without considering the sampled area (see Table 2 for a list of different types of qualitative methods frequently recommended or used by state and federal agencies).

Table 1. Federal agency programs encouraging or applying use of algae in stream regulation or monitoring.

Federal Agency Program	Application	Citation
USEPA/OST Biocriteria Development	States encouraged to use multiple assemblages including algae for biocriteria in streams	(USEPA 2011, 2013a)
USEPA/OST Nutrient Criteria Development	States encouraged to use primary producer measures to derive nutrient criteria for streams and rivers	(USEPA 2000)

USEPA Biological Assessment	Rapid Bioassessment Protocol for streams includes algal methods chapter for diatoms and non-diatoms	(Barbour et al. 1999)
USEPA ORD/OWOW EMAP and NARS	EPA national monitoring programs employ methods to collect algae in streams; NARS developed algal multimetric index to evaluate biological condition of nation's streams	(USEPA 2009, 2013b)
USGS NAWQA Program	USGS national monitoring program employs algal sampling methods for diatoms and non-diatoms	(Moulton et al. 2002)

Table 2. Quantitative and qualitative methods frequently used to sample and assess algae.

Quantitative Methods	
Method	**Description of method**
Quantitative multihabitat method	Scraping algae from known areas of several different habitats likely to support algae; samples may be held separate or combined.
Quantitative richest targeted habitat method	Scraping algae from known area of the habitat most likely to support the most algae at any site (e.g. rocks).
Passive periphytometer method	Deploying frame of glass slides upon which algae settle and grow.
Visual transect point-intercept method	Estimating percent cover at several locations along cross-sectional transects; measures at each point generally include some combination of percent algal cover or abundance, filament length, and periphyton thickness. Additional measures may include color, condition, and algal identification for soft algae.
Algal growth potential	Taking filtered stream water and measuring the growth of a single laboratory algal species in that water.
Qualitative Methods	
Method	**Description of method**
Qualitative multihabitat composite sample method	Taking scrapings of periphyton algal material from multiple habitats and combining them for identification.
Qualitative targeted habitat	General scrapings from specific targeted habitats.
Qualitative soft-algal method	Gathering a sample of representative soft algae for identification.
Qualitative point-transect composite method	Collect general scrapings from multiple substrates at points across multiple cross-section transects.

Twenty-three states were identified as having water quality programs that evaluate algae and a subset (11 states) of those were identified as having substantial program integration, including incorporation of algal measures into criteria exploration or development (USEPA 2002). The following discussion focuses on these 11 states first, providing a detailed review of each of them, followed by a general synthesis. A detailed table summarizing these state methods and applications is provided in Appendix I, along with references, for algal indices or other tools developed by states.

CALIFORNIA

California has developed standard methods for collecting diatom and non-diatom (soft algae) samples from streams for the purposes of biological assessment and numeric nutrient criteria or endpoint development (Fetscher et al. 2010). They use a mix of quantitative multihabitat methods for diatoms and non-diatoms, qualitative soft-algal methods, and visual transect point-intercept methods for percent benthic cover. The State estimates benthic biomass as chlorophyll *a* and AFDM, percent cover of algae, and identifies diatoms and non-diatoms in order to interpret that assemblage composition information with diatom/non-diatom multimetric index/indices.

In addition to the Statewide efforts, several regional water boards in California have explored development of diatom assemblage composition indices (multimetric and Observed/Expected [O/E] type models) for use in assessment. These have been explored for the San Diego, Lahontan, and Central Coast regions as well as the eastern Sierra Nevada mountains (Appendix I).

CONNECTICUT

Connecticut has an algal sampling program. They use USGS NAWQA methods, which consist of a quantitative richest targeted habitat sample to estimate algal biomass (chlorophyll *a* and AFDM) and quantitative diatom assemblage composition, as well as a qualitative targeted habitat sample from depositional habitats and a qualitative multihabitat composite sample. From the quantitative samples, the State estimates algal biomass and is developing an algal multimetric index. They have also used the raw diatom data to conduct stressor-response analyses to explore nutrient thresholds using TITAN analysis, change-point analysis, and boosted regression trees (Smucker et al. 2013).

FLORIDA

Florida has been using algal sampling for several years for biological assessment and criteria development. They measure benthic biomass (chlorophyll *a*) using quantitative multihabitat methods, water column algal biomass using quantitative volumetric filtration, percent benthic cover using a visual rapid periphyton survey of cover, thickness, and filament length based on the rapid bioassessment method (Barbour et al. 1999). In addition, the State measures benthic algal assemblage composition using a quantitative multihabitat method, a quantitative passive periphytometer method, and algal growth potential method. The State uses the water column biomass, percent cover, and species dominance in assessment as part of a combined criteria approach, explored development of an algal multimetric index for assessment, and used stressor-response relationships between nutrients and visual cover and biomass for criteria development.

IDAHO

Idaho samples benthic diatom assemblage composition using a quantitative richest targeted habitat method. They use these data to develop an algal multimetric index for potential use in biological assessment, and are conducting stressor-response analyses of nutrients versus diatom data for nutrient criteria exploration.

KENTUCKY

Kentucky has had an algal based sampling program for many years as well. This State samples benthic biomass (chlorophyll *a*) and benthic diatom assemblage composition using a quantitative richest targeted habitat method. They also measure percent benthic cover using a visual transect point-intercept method, and the State has separate qualitative targeted habitat methods and qualitative multihabitat composite sample methods to measure or detect additional algal species richness. The State has developed an algal multimetric index, which is used in biological assessment, and they are exploring stressor-response relationships between nutrients and algal data for use in criteria development.

MAINE

Maine has recently developed a substantial algal sampling program. They sample benthic biomass (chlorophyll *a*) and benthic algal assemblage composition using a quantitative richest targeted habitat sampling method as well as a passive periphytometer method. They also measure percent benthic cover using a visual transect point-intercept method. The State has developed total phosphorus (TP) and total nitrogen (TN) optima for algal taxa to develop tolerance values, used to construct some Maine-specific algal metrics. These were combined with other general algal metrics and are used to assign sites to aquatic life use tiers as part of their assessment program. They are also developing N and P inference models (inferring the nutrient conditions based on a weighted average of algal nutrient optima for taxa present) and used diatom data in stressor-response models to develop proposed numeric nutrient criteria.

MINNESOTA

Minnesota has developed and applied algal measures in setting criteria for rivers and streams. They measure benthic biomass (chlorophyll *a*) and benthic algal assemblage composition using the USGS NAWQA methods, primarily the quantitative richest targeted habitat sample method. They also measure water column biomass (chlorophyll *a*) using a standard quantitative volumetric filtration method. The State has used standard algal metrics for interpreting assemblage data and explored the use of these as response measures in stressor-response models for numeric nutrient criteria development.

MONTANA

Montana has long had a substantial algal sampling program. They sample benthic biomass (chlorophyll *a*) using a quantitative richest targeted habitat sampling method. They also measure percent benthic cover using a visual transect point-intercept method. The State also has qualitative algal assemblage composition methods using a qualitative multihabitat composite sample method from non-wadeables and a qualitative point-transect composite method for wadeables. The State uses a variety of diatom metrics including state-specific diversity, siltation, and pollution metrics. The indices are used in assessment and were used, along with biomass measures and visual cover measures, in stressor-response models to support numeric nutrient criteria development.

NEW JERSEY

New Jersey samples benthic biomass (chlorophyll a) and benthic algal assemblage composition using a quantitative richest targeted habitat sampling method. The State has developed TP and TN inference models (inferring the nutrient conditions based on a weighted average of algal nutrient optima for taxa present) and trophic diatom indices that are rescaled inference model values (0–100). The State uses the inference models and a diatom biological condition gradient (BCG) (Hausmann et al. 2016) to develop assessment tools and to support numeric nutrient criteria development. Rhode Island

Rhode Island has recently developed algal methods and analysis tools. They sample benthic biomass (chlorophyll *a*) and benthic algal assemblage composition using a quantitative richest targeted habitat sampling method as well as a passive periphytometer method. They also measure percent benthic cover using a visual transect point-intercept method. The State is using the raw diatom data to conduct stressor-response analyses to explore nutrient thresholds using TITAN and change-point analyses.

