

LABORATORY AND FIELD ASSESSMENTS OF BROMINATED
FLAME RETARDANTS AND OTHER ENVIRONMENTAL
CONTAMINANTS: EFFECTS IN FISHES

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ABSTRACT

Freshwater ecosystems have been the victim of many anthropogenic insults in the last century as consequence of industrial, agricultural and urban development. One type of industrial compound that has been of increasing concern in the last few decades is the brominated flame retardants (BFRs). BRFs have been added to a great variety of products and studies have shown that they are released into the environment and can be easily absorbed through the diet.

Polybrominated diphenyl ethers (PBDEs) have been the most commonly used BFR in Europe and the Americas. The chemical structure of PBDEs resembles the structure of thyroid hormones (THs), raising concerns about its potential for endocrine-disrupting activity. One of the most abundant PBDE congeners present in aquatic and terrestrial wildlife is BDE-47, a tetra-BDE, found in commercial mixtures of flame retardants or produced in the environment as the metabolic or degradation product of higher-brominated congeners.

Studies with teleosts have shown disruption of TH homeostasis, developmental neurotoxicity and reproductive toxicity after PBDE exposure. However, the mechanisms of PBDE action are still not well understood. The present study includes a laboratory and a field component. In the laboratory, the effects of BDE-47 on growth and reproductive development of juvenile fish were assessed using zebrafish (*Danio rerio*) as experimental model. In the field, the thyroid and health conditions of largescale sucker (*Catostomus macrocheilus*), a native species of the Columbia River, were assessed in the lower portion Columbia River near Portland (Oregon) and

Vancouver (Washington), an ecosystem where drastic increases of PBDEs have been recorded in the last two decades.

The effects of orally-administered BDE-47 on thyroid condition, somatic growth, and germ cell developmental stage were determined in zebrafish. The exposure started when fish were 35days post-hatching and lasted for 120 days, covering the juvenile-to-adult transition period (Chapter II). Fish were sampled at 40, 80 and 120 days after the onset of the exposure to 0-25 $\mu\text{g/g}$ BDE-47 in the diet. A decrease in body weight was observed in females exposed to 5 $\mu\text{g/g}$ BDE-47 after 40 days but not after 80 or 120 days or at any other concentration. In males, no apparent effects of BDE-47 on size were observed after 40 and 80 days, but fish exposed to the highest concentration (25 $\mu\text{g/g}$) showed a significant decrease in body weight at 120 days compared to control fish. No significant effects of BDE-47 exposure were found on thyroid condition or germ cell stage in either gender. These observations suggest that somatic growth but not reproductive development is affected by BDE-47 exposure in juvenile zebrafish, with males being more sensitive to long term exposure; and that the somatic effects occur independently of thyroid condition. In comparison with results of recent studies with other teleost species, such as fathead minnow, the thyroid endocrine axis of zebrafish seems to be relatively insensitive to PBDE exposure.

Three sampling sites on the lower Columbia River were chosen for the field study, following a contamination gradient: Longview (furthest downstream) and Columbia City, both with high levels of input from urban and industrial effluents from the Portland-Vancouver region; and Skamania (furthest upstream), considered the reference location having a relatively less disturbed environment. Fifteen males and

10 females per site were sampled in 2009 and 2010; in 2009, however, thyroid tissue was not collected from all females, therefore they were not included in this first study. The concentrations of plasma thyroxine (T_4) and triiodothyronine (T_3) were measured in addition to a number of thyroid histological variables; in addition, liver contaminant content was determined in males collected in 2010 (Chapter III). Plasma T_4 levels ranged from undetectable (LOD, 2.5 ng/ml) to 8.8 ng/ml in females and from undetectable to 10.4 ng/ml in males. Plasma T_3 levels ranged from 0.9 to 8.2 ng/ml in females and from undetectable (LOD, 0.7 ng/ml) to 9.1 ng/ml in males. The overall thyroid condition of male largescale sucker differed among collection sites in 2009 but not 2010. Plasma T_3 concentration and follicle cell density were the main drivers for the site discrimination in 2009, with Longview presenting the lowest and highest values, respectively. In females, thyroid condition not only differed among collecting sites, but also between fish from Skamania and the downstream sites; in these fish, colloid depletion and follicle cell density were the main drivers for site discrimination with Longview presenting the highest values for both variables. The contaminant content of male livers differed significantly among sites in 2010, with higher concentrations observed in the downstream sites relative to the reference site; hexachlorobenzene and dimethyl tetrachloroterephthalate contributed the most to the discrimination. However, clear associations between liver contaminant content, including PBDEs, and thyroid condition of male largescale sucker could not be established. Overall, the results of this study suggested that factors other than the ones considered here may be responsible for the differences in thyroid condition among sites.

The general health of largescale sucker from the lower Columbia River was also evaluated (Chapter IV). Fish length, weight, condition factor, gonadosomatic index and hematocrit were measured for males and females in 2009, and liver and gonad tissue were collected from males for histological analyses. In 2010, additional observations recorded for both males and females included a general assessment of the external condition, and collection of spleen, kidney and gills for histological analyses. In 2009, significant differences in biological traits were not observed in males or females. However, in 2010, when more variables were included in the analyses, males differed among sites mostly in regards to kidney and spleen histopathologies; whereas females could be separated primarily on the basis of kidney and liver histopathologies. However, despite the site discrimination by health condition and by liver contaminant profiles (Chapter III) in males, associations between these two variable sets were not significant.

The laboratory component of the present study reported that BDE-47 exposure in juvenile zebrafish causes a decrease in somatic growth, with males being more sensitive to long-term exposure than females. These effects, however, were independent of changes in thyroid condition. In the field, it was found that the thyroid and health condition of largescale sucker populations differ along the lower Columbia River Basin; however, factors other than those examined in the present study may be responsible for these differences. Further studies examining a broader group of contaminants in the area, in addition to laboratory exposure studies, may be required to better understand the potential effects of contaminant mixtures in this and other teleost species.

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CHAPTER I

INTRODUCTION

The thyroid endocrine axis of teleost fishes

The anatomy of the thyroid gland differs greatly between higher vertebrates and teleost fishes. In the former group the thyroid follicles are organized in compact organs, while in the latter the follicles are freely distributed in the mesenchyme of the ventral head area, in the vicinity of the anterior aorta (Genten 2009). The location of the follicles in teleosts is highly variable among different species; they are found alone or in aggregations of two or three embedded in connective tissue (Raine and Leatherland 2003). In spite of such differences at the gross anatomical level, the function of the thyroid follicle as the endocrine unit of the system is highly conserved through vertebrates (Paris and Laudet 2008).

A thyroid follicle consists of epithelial cells surrounded by a basement membrane that enclose an extracellular space into which the cells secrete a glycoprotein called thyroglobulin. Follicular cells extract inorganic iodide from the blood and transport it into the colloid where it is incorporated to the thyroglobulin to form L-thyroxine (T_4). In teleosts, thyroid peroxidase enzymes oxidize the thyroglobulin to release primarily T_4 into the blood stream where it is carried to target tissues bound to a T_4 -binding protein. The release of T_4 occurs under stimulation by thyroid-stimulating hormone from the pituitary, which is in turn regulated by regulatory factors from the hypothalamus (Blanton and Specker 2007). In peripheral and target tissues, a complex of deiodinase enzymes

(D1 and D2) remove one iodide atom from either of the two outer ring iodines, converting T_4 into the biologically active 3,5,3'-triiodo-L-thyronine (T_3) (Blanton and Specker 2007; Walpita et al. 2007). Inactivation of T_4 and T_3 occurs by inner ring deiodination catalyzed by a third group of deiodinase enzymes (D3) (Sutija and Joss 2005). It is T_3 that binds with high affinity to thyroid hormone (TH) receptors and is responsible for most biological activities (Carr and Patiño 2011) (Figure 1.1).