WEST VIRGINIA

Similar to many of the other states, West Virginia samples benthic biomass (chlorophyll *a*) and benthic algal assemblage composition using a quantitative richest targeted habitat sampling method. They also measure percent benthic cover using a visual transect point-intercept method. The State uses standard algal metrics as interpretive tools and is exploring the use of diatoms in numeric nutrient criteria development for aquatic life uses. In addition, a percent cover of 40% is used as a numeric translator of the narrative recreational use standard for a single transect.

EPA NATIONAL AQUATIC RESOURCES SURVEY (NARS) AND USGS NATIONAL WATER-QUALITY ASSESSMENT (NAWQA) PROGRAM

The NARS program at EPA and the NAWQA program at NASA both sample algal biomass (as chlorophyll *a* and AFDM) using quantitative methods. For benthic algal assemblage composition, EPA NARS uses a quantitative richest targeted habitat method at multiple fixed transect locations. USGS NAWQA uses a quantitative richest targeted habitat method as well. USGS NAWQA collects additional qualitative depositional and multihabitat samples, the former from pools and the latter from a variety of habitats, to search for additional algal taxa.

In addition to the states described above, twelve other states reported periphyton sampling to USEPA (USEPA 2002), but no further information could be identified elaborating on their methods or applications. These states are identified in Appendix I.

STATE SUMMARY

All of the states use quantitative active sampling methods that sample natural substrates in a manner that provides replicable estimates of diversity, abundance, and biomass. Algal biomass is typically estimated with chlorophyll *a*, and algal assemblage composition measured using microscopic identification of algae, most often to the species level. Most states sample from known areas of habitat believed to provide the best substrate for algal diversity and biomass (richest targeted habitat), which are often cobble in steep streams, and wood, macrophytes, or depositional materials (sand/silt) in low gradient systems. Most composite several samples from multiple substrates and/or transects into one sample to represent the site. Four states (FL, ME, NJ, and RI) also use passive periphytometer samplers, which are glass slides deployed in streams upon which a subset of stream algae grow. Eight states also conduct visual algal cover surveys, which consist of estimates or direct measurement of the extent and thickness of visual algal cover, as well as the length of any filaments. Most of these states use points along single or multiple transect for this measure. Kentucky also identifies large green and red algae as part of their visual method.

In terms of assemblage composition indicators, 8 of 11 states use algal metrics, which are measures of the diversity/richness, composition, traits, and autecology of the resident algae. Autecological measures include tolerance/sensitivity metrics to different pollutants including nutrients, pH, and oxygen. Most states (8) are measuring both diatoms and non-diatom algae, the others focus on diatoms alone. CT and RI use multivariate tools to measure thresholds in algal assemblage response to nutrient gradients for investigating nutrient concentrations that impact streams, but not for criteria development. New Jersey has developed inference models using algae. These are models that use taxon-specific nutrient optima to estimate the average nutrient concentrations at sites. New Jersey also uses diatom assemblage composition in the context of a Biological Condition Gradient (BCG) (Hausmann et al, 2016) to assess diatom community condition (pristine to severely disturbed).

Eight of the states are using or have used algal data in nutrient criteria development efforts. Most are using these data in stressor-response type analyses, looking for thresholds in ecological responses or interpolating values associated with desired biological conditions, most often based on reference condition. West Virginia is the only state identified that is currently using thresholds in visual cover as a recreational use criterion. The threshold for that was identified using a stressor-response model of user-perception survey data tied to their narrative aesthetic criterion (Responsive Management 2012). Montana has pursued development of user perception based algal cover endpoints in support of their nutrient criteria development efforts (Suplee et al. 2009).

EPA compiled a summary of state bioassessment and biocriteria programs in 2002 (USEPA 2002). That study documented an additional 12 states that have reported sampling algae, including periphyton, in their assessment programs, but not explicitly in criteria or criteria development (Appendix I - Summary table of U.S. state algal indicator endpoints, methods, interpretive tools, and use in criteria development and/or assessment.). Like the 11 states referenced above, these 12 primarily use quantitative active sampling of richest targeted habitat. Five of these also reportedly used passive periphytometer samplers and two (NM and SD) also collected a qualitative multihabitat sample. Most of these other states (8) focused on the entire algal assemblage, whereas the others focused on diatoms. None of these states reported any interpretive tools as part of this survey, so the extent to which these states still sample algae or have developed interpretive tools for use in criteria development is unknown. More recently, algal indicators have also been developed in Alaska for local application in urban streams and the Cook Inlet region (Rinella and Bogan 2007, Rinella and Bogan 2010).

In summary, 23 states were found in this synthesis to be evaluating algae routinely and a subset of those (11) are known to have developed interpretive tools or to have incorporated analysis of algal responses into nutrient criteria or biocriteria development. Major impediments to greater development likely include unfamiliarity, taxonomic expertise, and financial constraints. Algae are not as commonly applied in monitoring in this country and are an assemblage that may be less familiar than macroinvertebrates or fish, at least methodologically, to many state resource scientists. This can easily be overcome by disseminating the many method documents that exist and conducting training workshops. Taxonomic expertise is a limit because there are few labs and experts capable of identifying algal taxa, especially diatoms, to the species level. In addition, taxonomic consistency among labs is an issue, which may also fuel resistance (Besse-Lototskaya et al. 2011; Kahlert et al. 2012). These hurdles could be overcome by encouraging additional training of taxonomic experts, support for development of molecular identification techniques, and encouragement of taxonomic resolution among active labs, something that the European Union (EU) has been actively pursuing through the Water Framework Directive (Besse-Lototskaya et al. 2011; Besse-Lototskaya et al. 2006; Kahlert et al. 2012). Financial constraints are related to taxonomic expertise, since states that are already constrained by available funding, may be reticent to add an assemblage that requires additional sampling and taxonomic identification. The resolution for this constraint would be more investment in state monitoring programs for algal sampling and identification. The EPA is working to alleviate many of these impediments by providing monitoring support, training states in methods via NARS, working on a common taxonomy, and supporting research into molecular techniques for algal identification.

RESEARCH ON ALGAL ASSESSMENT AND INDICATOR USE IN STREAMS

This section will introduce independent (non-state and non-federal) studies and research conducted on algal indicators (e.g., diatom indices), in academia and the peer-reviewed literature, both in the U.S. and outside the U.S. More than 250 peer-reviewed manuscripts related to the use of algae in evaluating stream condition were reviewed. This information was organized into several general thematic areas, which are described here. These themes include:

- Geographic application
- Interpretive tools
- Indicator development
- Indicator comparisons
- Specific pollutant source application
- Novel insights from research related to chemical effects, habitat effects, variability, indicator analysis, and methods.

GEOGRAPHIC APPLICATION

Specific algal research was identified from at least 11 different unique states (CA, CT, ID, KY, ME, MI, NJ, NY, OH, OR, TN) (see Table 3 for state-specific studies). These include studies that involved specific analyses that could support state numeric nutrient criteria development, for example, threshold analyses of diatom response in CT (Smucker et al. 2013) and papers describing development of the algal indices included in the state review above, for example in California (Fetscher et al. 2014a), Idaho (Fore and Grafe 2002a), Maine (Danielson et al. 2012), and New Jersey (Ponader et al. 2007). As a whole, this research highlights the sensitivity of diatom and non-diatom algae to a variety of stressors (especially nutrients, sediment and acid mine drainage), their value as assessment tools across multiple states, and the variety of indicator options to use with algae. The last insight includes development of nutrient optima models with weighted averaging, predictive models (site specific metric predictions based on geomorphic predictors), multimetric models, percent model affinity models (comparing test sites to reference site composition), and ecosystem level measures of biomass and productivity.

Table 3. Breadth of states within which algal indicator research is being conducted.