Thyroid hormones are hydrophobic. Consequently, binding proteins are necessary to transport the hormones to their sites of action and, at the same time, to balance the hormone depletion resulting from active tissue uptake and metabolism (Schussler 2000). Transport proteins differ in their affinity for ligands. Some are highly specific (i.e. transthyretin), while others have lower affinities and generally lower specificities, but their plasma levels are high enough to bind significant amounts of ligand. Less specific binding proteins include albumin, α_1 -acid glycoprotein, and high (HDL), low (LDL) and very low (VLDL) density lipoproteins (Hervé et al. 1994).

Thyroid hormone-binding proteins include transthyretin (TTR), thyroxine-binding globulin, albumin (Morgado et al. 2007) and lipoproteins (Babin 1992). TTR is the main TH carrier in rodents and in humans the main transporter is thyroxine-binding globulin (Morgado et al. 2007; Zhou et al. 2001); whereas in fish TTR (Yamauchi et al. 2001; i.e. masu salmon) and lipoproteins (Babin 1992; i.e. trout) appear to be responsible for most plasma binding and transport of TH.

Thyroid hormone receptors belong to the nuclear receptor family and work as ligand-dependent transcription factors. Two TH receptors have been recognized: $TR\alpha$ and $TR\beta$ and both present a DNA-binding domain that bind specific sequences of the

DNA known as hormone response elements (Marchand et al. 2001). In the absence of the ligand (T_3), the receptors bind to co-repressors, inhibiting the transcription; while, in the presence of the hormone, the co-repressors are released, and the gene transcription takes place (Marchand et al. 2001).

The components of the vertebrate thyroid axis including thyroid hormones (THs – T_3 and T_4), precursor (thyroglobulin), peroxidase enzyme system, binding proteins, nuclear receptors, and the different synthesis, signaling and regulation pathways are highly conserved. However, the developmental and physiological events controlled by THs differ among vertebrates (Tan and Zoeller 2007). The range of actions of THs is extremely broad, including the regulation of gene expression, protein synthesis, development and growth, nervous system development, metabolic rate, homeostasis of cells and tissues; moreover, it constitutes an important stimulative and permissive factor to the actions of many other hormones (Franklyn 1994). In teleosts, THs are involved in the regulation of growth, early development (including gonadal development), metamorphosis, and some aspects of reproduction (Brown et al. 2004; Carr and Patiño 2011). Therefore, disruption of the thyroid endocrine axis by contaminants found in the aquatic environment can impact on the health and reproductive fitness of wild fish populations. A group of contaminants known to exhibit thyroid-disrupting activity are the brominated flame retardants.

Brominated flame retardants

Freshwater ecosystems have been the victim of many anthropogenic insults in the last century as consequence of industrial, agricultural and urban development. One type

of industrial compound that has been of increasing concern in the last few decades is the brominated flame retardants (BFRs). BFRs have been added to a great variety of products such as textiles, furniture, vehicle interiors and others in order to reduce fire-related injury and property damage. The use of these compounds certainly has reduced fire-related incidents; however, studies have shown that BFRs are released to the environment and can be easily absorbed through the diet (DeWit 2002).

For commercial purposes, BFRs can be incorporated into polymers (i.e., plastics) through two mechanisms: reaction and addition. A reactive combination implies the formation of covalent bonds between BFRs and the polymer; whereas an addition combination involves the mixing of the BFRs with the polymer resin (Kim et al. 2006). Additive BFRs, consequently, tend to continually leach out of the final product (DeWit 2002), entering the environment. The most used BFRs are: tetrabromobisphenol A, primarily reactive with some additive applications, used mainly in printed circuit boards; hexabromocyclododecane, primarily additive, used mainly in thermoplastic polymers and to a lesser extent in textile coating, cable, latex binders, and unsaturated polyesters; and polybrominated diphenyl ethers (PBDEs), additive, used in electrical and electronic equipment, textiles, plastic housings, office equipment and polyurethane foams (Birnbaum and Staskal 2003).

Polybrominated diphenyl ethers

PBDEs have been the most commonly used brominated flame retardants in Europe (Kuivikko et al. 2010) and the Americas (Birnbaum and Staskal 2003). In North America, particularly, the continuous use of PBDEs since the late seventies has led to

accumulation levels in some regions that are comparable to those of PCBs and organochlorines; and, given that North America is the major consumer of PBDEs in the world, it is probably the major contributor of these chemicals to the worldwide environment (Hale et al. 2001). In the Columbia River, an ecosystem of interest to the present study, there have been reports of drastic increases of PBDE concentrations in sediment and fish in the last two decades (Rayne et al. 2003), creating awareness of these contaminants in the area.

Two hundred and nine PBDE congeners can be potentially made based on the number and position of bromine atoms bound to their aromatic rings, although fewer are actually included in commercial mixtures (Birnbaum and Staskal 2003). Common mixtures include pentabromodiphenyl ether (penta-BDE), comprised of tetra-, penta-, and hexa-BDE congeners; octabromodiphenyl ether (octa-BDE), comprised of hexa- to nona-BDE congeners; and decabromodiphenyl ether (deca-BDE), comprised almost solely of the single deca-BDE congener (BDE-209) with a small amount of nona-BDE (US EPA 2009). Lower-brominated congeners (4 to 7 bromines) degrade slowly (Birnbaum and Staskal 2003) but congeners with more than 7 bromines are less stable and can undergo debromination when exposed to UV light (Olsman et al. 2002) or when metabolized by intestinal microbes or enzymes (Stapleton et al. 2004a; Vonderheide et al. 2006). Lower-brominated congeners tend to show higher toxicity than their higher-brominated counterparts (Usenko et al. 2011), a difference based on their lower metabolic and elimination rates that favor their bioaccumulation and bioconcentration (De Wit 2002).

Lower-brominated congeners tend to be found more frequently in humans and wildlife than other congeners, BDE-47 being the most common followed by BDEs 99,

100, 153 and 154. Conversely, in environmental media, such as house dust, sediments, and indoor air, higher-brominated congeners may be dominant (Environment Canada 2009). The environmental presence of PBDEs, however, is worldwide due to the capability of atmospheric and marine currents to transport PBDEs over relatively long distances (Environment Canada 2006). Thus, PBDEs have been found in tissues of ocean dwelling whales and marine mammals (Shaw and Kannan 2009), and in a variety of biota in remote areas far from anthropogenic sources, from the equator to the poles (Environment Canada 2006).

Given their persistence and bioaccumulative properties and concerns over their biological impacts, regulations for the use of PBDEs have been implemented in the last decade. The European Union was the first to ban the use of penta- and octa-BDEs in 2004 (Stoker et al. 2005) and the use of deca-BDE in 2008 (US EPA 2009). The ban applies to manufactured and imported electronics; however, deca-BDE is allowed for use in other applications (US EPA 2009).

In the United States, the company manufacturing the penta- and octa-BDE mixtures, Chemtura Corporation, voluntarily stopped production in 2004 (Birnbaum and Cohen Hubal 2006). In 2006, the State of California banned the manufacture, process or commercialization of products containing more than 0.1% penta- or octa-BDEs (US EPA 2009). Several states followed the banning including Hawaii, Illinois, Maine, Maryland, Michigan, Minnesota, New York, Rhode Island, Oregon and Washington. Washington also banned the use of deca-BDE in mattresses on 2008 and starting in January 2011, it prohibited the use of deca-BDE in televisions, computers and upholstered furniture. Maine also banned deca-BDE in mattresses, upholstered, furniture, televisions and other

plastic-encased electronics starting in 2008. In addition, the principal U.S. manufacturers and importers of deca-BDE will phase out production, importation and sales of deca-BDE for most uses by the end of 2012 and all uses by the end of 2013 (US EPA 2010).

Biological activity of PBDEs

Despite the trend to reduce or ban the manufacture and use of PBDEs, their persistence in the environment especially for the lower-brominated congeners is of ongoing concern. The chemical structure of PBDEs, two phenol rings connected by an oxygen atom (Stoker et al. 2005), resembles the structure of thyroid hormones (THs). In fact, PBDEs have an even closer structural relationship to TH than polychlorinated biphenyls (PCBs) (Boas et al. 2006) (Figure 1.2). This structural resemblance to TH raises concerns about the potential endocrine-disrupting activity of PBDEs. BDE-47 (Figure 1.3) is one of the most abundant congeners present in aquatic and terrestrial wildlife (Luross et al. 2002; Marsh et al. 2004; Stapleton et al. 2004b). It is a tetra-congener found in commercial mixtures of flame retardants or produced in the environment as a metabolic or degradation product of higher-brominated congeners (Stapleton et al., 2004b).

Studies in mammals have suggested that PBDEs compete with TH for binding proteins (Zhou et al. 2001) but not for receptor- (TR β)-binding (Suvorov et al. 2011). They also induce UDP-glucuronosyltransferase activity (phase II metabolic enzyme) in liver followed by an enhanced biliary excretion of TH glucuronides, thus leading to hyperplasia of the thyroid follicular cells as consequence of TH depletion (Ellis-Hutchings et al. 2006). Hypothyroidism as a consequence of PBDE exposure has been associated with alterations in the nervous system (Dufault et al. 2005). Reproductive

effects including impaired sperm quality (Abdelouahab et al. 2011) and failure to whelp (Zhang et al. 2009) have also been observed.

The presence of PBDEs in teleosts has been well documented in the last decade. Hale et al. (2001) examined fish muscle tissue collected in watersheds from the state of Virginia (USA). They reported total concentrations of PBDEs as high as 47.9 µg/g lipid, where BDE-47 contributed 40-70% of this amount. Other species surveyed include halibut (*Hippoglossus stenolepis*) and striped bass (*Morone saxatilis*) in San Francisco Bay (1.29 and 1.02 µg/g lipid, BDE-47, respectively; Lunder and Sharp, 2003), winter flounder (*Pseudopleuronectes americanus*) from the Massachusetts and Cape Cod Bays system (1.24±1.4 µg/g lipid, BDE-47; Montie et al., 2010), smallmouth bass (*Micropterus dolomieu*) and white sucker (*Catostomus commersonii*) of Penobscot River, Central Maine (6.49±3.0 and 4.7 µg/g lipid BDE-47, respectively; Anderson and MacRae, 2006), bloater chub (*Coregonus hoyi*) and deepwater sculpin (*Myoxocephalus thompsonii*) in Lake Michigan (0.244 µg/g lipid and 0.254 µg/g lipid BDE-47; Hahn et al., 2009).

In the Columbia River, the PBDE body burden of some species has also been analyzed, including whitefish (*Prosopium williamsoni*) (0.1 µg/g wet weight, Rayne et al. 2003), Chinook salmon (*Onchorynchus tshawytscha*), peamouth (*Mylocheilus caurinus*), northern pikeminnow (*Ptychocheilus oregonensis*) and largescale sucker (*Catostomus macrocheilus*) (5, 0.5, 0.8, and 1.2 µg/g lipid weight total PBDE, respectively; Sloan et al. 2010). Largescale sucker, particularly, has been sampled lately not only for contaminant-monitoring purposes, but also to evaluate the PBDE exposure of fish-eating birds in the area (Henny et al. 2009).

Studies with teleosts have shown some similarities with mammals regarding

PBDEs disruption of TH homeostasis, developmental neurotoxicity and reproductive toxicity (Muirhead et al. 2006). However, the mechanisms of PBDE action are still not well understood. A consistent decrease in plasma T₄ but not T₃ levels were observed in juvenile lake trout (*Salvelinus namaycush*) after oral exposure to 13 PBDE congeners at different concentrations (aprox. 2.5 and 25 ng/g) (Tomy et al. 2004). The same response in T₄ was observed in fathead minnows (*Pimephales promelas*) after oral exposure to BDE-47, and this was accompanied by elevated mRNA levels for TSH β in the pituitary and changes in the transcription of TH receptor α and β in the brain (body burden ranged from 11.43 $\pm$ 1.24 to 107.60 $\pm$ 29.40 μ g/g carcass) (Lema et al. 2008). Morgado et al. (2007) reported that lower brominated congeners (i.e., BDE-47) were more potent inhibitors of T₃ binding to transthyretin (TTR) in sea bream than higher brominated BDEs. However, the binding affinity of TTR for T₃ or T₄ depends on the species. In some species, TTR has a slightly greater affinity for T₃ than for T₄, while in others like sea bream, TTR binds both THs with similar affinity (Morgado et al. 2007).

Developmental and behavioral effects after PBDE exposure have been also attributed to impairments in the thyroid axis. Exposures during early stages of development can be a consequence of maternal transfer of PBDEs to the eggs (Nyholm et al. 2008). Studies with zebrafish (*Danio rerio*) focusing on the early stages of development found that embryonic exposure to BDE-47 led to deformities, behavioral abnormalities, cardiac impairments and a reversible body length decrease in larvae (293.28 $\pm$ 21.64 μ g/g ww composite; Lema et al. 2007). Zebrafish larval exposures resulted in an initial decrease in body weight (68.8 ng/g ww composite; Chen et al. 2010) and behavioral impairments in juveniles (409.68 $\pm$ 82.01 ng/g ww composite; Chou et al.

2010). Studies with killifish (*Fundulus heteroclitus*), after waterborne exposure to DE-71 (a tetra and penta- mixture), reported similar findings such as altered behavior and learning ability, accompanied by a slight developmental asymmetry of the tail curvature, right or left, effects more pronounced at higher doses (10 and 100 µg/l DE-71) (Timme-Laragy et al. 2006).

Reproductive impairments in teleosts due to exposure to PBDEs have been observed mainly in males. Adult male fathead minnow exposed to BDE-47 presented a 50% reduction in the amount of mature sperm which lead to a decrease in spawning activity (Muirhead et al. 2006), and a decrease in condition factor and impairment of gametogenesis (Lema et al. 2008).

Biological activity of PBDE metabolites

PBDEs are metabolized in several teleost species via debromination and formation of hydroxylated and methoxylated metabolites, which can potentially be more toxic than the parent compounds (Munschy et al. 2010). The transformation pathways of some species have been studied, for instance, in rainbow trout exposed to BDE-209 the primary transformation pathways are debromination and methoxylation (Feng et al. 2010). In medaka exposed to BDE-47, demethylation of 6-Me-O-BDE-47 is the primary transformation pathway in the formation of 6-OH-BDE-47, and both of these metabolites can be maternally transferred to eggs (Wan et al. 2010). In addition, it has been shown that MeO-PBDEs can occur naturally in marine organisms and, consequently, it can be an important contributor for the occurrence of OH-PBDEs in wildlife (Wan et al. 2009).

Interest in the biological effects of PBDE metabolites is beginning to rise but at the present time little is known about their mechanisms of action. Studies in mammal

cell lines suggest that some hydroxylated metabolites have higher affinity for the TH receptor (Kitamura et al. 2008) and the estradiol-17 β receptor (Mercado-Feliciano and Bigsby 2008) than the parent compounds.

The Columbia River

The Columbia River is the fourth largest river in the U.S. and the largest in the Pacific Northwest. It flows 1,955 km from the base of the Canadian Rockies in southeastern British Columbia to the Pacific Ocean at Astoria, Oregon, and Ilwaco, Washington. It drains approximately 670,800 km², which includes territory in seven states (Oregon, Washington, Idaho, Montana, Nevada, Wyoming, and Utah) and one Canadian province. After the Missouri-Mississippi River, the Columbia River has the largest average annual discharge (244 million dam³) and annual flow (7790 m³/s) (Center for Columbia River History 2011; Hinck et al. 2006).