State	Research	Citation
CA	Development and comparison of diatom and non-diatom algal indicators; comparison of response of ecosystem measures and community level indicators to nutrient pollution	(Fetscher et al. 2014a; Nelson et al. 2013)
CT	Application of regression tree and threshold analysis with algal metrics and biomass to develop numeric criteria	(Smucker et al. 2013)
ID	Development of algal indices for wadeable and non-wadeable streams using traditional multimetric and predictive multimetric models	(Cao et al. 2007; Fore and Grafe 2002b)

KY	Comparison of biomass responses of algae to nutrient gradients with criteria development implications	(Stevenson et al. 2006)
ME	Development of nutrient optima for algal species and state-specific algal metrics for use in assessing streams using those optima; Development of a model to rate sites into aquatic life use tiers using algal metrics	(Danielson et al. 2011; Danielson et al. 2012)
MI	Comparison of biomass responses of algae to nutrient gradients with criteria development implications	(Stevenson et al. 2006)
NJ	Development of weighted average nutrient optima for algal species and their use in state specific algal indicator creation for assessment and criteria derivation	(Ponader et al. 2007; Ponader et al. 2008)
NY	Development of algal index based on comparison of algal assemblage composition to that at reference sites	(Passy and Bode 2004)
OH	Development of a diatom index based on comparison to reference site composition and a diatom multimetric index to assess agriculture and acid mine drainage effects; Study of influence of acid-mine drainage on algal succession	(Smucker and Vis 2009, 2013; Zalack et al. 2010)
OR	Investigation of land use effects on diatom composition	(Weilhoefer and Pan 2006b)
TN	Comparative study of non-point source pollution on diatom assemblage composition, primary production, and excessive algal growth	(Lebkuecher et al. 2011)

There were also a number of regional studies (Table 4) that analyzed algal response in streams across broad regions including in the eastern U.S., Appalachians, Midwest and the Western U.S. (Black et al. 2011; Carlisle et al. 2008; Charles et al. 2006; Gillett et al. 2011; Griffith et al. 2002; Hill et al. 2000; Hill et al. 2001; Justus et al. 2010; Stevenson et al. 2008b; Walker and Pan 2006; Wang et al. 2005), as well as for large rivers (Kireta et al. 2012a; Kireta et al. 2012b; Reavie et al. 2010). In the same vein, there have been several studies published using the large national NAWQA periphyton dataset (Porter et al. 2008; Potapova and Charles 2007; Potapova and Charles 2002; Potapova et al. 2004). These broad regional and national studies identified strong regional controls of pH/alkalinity and hardness on diatom assemblage structure, helped develop algal sampling methods and indicators, and reinforced the sensitivity of algae and algal indicators to a variety of stressors, especially nutrients.

Table 4. U.S. areas within which broad regional algal indicator research is being conducted.

State	Research	Citation
Eastern U.S.	Exploration of major environmental controls on diatom distributions at multiple scales	(Charles et al. 2006)
Mid - Appalachians	Development of multimetric indices using algal richness, composition, and biomass measures; Comparison of genus and species level taxonomy on indicator response to stressors; Comparison of diatom to other assemblage response to land use	(Carlisle et al. 2008; Hill et al. 2000; Hill et al. 2001)
Interior Plateau Ecoregion (KY, TN, IN, and OH)	Diatom multimetric development and testing	(Wang et al. 2005)
Mississippi, Missouri, and Ohio Rivers	Evaluation of sampling methods for algae and response of metrics to stressors in large rivers; Development of diatom based indicators for	(Kireta et al. 2012a; Kireta et al. 2012b; Lane et al. 2007; Reavie

	large rivers; Comparison of benthic vs. planktonic algal indicators in large rivers	et al. 2010; Stevenson et al. 2006)
Ozark Region	Comparative study of algal indicators to other assemblages showing highest sensitivity of diatoms to nutrients	(Justus et al. 2010)
Rockies	Analysis of algal metric response to pollutant gradients	(Griffith et al. 2002)
Western U.S.	Development of novel diatom metrics including those based on algal weighted average optima for a variety of stressors and percent live diatoms; exploration of the relative importance of nutrients, land use, and habitat on diatoms and development of nutrient thresholds;	(Black et al. 2011; Gillett et al. 2011; Stevenson et al. 2008b)

Europe has a more extensive application of algal measures than the U.S., particularly in stream condition assessment (Birk et al. 2012). This is likely a function of the Water Framework Directive emphasis on biological monitoring of the entire community, and also the long use of algae for assessment of water quality in Europe starting in the early 1900s (Stevenson et al. 2010). Eighteen European countries have algal assessment methods, and the EU collaboration has supported development of indices and software to calculate a wide number of these indices (e.g. OMNIDIA software). Countries with active research, assessment, and indices for algae include: Austria, Belgium, Bulgaria, Finland, France, Germany, Hungary, Iceland, Italy, Latvia, Luxembourg, Norway, Poland, Portugal, Spain, Switzerland, Turkey, and the United Kingdom (Table 5). In addition to this work, the EU has put substantial effort into regional syntheses, which have focused on harmonizing assessments across countries, resolving issues related to reference condition, and taxonomic comparability (Almeida et al. 2014; Besse-Lototskaya et al. 2011; Besse-Lototskaya et al. 2006; Birk et al. 2012; Borics et al. 2007; Fisher et al. 2010; Hering et al. 2006; Johnson et al. 2006; Kahlert et al. 2012; Kermarrec et al. 2014; Kloster et al. 2014). The EU assessment (2011) found that the algal index score varied due to a lack of standardized methods, taxonomy, and counting consistency and are working to rectify this (Besse-Lototskaya et al. 2011). There are also a variety of indices used across Europe, and the EU is working to harmonize the metrics and indices using reference standardization (Almeida et al. 2014).

Many other countries have also embraced the use of algae in assessment (Table 5). In eastern Canada, extensive work has been done, especially focused on pollutant source impacts, but also on some of the first nutrient inference modeling for streams (Lavoie et al. 2006a; Lavoie et al. 2006b; Lavoie et al. 2014; Mazor et al. 2006; Winter and Duthie 2000; Wunsam et al. 2002). Additionally, one study in the Fraser River in western Canada compares multivariate assessment indices (Mazor et al. 2006). Algae have also been used in Mexico (Vazquez et al. 2011). No assessment studies in Central America were identified, although there are several research studies using algae in Costa Rica (e.g., Pringle and Hamazaki 1997). South American countries are represented by several assessment related studies using algae in Brazil and Argentina (Gómez and Licursi 2001; Lobo et al. 2004a; Lobo et al. 2004b; Salomoni et al. 2006). In Asia, diatoms are seeing increasing use in assessment, and several papers were identified related to algal assessment methods or applications in India, Iran, Japan, and especially China (Table 5). Similarly, in Africa, there have been several applications, including in Ethiopia, Kenya, Eastern Africa, and especially South Africa (Table 5). New Zealand has a long history of using algae in assessment and has developed comprehensive sampling protocols, indices, and even nutrient thresholds based on algal responses in streams (Biggs 2000; Biggs and Kilroy 2000; Schowe and Harding 2014). In addition, research from Australia also indicates the application of algal assessment measures there (Dela-Cruz et al. 2006).

Table 5. Sample breadth of countries outside the U.S. within which algal indicators are applied and/or research is being conducted.

Country/Region			
	(Rott and Schneider 2014)	Latvia	(Springe et al. 2006)
	(Triest et al. 2001)	Luxembourg	(Gevrey et al. 2004)
	(Passy 2007)	Norway	(Passy 2007; Rott and Schneider 2014; Schneider and Lindstrom 2011)
	(Eloranta and Soininen 2002; Mykra et al. 2012; Raunio and Soininen 2007; Soininen and Niemela 2002)	Poland	(Picinska-Faltynowicz 2009; Szulc and Szulc 2013)
	(Berthon et al. 2011; Blanco et al. 2012)	Portugal	(Adams et al. 2014; Feio et al. 2007, 2009; Mendes et al. 2012; Mendes et al. 2014)
	(Kloster et al. 2014)	Spain	(Delgado et al. 2010, 2012; Douterelo et al. 2004)
	(B-Beres et al. 2014; Stenger-Kovacs et al. 2013; Stenger-Kovacs et al. 2014)	Switzerland	(Solak and Acs 2011)
	(Gudmundsdottir et al. 2013)	Turkey	(Solak and Acs 2011)
	(Dell'uomo and Torrisi 2011; Gallo et al. 2013; Lai et al. 2014; Torrisi and Dell'Uomo 2006; Torrisi et al. 2008)	United Kingdom	(Solak and Acs 2011; Vinten et al. 2011)
	(Lavoie et al. 2006a; Lavoie et al. 2006b; Lavoie et al. 2014; Mazor et al. 2006; Winter and Duthie 2000; Wunsam et al. 2002)	Brazil Argentina	(Gómez and Licursi 2001; Lobo et al. 2004a; Lobo et al. 2004b; Salomoni et al. 2006; Salomoni et al. 2011)
	(Vazquez et al. 2011)		
	(Juttner et al. 2003; Karthick et al. 2010)	Japan	(Toda et al. 2002)
	(Atazadeh et al. 2007)	China	(Liu et al. 2013; Pignata et al. 2013; Tang et al. 2006; Wang et al. 2009; Wu et al. 2012)
	(Beyene et al. 2009; Beyene et al. 2014)	Eastern Africa	(Bellinger et al. 2006)

Kenya	(Beyene et al. 2009; Beyene et al. 2014; Triest et al. 2012)	South Africa	(de la Rey et al. 2008a; de la Rey et al. 2004; de la Rey et al. 2008b; Harding et al. 2005; Taylor et al. 2007)
Oceania			
Australia	(Dela-Cruz et al. 2006)	New Zealand	(Biggs 2000; Biggs and Kilroy 2000; Schowe and Harding 2014)

INTERPRETIVE TOOLS

As described earlier, algae have been used in biological monitoring for a long time (Stevenson 2014; Stevenson et al. 2010; Stevenson and Smol 2003). Since their earliest application in saprobien indices in Germany, algal assemblage composition data have frequently been interpreted using metrics and indices that combine information on the diversity of species, their traits, and what is known about individual species ecology (autecology) to evaluate water quality. This section splits interpretive tools into those that use taxonomic composition and those that use biomass.