There are more than 400 dams built in the river system, 11 on the main stem, generating more than 21 million kilowatts, which makes the Columbia River Basin the most hydroelectrically developed river system in the world. Unfortunately, the consequence for such development has been steep declines in several species of anadromous fish, especially salmonids. Fish hatcheries have become a major source of stocks for mitigating wild salmon declines, with hatchery-raised fish now making up more than 80 percent of commercially caught salmon in the river (Center for Columbia River History 2011).

The Columbia River also provides for cargo ship navigation up and downstream, constituting one of the main commerce tools for the area. The river and its tributaries

also have served as source of water for irrigation projects over the last century, benefiting the agriculture of the area (Center for Columbia River History 2011). Unfortunately, with the growth of agriculture, the amount and types of pesticides released into the river have also increased to the point of jeopardizing the wellbeing of the aquatic biota. Similarly, extracting industries such as mining, timber and commercial fishing have had a negative impact on the environment, resulting in contaminated water, fish consumption advisories, and threatened and endangered species (Hinck et al. 2006).

The Lower Columbia River Basin

The Lower Basin comprises the portion from Bonneville Dam to the mouth, which is 235 river-km in length and drains an area of about 46,620 km², all to the west of the Cascade Mountains. The Willamette River, the largest tributary in this section of the Columbia River, drains 65 percent of the area in the lower basin, and flows into the Columbia River at river mile 101, entering Portland, Oregon, a large city of nearly 600,000 people (U.S. Census Bureau, 2010).

Aquatic biota using or residing in the lower Columbia River are exposed to a variety of environmental contaminants from numerous sources including municipal and industrial discharges, atmospheric deposition, urban and industrial nonpoint pollution, accidental spills of oil and hazardous materials, and runoff from agricultural and forested areas (Fuhrer et al. 1996; Lower Columbia River Estuary Partnership 2007). In addition, contaminants may also be transported to the lower river from areas of known sediment contamination above the Bonneville Dam, such as Lake Roosevelt (Barton and Turney,

2010), the Yakima River (Fuhrer et al. 2004), the Snake River (Clark et al. 1998), and in areas of sediment deposition behind the dams (URS 2010).

The areas of the Lower Columbia River relevant to the present study, starting upstream, were Skamania (river mile 140), Columbia City (river mile 82) and Longview (river mile 66) (Figure 3.1). Skamania is located just downstream of Bonneville Dam, the lowermost dam on the Columbia River, and it is upstream of the urban areas of Portland (Oregon) and Vancouver (Washington). There are no known inputs of contaminants near the site except for what may be delivered from upstream and for a small urban development near this site, at the Columbia River Gorge National Scenic Area (Morace 2006).

The Columbia City site is in between two small Oregon cities, Columbia City on the downstream end and St. Helens on the upstream end. The Multnomah Channel, which branches off the Willamette River just before it enters the Columbia and travels along the west side of Sauvie Island, drains into the Columbia River at the upstream end of this site. Near the mouth of Multnomah Channel, there are four different areas of environmental remediation, targeting known past contamination issues with PCBs, PAHs, chlorinated dioxins/furans, mercury, arsenic, and petroleum hydrocarbons (Bailey 2010). The wastewater-treatment plant (WWTP) at St. Helens serves a population of only 10,000 people but has a fairly large design flow (45 million gallons per day) because of a paper mill aeration pond collocated with the treatment plant. Also, Lake River, which enters the Columbia River just upstream of the Multnomah Channel on the Washington side, drains Vancouver Lake, which receives much of the stormwater runoff from Vancouver, Washington. Past studies in the area have reported elevated concentrations

of contaminants and issues with salmon health (Fuhrer et al. 1996; Johnson et al 2006; Morace 2006).

The Longview site is located in the Port of Longview, consisting of eight marine terminals and waterfront industrial property dominated by forest products and steel industries. This site is influenced by the Cowlitz River, which enters the Columbia River at the upstream end of the site and drains the slopes of Mount Rainier, Mount Adams, and Mount Saint Helens. On the Oregon side at this location is the town of Rainier. Effluent from the local WWTPs (Three Rivers Regional and City of Rainier) contributes on both sites of the Longview collecting area (Morace, personal communication).

Largescale sucker life history

Largescale sucker (*Catostomus macrocheilus*) belongs to the family Catostomidae (order: Cypriniforme). This species occurs on most freshwater bodies on the west of the Rocky Mountains, on the northwestern North America, from the north of British Columbia (Canada) to the south of Oregon (Dauble 1986), including Montana, Idaho and Nevada (Montana Field Guide 2011).

Largescale suckers spend most of their time on the bottom of rivers and lakes. Sexual dimorphism is apparent during breeding season with the presence of nuptial tubercles on the rays of the anal and caudal fins in males. The anal and caudal fins are longer in males than in females and females tend to grow larger than males. Their life span is around 15 years and they reach sexual maturation in 5 to 7 years (Dauble 1986).

As larvae and juvenile, largescale suckers prey on zooplankton and periphyton, whereas as adults, they are considered opportunistic omnivores, with prey items that

include aquatic insects, gastropods, crayfish and small fish. They are in intimate contact with the sediment, as they ingest and expel it through the gills during food foraging (Dauble 1986). Largescale suckers also constitute a vital element on the diet of several species of fish (Nigro et al. 1983; Gray et al. 1984) and birds (Fitzner and Hanson 1979 Henny et al. 2009), being a key component of the nutrient cycling of this ecosystem (Schmetterling and McFee 2006).

Significance and objectives of the present study

Despite the trend to reduce or ban the manufacture and use of PBDEs, their persistence in the environment, especially for the lower-brominated congeners and their effects on terrestrial and aquatic biota, is of ongoing concern. Studies with teleosts have revealed potential impacts and mechanisms of action, but many questions are still pending. Therefore, a component of this dissertation was a study of PBDEs on growth and reproductive development of juvenile fish. I chose zebrafish for this study because it is an important laboratory animal for aquatic toxicity studies.

Zebrafish have been well-characterized in terms of their morphological, biochemical, genetic and physiological changes during their various stages of development. Their high fecundity and the transparency of the embryos allow for easy developmental staging and assessment of endpoints during toxicity testing. In addition, zebrafish size and husbandry practices allow easier maintenance at low cost (Hill et al. 2005)

Although laboratory studies allow the exploration of cause-effect relationships and potential mechanisms, field studies are necessary to understand what actually

happens to wild populations in the environment where multiple biotic and abiotic variables interact in a complex manner. The complexity of this scenario is what makes physiologically-oriented field studies relatively difficult and therefore uncommon. For this dissertation I had the opportunity to assess the thyroid status and the health condition of largescale sucker from the lower Columbia River where they are likely being exposed to a wide diversity of contaminants - including PBDEs. Largescale sucker, a key component of the foodweb, has been frequently used in contaminant assessments but its physiology has not been explored in depth.

The specific objectives of my dissertation are listed below and their rationale is described under their respective chapters:

1. To evaluate the effect of BDE-47 during the juvenile-to-adult transition in zebrafish using growth, gametogenesis and thyroid follicle status as endpoints (Chapter II).
2. To characterize the thyroid condition of largescale sucker collected along a contaminant gradient in the lower Columbia River (Chapter III).
3. To characterize the health condition of largescale sucker collected along a contaminant gradient in the lower Columbia River (Chapter IV).

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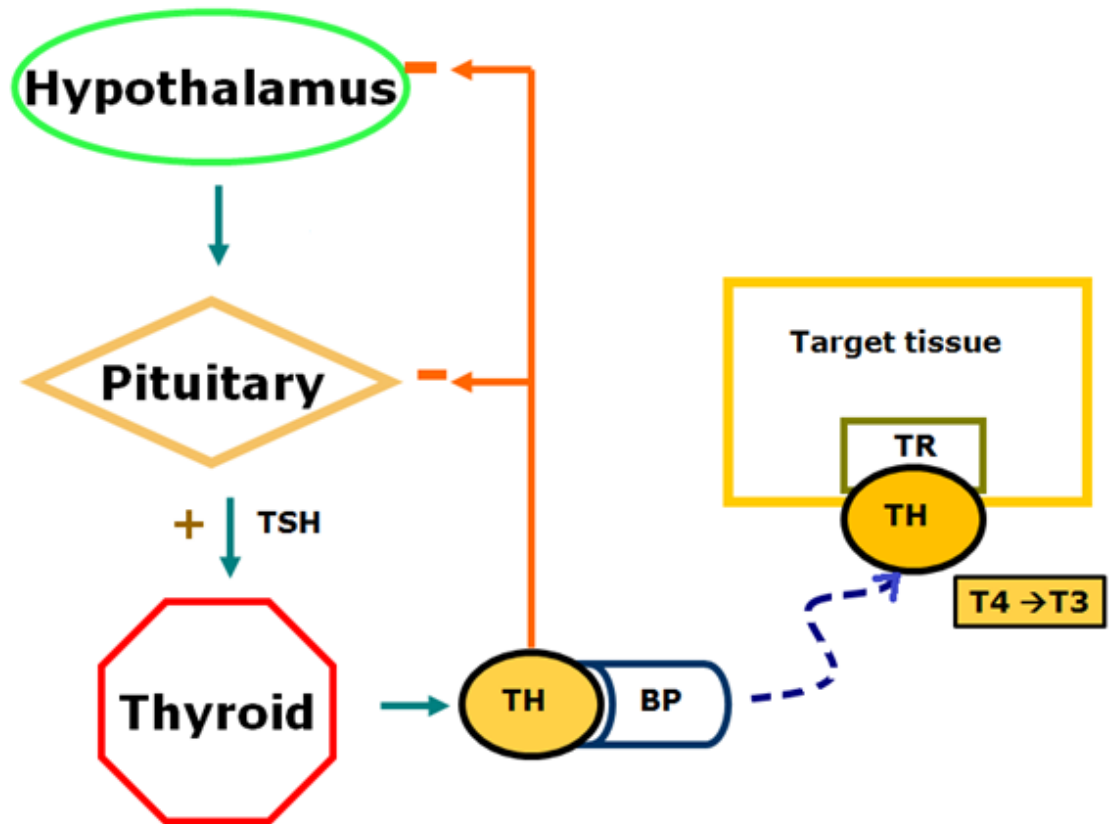


Figure 1.2. Thyroid axis pathway diagram. The hypothalamus controls the production of TSH in the pituitary, which in turn will stimulate the production of TH (T₄). TH binds to BP in the plasma and it is transported to the target tissue. In the target tissue, T₄ is deionidated to T₃, the active form of the hormone, which binds to the nuclear TR. See text for further explanation. TSH, thyroid stimulating hormone; TH, thyroid hormone; T₄, thyroxine; BP, binding proteins; T₃, 3,5,3'-triiodo-L-thyronine; TR, thyroid receptor.

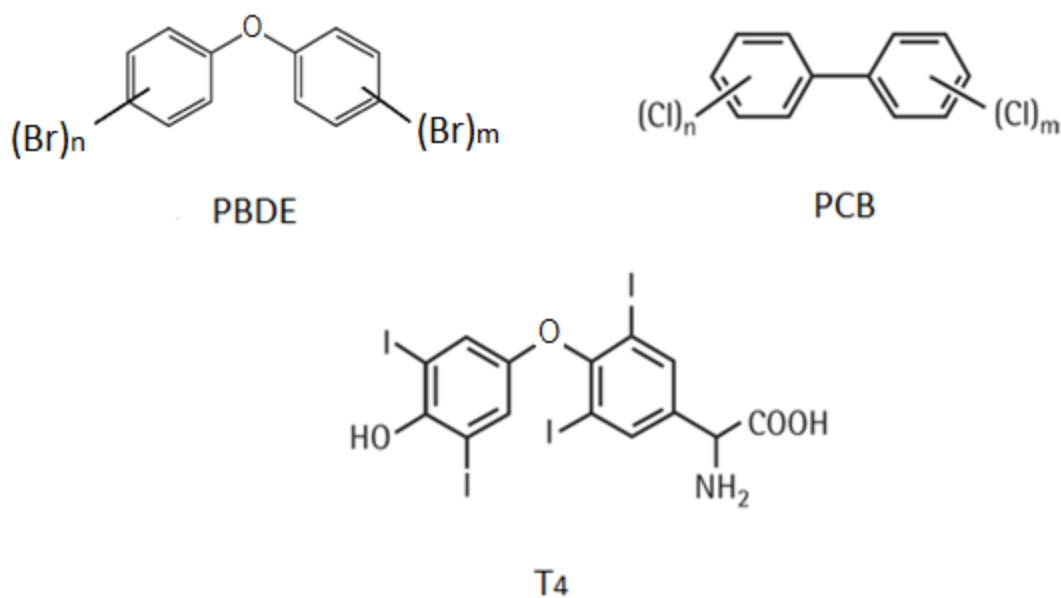


Figure 1.2. Diagram showing the structural similarities between PBDE (polybrominated diphenyl ether), PCB (polychlorinated biphenyl) and T₄ (thyroxine) (Reproduced with permission. Sightline Institute 2004)

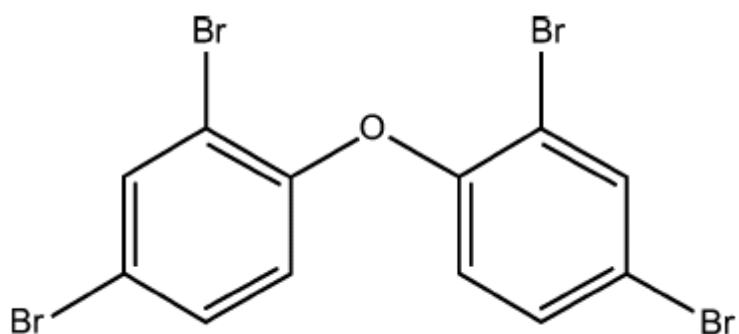


Figure 1.3. Diagram showing the chemical structure of the 2,2',4,4'-tetrabromodiphenyl ether (BDE-47), the most common PBDE found in biota (Reproduced with permission. Staskal et al. 2006).

CHAPTER II

ORAL EXPOSURE TO BROMINATED FLAME RETARDANT BDE-47 CAUSES GROWTH IMPAIRMENT IN JUVENILE MALE BUT NOT FEMALE ZEBRAFISH

Abstract

This study determined the effects of orally-administered brominated flame retardant, BDE-47, on thyroid condition, somatic growth, and germ cell stage of development of zebrafish during the juvenile-to-adult transition. Thirty-five-day-old fish were fed diets containing no BDE-47 (control), and 1, 5 or 25 $\mu\text{g/g}$ BDE-47/diet. Each treatment was conducted in triplicate 30-L tanks containing 50 zebrafish each. Fifteen fish per treatment (5 per tank) were sampled at days 40, 80 and 120. At the time of sampling, measurements were taken for body weight, standard length, and normalized head depth and length (corrected for standard length). Sex, germ cell stage and thyroid condition were histologically determined. Split-plot ANOVA was used to examine significant differences among treatments and between sexes in response to BDE-47 treatment. No significant effects were found on thyroid condition or germ cell stage in either sex. A decrease in body weight was observed in females fed the 5 $\mu\text{g/g}$ diet at 40 days but not at 80 or 120 days or after exposure to the other BDE-47

treatments at any time. In males, no apparent effects of BDE-47 were observed at 40 and 80 days, but fish exposed to 25 µg/g showed a significant decrease in body weight at 120 days compared to control fish. These observations suggest that (1) somatic growth is affected by BDE-47 exposure in juvenile zebrafish, with males being more sensitive to long term exposure; and (2) these effects occur independently of thyroid condition. Results of this study also emphasize the need to consider sex in studies of the impacts of brominated flame retardants in fishes.