COMPOSITION BASED TOOLS

The following tools (Table 6) employ benthic taxonomic presence/absence and abundance data collected using standardized methods to develop indicators that are used as measures of condition. These include multimetric indices, O/E or taxonomic completeness indices.

MULTIMETRIC INDICES

Multimetric indices (MMI) are by far the most common tool used around the world (Table 6). These consist of metrics reflecting diversity (number of taxa), composition, traits (e.g., growth form and motility) and autecological properties of individual taxa (e.g., tolerance/sensitivity to different pollutants). Individual metrics are selected based on a number of factors (e.g., responsiveness to stressors, redundancy, precision, range, etc., (Barbour et al. 1999) and scored using reference sites or ranges of conditions, standardized, and then combined into an overall MMI. Representative values are then used as boundaries for condition assessment. A proliferation of regional indices has developed around the world and while indices vary in their portability to different areas, some indices and especially component metrics, exhibit broad applicability (Pignata et al. 2013).

Table 6. Methods to interpret algal assemblage composition data used in biological condition assessment, monitoring, and criteria development

Index	Citation
Multimetric	(Alvarez-Blanco et al. 2013; Atazadeh et al. 2007; B-Beres et al. 2014; Bellinger et al. 2006; Blanco et al. 2012; Cao et al. 2007; de la Rey et al. 2008a; de la Rey et al. 2004; Delgado et al. 2010, 2012; Dell'uomo and Torrisi 2011; Eloranta and Soininen 2002; Fetscher et al. 2014a; Fore and

	Grafe 2002b; Gallo et al. 2013; Gómez and Licursi 2001; Harding et al. 2005; Hill et al. 2000; Kelly et al. 2008; Lai et al. 2014; Lebkuecher et al. 2011; Lobo et al. 2004a; Mendes et al. 2012; Picinska-Faltynowicz 2009; Pignata et al. 2013; Potapova et al. 2004; Rott et al. 2003; Salomoni et al. 2011; Schneider and Lindstrom 2011; Schowe and Harding 2014; Smucker and Vis 2009, 2011; Solak and Acs 2011; Stevenson et al. 2008b; Tan et al. 2013; Tang et al. 2006; Taylor et al. 2007; Torrisi and Dell'Uomo 2006; Triest et al. 2001; Triest et al. 2012; Wang et al. 2005; Wu et al. 2012; Zalack et al. 2010)
O/E Type and Multivariate	(Carlisle et al. 2008; Feio et al. 2007, 2009; Kelly et al. 2009; Lavoie et al. 2006a; Lavoie et al. 2006b; Lavoie et al. 2014; Mazor et al. 2006; Mendes et al. 2014)
Inference Models	(Alvarez-Blanco et al. 2013; Kireta et al. 2012b; Ponader et al. 2007; Ponader et al. 2008; Soininen and Niemela 2002; Wang et al. 2009; Winter and Duthie 2000)
Biological Condition Gradient (BCG)	(Danielson et al. 2012)
Multivariate Analysis	(Beyene et al. 2009; Beyene et al. 2014; Charles et al. 2006; Fisher et al. 2010; Griffith et al. 2002; Korhonen et al. 2013; Lavoie et al. 2006a; Lavoie et al. 2006b; Lavoie et al. 2014; Pan et al. 2006; Potapova and Charles 2002; Salomoni et al. 2006; Smucker and Vis 2009; Stancheva et al. 2011; Walker and Pan 2006; Weilhoefer and Pan 2006b; Winter and Duthie 2000)

TAXONOMIC COMPLETENESS INDICES

In terms of interpretive index type tools, taxonomic completeness indices, including Observed/Expected (O/E) type, some multivariate models, and similarity indices were the next most commonly used to MMI (Table 6). In contrast to multiple metrics, taxonomic completeness models compare the completeness of observed taxa richness to that expected under least disturbed conditions. The most common tool in this group is the O/E index. With an O/E index, one compares the expected taxa richness (E) modeled based on biogeographic predictors to that observed (O) at the site. A deviation from 1 indicates impact. Multivariate models (e.g., Benthic Assessment of SedimenT, BEAST) are similar to O/E type models as they measure how similar the species composition at a test site is to the population of regional reference sites. A significant departure from the natural variability in species composition is interpreted as an impact. In a similar vein, percent model affinity indices are simple multivariate models, and are cited in Table 6 in the multimetric category. Percent model affinity indices compare the similarity of composition of any test site to the average composition expected in reference sites but not using complex multivariate statistics but rather a similarity index. New York uses percent model affinity indices for macroinvertebrates and algae (Passy and Bode 2004).

INFERENCE MODELS

In addition to these index models, a number of studies have developed nutrient inference models (Table 6). Nutrient inference models use the observation that individual taxa have nutrient conditions under which they achieve their highest abundances or nutrient *optima*. These optima exist as a result of competitive differences for nutrients among taxa. Once these optima are known, then the taxa present at a site can be used to infer the likely average nutrient conditions based on averaging the taxa nutrient optima often weighted by relative abundances. This is arguably a better potential measure of the true average nutrient conditions since these taxa integrate nutrient concentrations over a longer time period than a single or few water quality grab samples. The trophic diatom index developed for use in New Jersey is an inference model re-scaled from 0–100 (Ponader et al. 2007; Ponader et al. 2008).

BIOLOGICAL CONDITION GRADIENT (BCG) MODELS

Maine has developed a biological condition gradient (BCG) type model for use with algae akin to the one they have developed for use with macroinvertebrates (Table 6) (Danielson et al. 2012). As opposed to a single aquatic life use, Maine has multiple aquatic life use classes organized along a gradient in biological condition (tiered aquatic life uses) used to protect high quality waters and to improve waters incrementally (USEPA 2011). The algal BCG model was constructed by asking experienced algal and water quality experts to classify sites into the appropriate aquatic life use tiers using the state narrative tier descriptions and algal metric data for each site. These decisions were then automated using discriminant function analysis so future sites can be classified based on their algal metric values alone.

MULTIVARIATE ANALYSIS

A number of studies used traditional multivariate analyses (including canonical correspondence analysis, non-metric multidimensional scaling, two-way indicator species analysis, and classification and regression trees) to analyze and interpret algal assessment data (Table 6) (Beyene et al. 2009; Beyene et al. 2014; Charles et al. 2006; Fisher et al. 2010; Griffith et al. 2002; Korhonen et al. 2013; Lavoie et al. 2006a; Lavoie et al. 2006b; Lavoie et al. 2014; Pan et al. 2006; Potapova and Charles 2002; Salomoni et al. 2006; Smucker and Vis 2009; Stancheva et al. 2011; Walker and Pan 2006; Weilhoefer and Pan 2006b; Winter and Duthie 2000). These techniques are commonly used to interpret ecological community data and to relate environmental gradients to patterns in species occurrence and abundance. They are common interpretive tools, for analytical purposes, and frequently used to inform the development of the most common multimetric and taxonomic completeness indices.