Introduction

Over the past several decades, brominated flame retardants (BFRs) have been added to a variety of products (i.e., textiles, furniture, vehicle interiors, etc.) in order to reduce fire-related injury and property damage. These compounds can be released to the environment and easily ingested through the diet (De Wit 2002). The most commonly used BFRs are the polybrominated diphenyl ethers (PBDEs). Commercial PBDEs are found as mixtures composed of about a dozen prominent congeners. Their hydrophobic nature leads to considerable bioaccumulation, bioconcentration (De Wit 2002) and biomagnification in the biota (Burreau et al. 2004; De Wit 2002; Isosaari et al. 2005). In addition, the lower-brominated congeners typically have higher levels of toxicity than their higher-brominated counterparts (Usenko et al. 2011). The congener, 2, 2', 4, 4'- tetrabromodiphenyl ether (BDE-47) is found in commercial mixtures or as metabolic product of higher brominated PBDEs (Stapleton et al. 2004b) and is considered one of the most prominent in aquatic and terrestrial wildlife (Luross et al. 2002; Marsh et al. 2004; Meng et al. 2008; Stapleton et al. 2004a).

The structural similarity of PBDEs and their hydroxylated metabolites to thyroid hormones (TH) qualify them as potential endocrine disruptors (Boas et al. 2006; Yang et al. 2011). Studies with mammals have indicated that PBDEs compete with TH for binding proteins (Zhou et al. 2001; Yang et al. 2011). In addition, PBDEs are able to induce UDP-glucuronosyltransferase (UGT) activity (phase II metabolic enzyme) in the liver followed by an enhanced biliary excretion of TH glucuronide conjugates, resulting in increased stimulation and hyperplasia of thyroid follicular

cells as consequence of TH depletion (Ellis-Hutchings et al. 2006). In developing rats, hypothyroidism and consequent alteration in the development of the nervous (Dufault et al. 2005) and reproductive (Kuriyama et al. 2004; Lilienthal et al. 2006) systems have been reported after PBDEs exposure.

Studies in teleosts have shown some similarities with mammals regarding the disruption of TH homeostasis (Muirhead et al. 2006; Tomy et al. 2004), developmental neurotoxicity (Chou et al. 2010; Lema et al. 2007; Timme-Laragy et al. 2006; Usenko et al. 2011) and reproductive toxicity (Muirhead et al. 2006); but the mechanisms are still unclear. In adult male fathead minnow, exposure to BDE-47 caused a decrease in condition factor and impairment of gametogenesis (Lema et al. 2008) and reproductive performance (Muirhead et al. 2006). Studies with zebrafish focusing on the early stages of development found that embryonic exposure to BDE-47 led to deformities, behavioral abnormalities (Usenko et al. 2011), cardiac impairments and an initial body length decrease in larvae (Lema et al. 2007). Larval exposures, on the other hand, resulted in an initial decrease in body weight (Chen et al. 2010) and behavioral impairments in juveniles (Chou et al. 2010). Impacts of PBDEs during the juvenile-to-adult transition, which includes the late stages of body growth and development of the reproductive system prior to adulthood, have not been previously examined in teleosts.

Levels of TH (triiodothyronine, thyroxine) in plasma – or, in small organisms, whole-body extracts – have been commonly used as biomarkers of thyroid dysfunction. However, TH levels are now considered to be an inconsistent biomarker

of thyroid disruption (Blanton and Specker 2007; Eales and Brown 1993; Kuiper et al. 2008; Mukhi et al. 2005; Tan and Zoeller 2007; Zoeller et al. 2007). A recent review of the literature concluded that histological evaluation of the thyroid follicle yields more reliable information (Carr and Patiño 2011). In zebrafish, thyroid histological features that are sensitive to disruption include the increased vascularization within and around the thyroid epithelium and hypertrophy of the follicular epithelium (Mukhi et al. 2005). It has been reported that TH can stimulate growth hormone production in mammals (Müller et al. 1999) and teleosts (Melamed et al. 1995) and, therefore, growth may also be a useful apical endpoint for studies of thyroid endocrine disruption.

The objective of the present study is to evaluate the effects of BDE-47 exposure in zebrafish during the juvenile-to-adult transition. Exposures were applied through the diet and treatments were designed to achieve environmentally relevant whole-body concentrations of BDE-47. Endpoints examined were growth, gametogenesis and thyroid follicle status. Results were assessed taking into account sex, because male and female individuals seem to react differently to certain anthropogenic contaminants (Burger et al. 2007; Filby et al. 2007), even at a young age (Sharma and Patiño 2010).

Use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Methods

Fish husbandry

Protocols for the use of animals in this study were reviewed and approved by the Texas Tech University Animal Care and Use Committee (Lubbock, TX, USA). Juvenile zebrafish (hatch date: August 29, 2007) were obtained on October 3, 2007 from Aquatic Research Organisms, Inc. (Hampton, NH, USA). Mixed-sex groups of 50 juveniles were placed in 30-L glass aquaria containing reconstituted water (31 g of Kent R/O Right for each 100 L of RO water) (Central Garden & Pet Company, Walnut Creek, CA, USA) fitted with a submersible heater and two airflow-driven internal biofilters (glass beakers containing glass marbles and wool). Fish were acclimated to aquaria conditions for two weeks before starting the exposures. Water temperature, pH and photoperiod were maintained at 28.5°C, 7.0 and 12-h light:12-h dark, respectively. Ammonia, nitrites and nitrates were monitored daily during the first two weeks of acclimation, and every three days afterwards. One third of water volume was exchanged twice weekly or as often as needed to maintain water quality. Tanks were treated with Stress Zyme® (Mars Fishcare Inc., Chalfont, PA, USA) after every water exchange, to help the biofilter with the breakdown of ammonia and nitrite compounds.

Fish were fed Tetramin® Tropical Flakes with balanced nutrition ProCare clear water formula (Tetra US Inc., Blacksburg, VA, USA) twice a day (morning and afternoon) to satiation during the acclimation period. The afternoon meal was replaced by Bio-Pure® brine shrimp (Hikari Sales USA Inc., Hayward, CA, USA)

twice a week to enhance the overall nutritive value of their diet. Treatment diets were prepared by adding the appropriate amount of BDE-47 to the commercial preparation and the feeding regimen during treatment was the same as during acclimation: twice daily to satiation with the afternoon meal replaced with Bio-Pure® brine shrimp twice weekly. Dietary exposures started on 18 October 2007, at 8 weeks postfertilization. Five treatments were applied: untreated diet (no BDE-47 added), treatment control, and 1, 5 and 25 µg/g BDE-47/diet. Each treatment was conducted in triplicate tanks.

Preparation of fish flake diet with BDE-47

Ninety-gram batches of the commercial diet preparation (see previous section) were placed in half-gallon jars fitted with Teflon-lined lids, spiked with BDE-47 or solvent control, and tumbled for 18-48 hours to uniformly disperse the chemical and to reduce the size of the flakes to small uniform pieces suitable for the fish diet. Strips of solvent-rinsed aluminum foil were added to the jars to aid in breaking up of the flakes. Solutions of BDE-47 were prepared in chromatography-grade acetone using 99% pure standard BDE-47 (AccuStandard; New Haven, Connecticut, U.S.A.). A BDE-47 acetone stock solution of 1,000 µg/mL was prepared and quantitatively diluted to obtain 10, 50 and 250 µg/mL solution for spiking the fish flakes at 1, 5 and 25 µg/g. A clean 0.5-mL glass syringe was used to deliver 9 mL of each BDE-47 solution by carefully wafting 18 separate 0.5-mL aliquots onto the flakes in the jar, each time manually tumbling for about 30 seconds to disperse the BDE-47 to the flakes. A blank control Tetramin batch was prepared with 9 mL of acetone. Each 90-g batch of

spiked flakes was then homogenized by tumbling on a motorized roller for 18-48 hours and any residual acetone was evaporated. After tumbling, the aluminum foil strips were removed and the flakes were transferred to clean glass storage jars with Teflon lids. A portion of each spiked batch of flakes was used for quantitative analysis of PBDE concentrations.