BIOMASS

A frequently used algal measure in streams is biomass, which is measured by chlorophyll a, ash-free dry mass or biovolume methods (Barbour et al. 1999, USEPA 2000). The most commonly used of the biomass indicators is chlorophyll *a*, one of the photosynthetic pigments found in algae (USEPA 2000). It has a long history of application for estimating algal biomass in aquatic systems, despite the fact that chlorophyll cell content among algae varies due to physiological and genetic factors. In streams, both water column (sestonic) as well as bottom (benthic) measures of chlorophyll are used (USEPA 2000). For the former, water column samples of suspended algae are filtered onto glass fiber filters and chlorophyll extracted to estimate volumetric chlorophyll *a* biomass. For the latter, a known area of substrate is scraped and the resulting periphyton filtered onto glass fiber filters and chlorophyll extracted to estimate areal chlorophyll *a* biomass.

Ash free dry mass is another measure used (USEPA 2000). Ash free dry mass is measured similarly, from the water column or benthic samples. Instead of chlorophyll *a* extraction, to estimate ash free dry mass, the water sample or benthic sample is filtered onto pre-weighed glass fiber filters and the organic content estimated by subtracting the mass after combustion at 500 degree C (to remove all organic matter) from the mass after drying the sample (to remove water). This difference is the ash free dry mass. It is a less accurate measure of algal biomass since it also contains non-algal detritus, microbes, small invertebrates, etc. that all contribute to ash free dry mass.

Biovolume is often used to estimate algal biomass (Barbour et al. 1999). For this estimate, the approximate dimensions of living cells are taken using microscopy and geometric equations used to estimate the volumetric mass of organic matter. Biomass can be estimated using published cell volume to biomass conversions.

INDICATOR DEVELOPMENT

Developing indicators, especially multimetric indicators, relies on developing metrics using trait and autecological information about different taxa (e.g., growth forms, motility, and pollutant tolerance/sensitivity). Much of the information on the former two (growth forms/motility) is available in ecological and taxonomic texts, but much of the latter (pollutant sensitivity) is developed using inference models. Weighted-averaging partial least squares models are among the more common technical approaches to do this, and these models have been applied to develop such information for algal taxa (Alvarez-Blanco et al. 2013; Danielson et al. 2011; Dela-Cruz et al. 2006; Kireta et al. 2012b; Potapova et al. 2004; Stevenson et al. 2008b). These weighted-average models help identify optima, which can then be relativized to infer sensitivity or tolerance. These models perform best when pollutant responses are unimodal, an assumption that is not always met and needs to be considered in developing optima (Potapova et al. 2004). Simple regression models of abundance to pollutant gradients have also been used to infer sensitivity/tolerance (Stevenson et al. 2008b).

Once identified using approaches like those described above, metrics are generally constructed to be sensitive to pollutants, especially nutrients. Some very unique metrics have been developed and have been found to be especially sensitive on a national scale. For example, the relative abundance of nitrogen fixers was inversely proportional to nitrogen concentration, and high dissolved oxygen taxa were inversely proportional to nitrogen to phosphorus (N:P) ratio across the U.S. using the NAWQA dataset (Porter et al. 2008). Since diatoms are identified by their silicate cases (or frustules) that do not necessarily reflect live individuals, another example explored the use of percent live diatom metrics and found them of mixed performance (Gillett et al. 2011). It is quite likely that novel metrics will continue to be developed and prove valuable in various applications.

Recent research has compared and contrasted diatom only and hybrid diatom and non-diatom algal models. These studies have found excellent performance from both diatom only, hybrid, and even non-diatom only models (Fetscher et al. 2014a; Stancheva et al. 2012). There appears to be a tendency to incorporate more non-diatom taxa into assessment models, especially since as many of the nuisance stream taxa are not diatoms.

Metrics have been used in assessment models alone or as multimetrics. In some instances, single metric indices have been found to be sufficient as indicators (Ponader et al. 2007; Schowe and Harding 2014), in other cases MMIs have been found to be superior to single metrics (Delgado et al. 2010). Multimetrics are far more common, but testing is routinely performed to evaluate the performance of any of these model options.

INDICATOR COMPARISONS

In many cases, indicators and even metrics and multimetrics have been found to be transferable to other regions and to perform well. For example, European indices have been applied in many countries, facilitated by the availability of software. European indices have been tested and found to work in China (Pignata et al. 2013; Tan et al. 2013), India and Nepal (Juttner et al. 2003), Iran (Atazadeh et al. 2007), and Eastern and South Africa (Bellinger et al. 2006; Taylor et al. 2007). Across Europe, comparison of indices has shown some promise as well (e.g., Dell'uomo and Torrisi 2011; Rott et al. 2003). While models using default European data often work, the most common observation has been that the regionally calibrated models generally outperform uncalibrated models, emphasizing the need for local ecological information to optimize model performance (Danielson et al. 2012; Delgado et al. 2010, 2012; Mendes et al. 2012; Potapova and Charles 2007; Rott et al. 2003).

Europe has undergone great analytical efforts to evaluate performance and comparability of metrics and indices across countries. This effort has identified effects of methods and taxonomy on index scores as well as differences in reference conditions that affect comparability of attainment boundaries and, therefore, general condition assessments (Besse-Lototskaya et al. 2011; Besse-Lototskaya et al. 2006; Birk et al. 2012; Juttner et al. 2003). European scientists are working to harmonize such differences across entities to improve the comparability of their stream algal assessments (Almeida et al. 2014).

In addition to comparison across regions, comparison across assemblages have also been conducted, for example, assessments based on algae versus invertebrates, fish or macrophytes. There is no obvious *a priori* reason to expect that all assemblages would respond the same to any given stressor and they often do not (Carlisle et al. 2008), but all assemblages have been demonstrated to be successful indicators of environmental condition, although some are more sensitive, and therefore, better than others (Johnson et al. 2006; Resh 2008; Zalack et al. 2010). Researchers have found algae to be more sensitive to nutrients than invertebrates (de la Rey et al. 2008a; de la Rey et al. 2008b; Feio et al. 2007; Gallo et al. 2013; Gudmundsdottir et al. 2013; Hering et al. 2006; Justus et al. 2010; Pignata et al. 2013; Smucker and Vis 2009; Triest et al. 2001). Other studies show that invertebrates have been found to be more sensitive to habitat impacts (Feio et al. 2007; Hering et al. 2006; Pignata et al. 2013; Triest et al. 2001). Some studies have found that algae may be more variable than invertebrates (Mazor et al. 2006; Mykra et al. 2012), or at least more variable at larger spatial scales (Springe et al. 2006). Finally, one analysis looking at a combined assessment model found that an O/E type model in which the algae and invertebrates were combined into one assessment indicator outperformed, in terms of sensitivity to disturbance, either assemblage alone (Mendes et al. 2014).

Comparisons of algal assemblages with fish were less common. The few studies that were identified found similar results to invertebrates, namely that there was weak concordance among assessments using algae and fish (Carlisle et al. 2008), that diatoms generally were more sensitive to nutrients than fish, and that fish were more sensitive to habitat impacts (Hering et al. 2006; Johnson et al. 2006; Justus et al. 2010; Smucker and Vis 2009). They also found a better signal with fish to gradients along large spatial scales (whole basin) than diatoms, presumably due to greater natural within basin variability of diatoms versus fish (Hering et al. 2006; Springe et al. 2006).

SPECIFIC POLLUTANT SOURCE APPLICATIONS

Algae have been used to study a variety of pollutant sources. They have been found to be sensitive to pollutants derived from acid mine drainage (Schowe and Harding 2014; Smucker and Vis 2013; Zalack et al. 2010), which likely includes both pH and metal sensitivities (Charles et al. 2006; Wunsam et al. 2002). They have also been shown to be sensitive to urbanization, which may reflect the strong effect of conductivity per se on algae (Charles et al. 2006; Ponader et al. 2008) as well as increased nutrient concentrations frequently associated with urbanization (Paul and Meyer 2001; Walker and Pan 2006). Algal nitrogen isotopic signatures have also been used to infer the source of nitrogen in urban settings, which may prove valuable in source tracking and, ultimately, nutrient pollution management. In Japan, for example, the isotopic signature of algal nitrogen in an urban stream was more closely related to sewage than fertilizer (Toda et al. 2002). Finally, algae have been shown to be sensitive to agricultural land use, due to their sensitivities to nutrients as well as to sediment, since algal species differ in their motility and abilities to move among fine substrates to access light (Black et al. 2011; Smucker and Vis 2011; Vazquez et al. 2011). Nutrient sensitive taxa and overall diversity decreased, the relative abundance of nutrient tolerant taxa increased, and motile species increased with increases in nutrients and sediment in these landscapes (Smucker and Vis 2011; Vazquez et al. 2011).