Sampling

Fifteen fish per treatment (5 per replicate aquarium) were sampled and euthanized in tricaine mesylate (MS-222, 1 g/L in PBS) (Sigma-Aldrich®, St. Louis, MO, USA) at days 40, 80 and 120 of treatment. Fish were measured for body weight, standard length, head depth (across the center of the eye from dorsal to ventral edges), and head length (from tip of snout to the edge of the operculum). Whole fish were fixed in Bouin's solution and stored in 70% ethanol until histological analyses of the thyroid and gonads were performed.

An additional fifteen fish per treatment (5 per aquarium) were taken during the third sampling date (120 days), measured for morphometric traits and snap-frozen and stored at -80°C for BDE-47 content analysis. The morphometric measurements of these fish were included in the statistical analysis for the 120-day sampling.

Histology

Gonads

All sampled fish, except for those used for BDE-47 screening, were processed for these analyses. The trunk region of the fish was cross-sectioned at the level where kidney, gas bladder and the posterior edge of the liver were visible in the same section (paraffin, 7 µm) and stained (hematoxylin-eosin). Gonadal sex and the most advanced stage of germ cell development were determined. Females were classified as *juvenile* if only oocytes in primary growth were present, *pubertal* if more advanced stages of development were present except mature oocytes, or *adult* if mature oocytes and/or post-ovulatory follicle complexes were present (Grier et al. 2009). Males were categorized as *juvenile* if only spermatogonia were present, *pubertal* if more advanced stages of development were present except sperm, or *adult* if sperm were present (Grier and Uribe Aranzábal 2009).

Thyroid follicles

Analyses of thyroid follicles were performed only on fish sampled at the end of the exposure (120 days). The head of fixed fish was severed behind the operculum and preparations were made using standard paraffin procedures (6-µm-sections; hematoxylin and eosin staining). Histological images were acquired and processed for data collection and analyses as previously described (Mukhi et al. 2005). Briefly, three to five follicles per fish were randomly chosen for all analyses. Follicle cell height (index of hypertrophy) was determined at four specific locations around each

follicle (12, 3, 6, 9 o'clock). The average height was determined for each follicle, and the average value of the five follicles was used as the individual fish value. The number of blood vessels within the thyroid epithelium was determined and standardized to 100 μm of follicular perimeter, and the average for the five follicles was reported as the fish value. Colloid depletion ranks for each follicle were assigned as follows: 0, no colloid depletion; 1, up to one-third of colloid depleted; 2, up to two thirds of colloid depleted; and 3, up to full colloid depletion. The average rank of the five follicles was reported for each fish.

PBDE analysis

Fifteen zebrafish (5 per replicate) per treatment were sampled at 120 days of exposure for BDE-47 analysis. PBDE congeners 28, 66, 85, 99, 100, 153, 154, and 183 were also measured. Prepared diets were screened for the same compounds, organochlorine pesticides and total PCBs. Due to the relatively high concentration of BDE-47, a simplified clean-up procedure was sufficient to purify the samples for analysis by dual-column capillary gas chromatography (GC) with electron capture detection (ECD). Fish and fish-flake diet were weighed to 0.0001 g accuracy, homogenized and dehydrated with anhydrous sodium sulfate, and the method recovery standard PCB 155 was added. Samples were column extracted with dichloromethane and concentrated by rotary evaporation and nitrogen blow-down to a specified volume. For percent lipid determination, small aliquots of the extracts were dried overnight, and weighed. Lipids and other biogenic compounds contained in the

remaining extract for PBDE analysis were removed by passage through a multi-layer column containing activated silica-gel, potassium hydroxide-treated silica-gel, and sulfuric acid-treated silica-gel. The final extract was brought to an appropriate final volume to match the quantitation range of the BDE-47 GC/ECD calibration curve, and the instrumental internal standard (PCB 30) was added.

A nine-component 2000 ng/mL PBDE stock solution obtained from Wellington Laboratories (Guelph, ON, Canada) was used to prepare the calibration standards. The analytical GC columns were a 60 m x 0.25 mm DB-5 phase (0.25- μ m film of 5% phenyl- 95% methylsilicone, Agilent, Palo Alto, CA) and a DB-17HT (0.25- μ m film, 50% phenyl-, 50% methylsilicone high temperature, Agilent). BDE-47 was quantified on the DB-5 column. Using an Agilent 6890N GC with cool on-column capillary injection systems and Agilent model 7683D autosamplers, 1 μ L of the extract was injected. A 3-m section of 0.53-mm i.d. uncoated and deactivated capillary retention gap (Agilent) was attached to the analytical column by a Press-Tight® (Restek Corp., Bellefonte, PA) union. Hydrogen carrier gas was regulated at 25 psi for the DB5 column and 14.8 psi for the DB17HT column. The GC oven temperature was initially 60 °C, then ramped to 150 °C at 15 °C/min, and to 240 °C at 1 °C/min, and finally to 330 °C at 10 °C/min, and held for 15 min. The ECD temperature was 330° C. GC/ECD data were collected, archived in digital form, and processed using a PerkinElmer chromatography data system, with model 970 interface and version 6.2 of Totalchrom Workstation chromatography software, on a Windows XP microcomputer.

Data analysis

Data were analyzed for each sampling event at 40, 80 and 120 days of exposure. The variables, head depth and head length, were corrected (normalized) for standard length prior analysis (normalized variable = original value/standard length). T-test and Mann-Whitney U test (as appropriate) were performed between the untreated diet and the control (solvent-treated) diet to determine possible differences in the nutritional value of the diet after treatment with solvent. The intact diet (solvent-untreated) was not included in the analyses of treatment effects because it falls outside any theoretical or expected gradient of effects. Differences in survival rates were analyzed using Kruskal-Wallis test, since they did not meet the parametric assumptions.

Split-plot ANOVA was used to determine whether significant differences exist among treatments (blocks) and between sexes (subplots) in response to treatments. Morphometric and ratio variables were log-transformed and proportions arcsine-square-root-transformed prior to the analyses to better fit the ANOVA assumptions. If treatments, sexes and/or treatment-sex interactions were significant, Dunnett's multiple comparison tests, using the control as the reference group, were carried out for males and females separately. It is not recommended to test for interactions using non parametric methods (Seaman Jr. et al. 1994); thus, categorical variables (i.e. stage of germ cell development and colloid depletion) were transformed to ranks prior analysis using nested ANOVA– in males and females separately – to examine differences among treatments, followed by Dunnett's multiple comparison tests.

Split-plot ANOVA, nested ANOVA and Dunnett's comparisons were conducted using SAS software, Version 9.2 (SAS Institute Inc. SAS/STAT[®], Cary, NC, USA), t-test, Mann-Whitney U test and rank data-transformation were conducted using STATISTICA data mining software, Version 8 (StatSoft, Inc., Tulsa, OK, USA). The overall level of significance α was 0.05 for all analyses.

Results

At day 91, the heater in control-tank 3 malfunctioned sometime between the morning meal (9:00 am) and 3:00 pm, when the temperature was recorded at 39.3 °C. In order to bring the temperature back to 28.5°C, the heater was removed immediately and 50% of the water was replaced with water at 25°C. Normal temperature was restored by the end of the day. No mortalities were related to this event. A one-way ANOVA and Kruskal-Wallis ANOVA (as appropriate) were used to test for significant differences for all response variables among the three tank replicates of the control treatment; and, given that no significant differences were found, data from control-tank 3 were included in subsequent statistical analyses.