ADDITIONAL RESEARCH HIGHLIGHTS

This section describes additional research highlights related to this algal indicator review that may be of value to readers, including specific chemical applications, habitat applications, effects of variability, specific indicator analyses, and methods. While this review did not focus on specific chemical effects, a number were reported in the literature. For example, in addition to nutrients, algae are known to respond to a wide variety of conditions, especially changes in pH, conductivity, and dissolved oxygen, and this sensitivity was reflected in several of the studies reviewed (Charles et al. 2006; Lebkuecher et al. 2011; Ponader et al. 2008; Wunsam et al. 2002). Another study found that sewage discharges of soluble phosphorus resulted in large increases in the cyanobacteria, especially the Oscillatoriales and Nostocales (Douterelo et al. 2004). In contrast, nitrogen fixing algal forms (heterocystous cyanobacteria and diatoms with N-fixing symbionts) in California were observed to decline with increasing nitrogen, but at relatively low concentrations (see above, Stancheva et al. 2013).

Findings related to habitat response are mixed. Most studies comparing assemblage responses (e.g., invertebrates versus algae) found that diatoms do not appear to respond to habitat gradients as well as invertebrates or fish. However, other studies have found significant algal responses to habitat change. This is especially true with increases in fine sediments, which tend to favor more motile diatom taxa (Pan et al. 2006). The latter study did not compare the algal response to other assemblages, so it may be that algae do indeed respond, just not as strongly as other taxa.

Algae vary spatially (Charles et al. 2006; Passy 2007; Weilhoefer and Pan 2006b), given differences in limiting factors like light, flow, and substrate, even within one reach, not to mention across basins. This variability makes classification for metric and index development, either explicitly (in MMI indices) or implicitly (in O/E type models), critical (Charles et al. 2006), and this is a big component of many index development studies (Charles et al. 2006; Fisher et al. 2010; Gevrey et al. 2004). Algae also vary over time (Taylor et al. 2007), and this variability can increase with eutrophication (Korhonen et al. 2013). The time scale to which an assemblage responds to nutrients may be several weeks long. Nutrient concentrations in streams in South Africa monitored one month to six weeks prior to algal sampling were better predictors of algal response than grab samples taken during algal sampling (Taylor et al. 2007). An area in need of more research is the degree to which biomass and assemblage structure vary over time and whether the latter is less variable, emphasizing a potential further benefit of assemblage response measures (Stevenson 2014).

As the proliferation of algal metrics and indices has increased across the globe, so too has analysis of metrics and their performance. The first observation is that there is some general consensus that, for streams, biomass measures are frequently highly variable in their response to nutrients (Porter et al. 2008; Stevenson et al. 2006). However, one of the same studies also found that chlorophyll *a* and *Cladophora* biomass were related to nutrient concentrations, so the highly variable responses are by no means universal and may depend on the biomass measure chosen (Stevenson et al. 2006).

Biovolume responses are sometimes related to nutrient concentration, depending on the type of measure used (e.g., relative versus total biovolume, Reavie et al. 2010), but more frequently not (Raunio and Soininen 2007; Stancheva et al. 2011; Stancheva et al. 2012). In Canada, relative abundance was found to be better than biovolume, which did respond to nutrients, but was more variable (Lavoie et al. 2006a; Lavoie et al. 2006b).

Diversity and autecological metrics also differ. Diversity metrics, described above, among the first algal metrics used in assessment in the U.S. (Stevenson et al. 2010), vary in performance. Diversity metrics have sometimes exhibited poor correlation with nutrient concentration (Stancheva et al. 2011; Stancheva et al. 2012); this may be due, somewhat, to the Gaussian response of diversity to nutrient concentrations, tending to increase at intermediate concentrations (Wang et al. 2009). Other studies have found stronger responses of diversity metrics (Gudmundsdottir et al. 2013). Overall, the majority of studies have found that autecological (tolerance, sensitivity) and trait-based (growth form, motility) metrics generally exhibit better correlations and responses to nutrients than diversity or richness metrics (Berthon et al. 2011; Blanco et al. 2012; Danielson et al. 2011; de la Rey et al. 2008a; Griffith et al. 2002; Stenger-Kovacs et al. 2013).

Lastly, there are a number of method improvements and novel methods that may help increase the application of algae in water quality assessment. There have been improvements in microscopy that may reduce variability in microscopic identification and increase the processing time for these types of analyses. These include applications of confocal laser scanning microscopy in what is labeled "spectral fingerprinting" using spectral emission signatures of algae (Larson and Passy 2005) to identify taxa, as well as new image analysis software (e.g., SHERPA) that has improved on previous efforts and can automate identification (Kloster et al. 2014). There also continues to be development of novel molecular method applications, including application of DNA microarrays using phylochip technology as well as next-gen sequencing of diatoms, which could, in theory, decrease processing time and taxonomic variability, and increase sample sizes (Kermarrec et al. 2014; Metfies et al. 2007).

CONCLUSIONS

Algae are critical components of stream ecosystems, are relatively cheap and easy to measure, and sensitive to nutrient pollution, making them a potentially useful indicator of ecosystem change. Their population and biomass dynamics affect the food web of the entire stream ecosystem. Algal species composition and biomass are, in turn, also affected by water quality and habitat alteration and can be informative indicators of environmental condition. Because of these facts, algae affect aquatic life, recreational, and drinking water source uses, and are excellent assessment endpoints that have been applied globally as ecological indicators. A wide variety of methods and tools exist for sampling and assessing algae in streams that continue to proliferate and improve each year. While algal indicators are promising tool for managers, the use of these indicators is not currently widespread. Less than 50 percent of U.S. states appear to evaluate algae regularly. However, those using the algal assemblages have applied them to both the development of nutrient and biocriteria, as well as assessment and stressor diagnosis. Maine, Montana, and Kentucky appear to have the most comprehensive, current application of algal sampling, incorporating both species composition and biomass measures into their assessment programs and into nutrient criteria development. From the literature, it appears algal indicators are more widely employed routinely in Europe, where they are used to assess water quality, biological condition, and identify water quality stressors like nutrients and acidity. The European Union (EU) is ahead of the U.S. not only in applying this assemblage, but also in working across jurisdictions to resolve methodological and interpretive differences in algal assessment information. EU methodologies and their application are well documented, which should help the U.S. in developing consistent application.

ACKNOWLEDGMENT

Stefanie Gera (Tetra Tech, Inc.), Dacia Mosso (Tetra Tech, Inc.), and Kristen Parry (Tetra Tech, Inc.) helped identify state documents and peer-reviewed manuscripts for consideration and their contributions were integral to this project. Yukiko Ichishima (Tetra Tech, Inc.) helped with editing and reformatting.

The authors also wish to gratefully acknowledge the efforts and input of individuals who reviewed earlier drafts, including: Sophie Greene, John William Carroll, Lesley D'Anglada, Ana-Maria Murphy-Teixidor, Mario Sengco, Toby Stover, Izabela Wojtenko, Mark Barath, Bill Richardson Lauren Petter, Robie Anson, Forrest John, Mike Bira, Gary Welker, Tina Laidlaw, Alfred Basil, Suesan Saucerman, Rochelle Labiosa, Hillary Snook, Diane Switzer, Margherita Pryor, James Kurtenbach, Greg Pond, Louis Reynolds, David Melgaard, Chris Decker, Edward Hammer, Robert Cook, Catherine Wooster-Brown, Terrence Fleming, Gretchen Hayslip, Robert Angelo, Kevin Berry, Steve Kroeger, Kenneth Weaver and the Association of Clean Water Administrators - Monitoring, Standards and Assessment Committee

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APPENDIX I - SUMMARY TABLE OF U.S. STATE ALGAL INDICATOR ENDPOINTS, METHODS, INTERPRETIVE TOOLS, AND USE IN CRITERIA DEVELOPMENT AND/OR ASSESSMENT[1].

State	Endpoints	Methods	Interpretive Tools	Use in Criteria Development/ Assessment	Citations
	Benthic biomass (AFDM and Chl a)	Quantitative multihabitat	Biomass estimate		(Busse 2009; Fetscher et al. 2010; Fetscher et al. 2014a; Fetscher et al. 2014b)
	Percent benthic cover	Visual transect point intercept	Percent cover		
	Benthic algal assemblage composition	Quantitative multihabitat; diatom and non-diatom	Diatom/Non-Diatom MMI	Analyzing thresholds of algal MMI and component metrics. Potential to add in statewide aquatic life use (ALU) assessment.	
		Qualitative soft-algae method			
	Benthic diatom assemblage composition		MMI		(Bernstein 2014)
	Benthic algal assemblage composition		Multidimensional ordination Diversity Index		(Blinn and Herbst 2003)
	Benthic diatom assemblage composition		MMI and taxonomic completeness (Observed/Expected) models		(Rollins et al. ND)
	Benthic diatom assemblage composition		MMI		(Herbst and Blinn 2008)
	Benthic biomass (Chl a)	USGS NAWQA methods	Biomass estimate	Unknown	

[1]This review was likely not fully comprehensive, but was based on available literature and documents.

State	Endpoints	Methods	Interpretive Tools	Use in Criteria Development/ Assessment	Citations
	Benthic diatom assemblage composition		MMI in development TITAN analysis with relative abundance data Change-point analysis of metrics Boosted regression trees of NMS axes		(Association of Clean Water Administrators 2012; Becker 2012; Smucker and Vis 2013)
FL	Benthic biomass (Chl a)			Use water column biomass in assessment; Explored stressor response relationships with percent cover and biomass; Use biomass, percent cover, and species dominance in combined criteria approach	(Florida Department of Environmental Protection (FL DEP) 2014; Fore 2010; Stevenson and Wang 2001)
	Water column biomass (Chl a)				
	Percent benthic cover	Visual rapid periphyton survey (cover, thickness and length)			
	Benthic algal assemblage composition	Quantitative multihabitat method; Passive periphytometers	Diatom/Non-diatom MMI and biological condition gradient (exploratory);		
	Algal growth potential				
ID	Benthic diatom assemblage composition	Quantitative richest targeted benthic habitat	MMI	Exploring diatom data use in criteria development	(Fore and Grafe 2002b; Idaho Department of Environmental Quality (IDEQ) 2002)
KY	Benthic biomass (Chl a)	Quantitative richest targeted benthic habitat (known area)			(Association of Clean Water Administrators 2012; Kentucky Department of Environmental Protection (KDEP) 2009a, 2009b, 2010)
	Percent benthic cover	Visual transect point-intercept (cover and thickness, identify green and red algae, general abundance)			
	Benthic diatom assemblage composition	Quantitative richest targeted benthic habitat (known area) Qualitative targeted habitat Qualitative multihabitat composite	MMI	Diatom MMI used in assessment; Exploring stressor-response modeling of diatom metrics for use in criteria development	

State	Endpoints	Methods	Interpretive Tools	Use in Criteria Development/ Assessment	Citations
ME	Benthic biomass (Chl a)	Quantitative richest targeted habitat; Passive periphytometers			(Danielson et al. 2011; Danielson et al. 2012; Maine Department of Environmental Protection (MDEP) 2009, 2014)
	Percent benthic cover	Visual transect point-intercept with viewing bucket (cover, length, and thickness)			
	Benthic algal assemblage composition	Quantitative richest targeted habitat; Passive periphytometers	Total P and Total N optima used to develop tolerance values (TVs); TVs used to develop some ME specific diatom metrics along with general metrics; Diatom metrics used to assign sites to ALU tiers; Also developing N and P inference models;	Assess sites with diatom metrics; Used diatom data in stressor-response models to develop numeric criteria	
MN	Benthic biomass (Chl a)	USGS protocols			(Heiskary et al. 2013)
	Water column biomass (Chl a)	Standard quantitative water column sample			
	Benthic algal assemblage composition	USGS Protocols	Exploratory analysis of stock metrics	Exploratory use in NNC Technical Document	
MT	Benthic biomass (Chl a)	Quantitative richest targeted benthic habitat (known area)			(Suplee et al. 2009, Montana Department of Environmental Quality 2011a, 2011b, 2011c, 2011d)
	Percent benthic cover	Visual transect (cover, color, condition, length, and thickness)			
	Benthic algal assemblage composition	Qualitative multihabitat composite; Qualitative multihabitat point transect composite	Metrics including MT specific diversity, siltation, and pollution indices	Indices used in assessment; Metrics were used in stressor-response analysis to support adopted NNC development.	

State	Endpoints	Methods	Interpretive Tools	Use in Criteria Development/ Assessment	Citations
NJ	Benthic biomass (AFDM and Chl a)	Quantitative richest targeted benthic habitat (known area)			(New Jersey Department of Environmental Protection 2007; Ponader and Charles 2003; Ponader and Charles 2005; Ponader et al. 2007; Ponader et al. 2008)
	Percent benthic cover	EPA Rapid Bioassessment Protocol (RBP) view bucket			
	Benthic algal assemblage composition	Quantitative richest targeted benthic habitat composite (known area); Qualitative targeted habitat composite; Passive periphytometers;	TP and TN Inference Models using weighted averaging - partial least squares; Trophic Diatom Indices are rescaled inference model values (0-100)	Using models to develop assessment tools and to support NNC development	
RI	Benthic biomass (Chl a)	Quantitative richest targeted benthic habitat (known area) Periphytometers			(Rhode Island Department of Environmental Management 2011a, 2011b, 2011c)
	Percent benthic cover	Visual transect point-intercept with viewing bucket (cover, length, and thickness)			
	Benthic algal assemblage composition	Quantitative richest targeted benthic habitat (known area); Passive periphytometers	TITAN and Classification and Regression Tree (CART) analysis of metric response to nutrients	Unknown	
WV	Benthic biomass (Chl a)	Quantitative richest targeted benthic habitat (known area)			(West Virginia Department of Environmental Protection 2014a, 2014b)
	Percent benthic cover	Visual transect segments (cover, thickness)		Percent cover of >40% is used as numeric translator of narrative recreational use standard for a single transect	
	Benthic algal assemblage composition	Quantitative richest targeted benthic habitat (known area)	Standard algal metrics	Exploring use of diatoms in NNC development for ALU	
USGS	Benthic biomass (AFDM and Chl a)	Quantitative richest targeted benthic habitat (known area)			(Moulton et al. 2002; Porter et al. 2008; Potapova and Charles 2007)
	Water column biomass (Chl a)	Quantitative water column sample			

State	Endpoints	Methods	Interpretive Tools	Use in Criteria Development/ Assessment	Citations
	Benthic algal assemblage composition	Quantitative richest targeted benthic habitat (known area); Qualitative depositional habitat composite; Qualitative multihabitat composite	Standard algal metrics		
EPA NARS	Benthic biomass (AFDM and Chl a)	Quantitative multiple transect composite			(USEPA 2013b)
	Benthic algal assemblage composition	Quantitative multiple transect composite	Diatom MMI		
The following states reported assessing algae to USEPA in 2002. They were not included above based on understanding of current application, but this information is included for completeness.					
AZ	Benthic algal assemblage composition	Quantitative richest targeted benthic habitat (known area); Passive periphytometers			(USEPA 2002)
IN	Benthic algal assemblage composition	Pilot project with USGS			(USEPA 2002)
KS	Benthic biomass (Chl a)	Quantitative richest targeted benthic habitat (known area)			(USEPA 2002)
	Benthic diatom assemblage composition	Quantitative richest targeted benthic habitat (known area); Passive periphytometers	Limited taxa identification		
MA	Benthic biomass (Chl a)	Quantitative richest targeted benthic habitat (known area)			(USEPA 2002)
	Benthic algal assemblage composition	Quantitative richest targeted benthic habitat (known area); Passive periphytometers			

State	Endpoints	Methods	Interpretive Tools	Use in Criteria Development/ Assessment	Citations
NM	Benthic diatom assemblage composition	Quantitative richest targeted benthic habitat (known area); Qualitative multihabitat; Passive periphytometers			(USEPA 2002)
NC	Benthic algal assemblage composition	Quantitative richest targeted benthic habitat (known area)			(USEPA 2002)
ND	Benthic diatom assemblage composition	Quantitative richest targeted benthic habitat (known area)			(USEPA 2002)
NY	Benthic diatom assemblage composition	Quantitative richest targeted benthic habitat (known area)			(USEPA 2002)
OR	Benthic algal assemblage composition	Quantitative richest targeted benthic habitat (known area)			(USEPA 2002)
SD	Benthic biomass (Chl a)	Quantitative richest targeted benthic habitat (known area)			(USEPA 2002)
	Benthic algal assemblage composition	Quantitative richest targeted benthic habitat (known area); Qualitative multihabitat; Passive periphytometers			
WA	Benthic algal assemblage composition	Quantitative richest targeted benthic habitat (known area)			(USEPA 2002)
WI	Benthic biomass (Chl a)	Quantitative richest targeted benthic habitat (known area)			(USEPA 2002)
	Benthic algal assemblage composition	Quantitative richest targeted benthic habitat (known area)			

Note,
"Chl a" = chlorophyll *a*;
"NNC" = numeric nutrient criteria;
"MMI" = multimetric index

APPENDIX II - SAMPLE OF NUTRIENT THRESHOLDS DERIVED FROM VARIOUS ALGAL ENDPOINTS

A number of studies in the U.S. and beyond, have explored the presence of thresholds in nutrient concentrations associated with algal conditions for ecological phenomenological reasons (e.g., what nutrient concentrations represents a change in ecosystem state) or for more applied reasons (e.g., at what nutrient concentrations do users find algal response conditions that are unsuitable for recreation). This appendix reviews a sample of these numeric nutrient thresholds for a variety of different endpoints.

A few studies were found that examined nutrient thresholds associated with algal responses, but this search was far from exhaustive. One group of studies focused on identifying trophic state boundaries for streams. Trophic boundaries are a reflection of primary production among other factors, and therefore reflect algal growth. Boundaries associated with trophic states suggested thresholds at 20 µg/L TP and 300–600 µg/L TN for oligo-mesotrophic streams and 50–60 µg/L TP and 600–750 or even 1500 µg/L TN for meso-eutrophic streams (Table 7). Other studies put eutrophic streams at 70 µg/L TP and 1500 µg/L TN.

Other studies examined nutrient conditions associated with specific algal biomass levels, many of which have been tied to adverse effects. These found maximum benthic chlorophyll levels in streams below 50–60 mg/m^2 with TP less than 16 or 25 µg/L and TN less than 115–145 or 700 µg/L (Table 7). Maximum chlorophyll was below 100 mg/m^2, when TP was less than 35–38 or 46 µg/L and TN less than 252–275, 470 or 1800 µg/L. Maximum chlorophyll was below 200 mg/m^2 with TP less than 75 or 90 µg/L and TN less than 650 or 1500 µg/L. Mean chlorophyll levels below 50 mg/m^2 were associated with TP concentrations of 60 and 62–65 µg/L and TN of 450–470 µg/L. Mean chlorophyll levels below 100 mg/m^2 were associated with TP concentrations of 197–221 µg/L and TN of 1423–1600 µg/L. Finally, mean chlorophyll levels below 200 mg/m^2 were associated with TP concentrations of 415–1020 µg/L and TN of 3000–7570 µg/L. Thresholds in suspended chlorophyll (sestonic chlorophyll *a*) response were identified at TP concentrations of 21, 64, and 70 µg/L TP and 927, 945, and 1169 µg/L TN (Table 7).

Studies were identified that looked at growth responses. These identified nutrient limitation for diatoms occurring from 10–30 µg/L TP and for algal biomass at 30 µg/L TP and 1000 µg/L TN. Saturated growth and biomass accrual were identified at 50 and 82 µg/L TP, respectively (Table 7).

In terms of taxonomic changes that have been observed, the nuisance taxa *Lyngbya* and *Vaucheria* were found to increase above TP concentrations of 33 and 26 µg/L TP and 250 and 284 µg/L TN respectively, in spring streams in Florida (Table 7). In Texas, nuisance algal growth occurred at 200 µg/L TP. In contrast, heterocystous cyanobacteria forms (N-fixing) and diatoms with N-fixing symbionts declined above 40 µg/L NH_4-N and 75 µg/L NO_3-N in another study, indicating the sensitivity of these taxa or growth forms to even low concentrations on nitrogen. Other studies found that sensitive algal taxa began to decline at 20 µg/L TP, continued to decline at 40 µg/L TP coincident with other assemblage changes, and then sensitive taxa were lost from 40–65 µg/L, when tolerant taxa began to increase (Table 7). These thresholds were reflected in index and metric nutrient thresholds which were variously identified at 10 to 30 µg/L TP for some sensitive metrics, 50 µg/L TP for a diatom index, and 280 µg/L TP for a tolerant metric response. Equivalent TN thresholds were identified at 590 and up to 1790 µg/L TN (Table 7).

Table 7. Summary of nutrient threshold analyses.

Study Location	Response	TN (µg/L)	TP (µg/L)	Citation
Multiple	Stream oligo-mesotrophic boundary	285–375	23–29	(Dodds 2006)
Multiple	Stream meso-eutrophic boundary	659–714	48–71	(Dodds 2006)
Multiple	Chl a < 200 mg/m^2	<3000	<400	(Dodds 2000)
Multiple	Chl a of 50 mg/m^2 (< 100 mg/m2 most of the time)	470	60	(Dodds 2000)
Multiple	Stream oligo-mesotrophic boundary (60 mg/m^2 Chl a max)	700	25	(Dodds et al. 1998)
Multiple	Stream meso-eutrophic boundary (200 mg/m^2 max)	1500	75	(Dodds et al. 1998)
California	Heterocystous cyanobacteria and diatoms with N-fixing symbionts decline	75 (NO_3 –N) 40 (NH_4 –N)		(Stancheva et al. 2013)
Florida	*Lyngbya wollei*	110 (NO_3 –N)		(Albertin 2009)
Florida	*Lyngbya wollei*	230 (NO_3 –N) 250	28 (PO_4-P) 33	(Stevenson et al. 2007)
Florida	*Vaucheria* sp.	261 (NO_3 –N) 284	22 (PO_4-P) 26	(Stevenson et al. 2007)
Mid-Atlantic region	Diatom nutrient limitation		10–30	(Stevenson et al. 2008a)
Michigan and Kentucky/Indiana	Algal biomass	1000	30	(Stevenson et al. 2006)
Montana	50 mg/m^2 mean Chl a target	450–470	62–65	(Dodds et al. 1997)
Montana	50 mg/m^2 max Chl a target	115–145	16–20	(Dodds et al. 1997)
Montana	100 mg/m^2 mean Chl a target	1423–1600	197–221	(Dodds et al. 1997)
Montana	100 mg/m^2 max Chl a target	252–275	35–38	(Dodds et al. 1997)

Study Location	Response	TN (µg/L)	TP (µg/L)	Citation
	200 mg/m^2 mean Chl a target	3000–7570	415–1020	
	200 mg/m^2 max Chl a target	650	90	
New Jersey	Diatom index response	200 (NO_3 –N)	50	(Ponader and Charles 2003)
Oak Ridge National Lab, Tennessee	Benthic Algal Growth Saturation		50	(Hill et al. 2009)
Ohio	Sensitive algal taxa decline		20	(Smucker et al. 2013)
	Sensitive taxa loss and assemblage change		40	
	Sensitive diatoms lost, tolerant taxa increase		65	
	Saturation		82	
Texas (Aggregate Nutrient Ecoregion IX)	Decline in biological integrity (loss of algal, macrophytes, and macroinvertebrate species), decline in DO below levels suitable for native fauna during low flows, and increasing nuisance algal growth		20 (second degradation tier at 200)	(King et al. 2009)
Washington State, Nebraska	Algal metric responses	590–1790	30–280	(Black et al. 2011)
Wisconsin Non-wadeable Streams	Suspended Chl a increases	927	64	(Robertson et al. 2008)
Wisconsin Wadeable Streams	Suspended Chl a increases	1169	70	(Robertson et al. 2006)
	Benthic Chl a	609–1106	90	
Ontario/Quebec Canada	Eutrophic boundary	1500	75	(Chambers et al. 2008)
	Suspended Chl a	945	21	
	Benthic Chl a < 100 mg/m^2	1800	46	

Study Location	Response	TN (µg/L)	TP (µg/L)	Citation
Norway	Diatom multimetric threshold begins		10	(Schneider and Lindstrom 2011)
	Large diatom multimetric responses occur		10–30	

Note,
"Chl a" = chlorophyll *a*