To minimize disease outbreaks during the exposures, any fish exhibiting erratic swimming behavior were removed from their tanks and included in the mortality record. Percent survival was calculated for each sampling period based on the number of fish present at the conclusion of the preceding period; namely, at 0-40, 41-80 and 81-120 days. No significant differences were found in survival rates among treatments at any of the sampling dates ($p > 0.05$). Mean ($\pm$ SEM) survival rates were

100.0 (± 0)%, 99.3 (± 0.1)%, 98.0 (± 0.2)%, 99.3 (± 0.1)% and 97.3 (± 0.4)% at 0-40 days for the untreated diet, control, 1 $\mu\text{g/g}$, 5 $\mu\text{g/g}$ and 25 $\mu\text{g/g}$ BDE-47 treatments, respectively; 100.0 (± 0)%, 100.0 (± 0)%, 99.3 (± 0.1)%, 100.0 (± 0)% and 100.0 (± 0)% at 41-80 days; and 99.3 (± 0.1)%, 98.7 (± 0.1)%, 100.0 (± 0)%, 100.0 (± 0)% and 100.0 (± 0)% at 81-120 days.

BDE-47 concentrations in diet and fish

Measured concentrations of BDE-47 in the diet were 0.003, 0.69, 3.59, and 17.8 $\mu\text{g/g}$ (wet weight) for the nominal treatment concentrations of 0, 1, 5 and 25 $\mu\text{g/g}$, respectively. BDE-47 analyses were done only on fish sampled at 120 days of treatment. Whole-body concentration of BDE-47 was measured in composite samples of males ($n=2$) and females ($n=3$) for untreated diet, control, 1 and 5 $\mu\text{g/g}$. For the 25 $\mu\text{g/g}$ treatment, the samples were analyzed individually (females, $n = 9$; males, $n = 4$). Concentrations are corrected for lipid content; lipid content between males and females did not differ significantly ($p>0.05$). Concentrations of BDE-47 were 0.534 $\mu\text{g/g}$ (lipid weight) in males and 0.236 $\mu\text{g/g}$ in females fed untreated diet; 0.489 $\mu\text{g/g}$ in males and 0.455 $\mu\text{g/g}$ in females fed control diet; 5.53 $\mu\text{g/g}$ for males and 6.28 $\mu\text{g/g}$ for females fed the 1 $\mu\text{g/g}$ -treatment; and 34.0 $\mu\text{g/g}$ for males and 27.2 $\mu\text{g/g}$ for females fed the 5 $\mu\text{g/g}$ -treatment. In the 25 $\mu\text{g/g}$ -treatment, they were 129 ± 28.9 $\mu\text{g/g}$ (mean $\pm$ SEM) in males and 119 ± 34.4 $\mu\text{g/g}$ in females.

Morphometrics

No significant differences among treatments were detected after 40 days of exposure ($p>0.05$), but significant differences in body weight were found between sexes ($p=0.0286$). Dunnett's test showed significant differences between the 5 $\mu\text{g/g}$ treatment and the control in females only (Figure 2.1). No treatment or sex effects were observed after 80 days of exposure ($p>0.05$), but treatment - sex interaction was highly significant for normalized head length ($p=0.01$), suggesting that males and females responded differently to the treatment (Figure 2.1). This was confirmed by Dunnett's test, showing that the three concentrations of BDE-47 had significantly lower normalized head length than the control treatment in females only.

At 120 days of exposure, significant differences among treatments ($p=0.045$) and between sexes ($p<0.001$) were observed in body weight, but the treatment-sex interaction was not significant ($p>0.05$); Dunnett's test, however, showed significant differences between the control and the 25 $\mu\text{g/g}$ -treatment only in males (Figure 2.1). Standard length was significantly different between sexes ($p=0.002$), but no effects of treatment were detected ($p>0.05$); Dunnett's test was not significant in either males or females (Figure 2.1).

Histological observations

Gonads

Casual observations indicated that, independently of the treatment, some of the fish exhibited reproductive behavior after about 70 days of the exposure. Larvae were in fact observed in the biofilters of some of the tanks; e.g., at day 75 of exposure in one of the control tanks and at day 81 of exposure in one of the 25 µg/g treatment tanks.

Rank-transformed data for germ cell stage were analyzed separately for males and females. No significant differences were found in gametogenetic stage among treatments at any time during the exposure ($p>0.05$) (Table 2.1).

Thyroid follicles

Thyroid follicles were analyzed only on the fish collected after 120 days of exposure. No significant differences were found in follicular cell height and angiogenesis among treatments or between sexes ($p>0.05$); similarly, colloid depletion was not significantly different among treatments in either males or females ($p>0.05$) (Table 2.2).

Discussion

The effects of BDE-47 on growth differed between males and females. In females, an inhibitory effect on body weight was observed at 40 days in individuals

fed the 5- $\mu\text{g/g}$ treatment diet but not the other treatments or at later exposure times. After 80 days, normalized head length was reduced in females exposed to all treatment levels. Finally, after 120 days, no significant statistical differences or tendencies were observed among treatments. This suggests that if it was an initial effect of BDE-47 in females early on the exposure, a potential compensation mechanism may occurred. In males, no significant effects of BDE-47 were observed on any of the endpoints examined after 40 and 80 days of exposure; however, after 120 days, fish exposed to the highest BDE-47 concentration (25 $\mu\text{g/g}$) showed a significantly decreased body weight compared to the control. These observations suggest that females, but not males, may show an early response to PBDE exposure that is reversible; and that males may be more susceptible to long term exposure to BDE-47.

Other recent studies of zebrafish have included growth as an endpoint of analysis but their results cannot be directly compared to those of the present study because they used a different exposure path or focused on a different stage of development. For example, Lema et al. (2007) exposed zebrafish embryos to high concentrations of waterborne BDE-47 and found a significant decrease in body length at 72 and 96 hpf, in addition to a series of trunk deformations and cardiac impairments. The exposure approach and the waterborne concentrations used (range, 100 - 5000 $\mu\text{g/l}$) by Lema et al. (2007) are, nevertheless, outside what is considered environmentally relevant conditions. Chen et al. (2010) focused on the effect of BDE-47 on zebrafish larvae after 40 days of oral exposure (whole body concentration, 369.9 ng/g lipid weight). They found that larvae may experience an initial decrease in body

weight and length at 38 dph that was later reversed by 60 dph; however, they did not factor sex into the analysis. Chou et al. (2010), on the other hand, did not report any significant effects or trends of BDE-47 on body size after exposing zebrafish larvae (21 dph) through sexual maturity (90 dph) (whole body concentration in the highest treatment dose, $1,924.2 \pm 289.4$ ng/g lipid). Note that the whole body BDE-47 concentration in Chen et al. (2010) and Chou et al., (2010) studies were much lower than the ones reported here; also, their time of exposure was shorter. This might imply that the concentrations they used and the length of exposure in their studies were not high or long enough to lead to a statistically significant effect.

Few studies with teleosts have addressed the effects of oral exposures to BDE-47 on reproductive function. Muirhead et al. (2006) reported impairments on the reproductive performance of male but not female adult fathead minnow exposed to BDE-47 for 25 days, even when the body burden of females ($50\text{--}60$ $\mu\text{g/g}$) at the end of the exposure was 4 to 5 fold higher compared to males (15 $\mu\text{g/g}$). Lema et al. (2008) also observed impaired spermatogenesis, but not oogenesis, in adult fathead minnow after 21 days of oral exposure to BDE-47. The whole-body concentrations of BDE-47 reported by Lema et al (2008) were 64.62 ± 6.10 $\mu\text{g/g}$ carcass in males and 107.60 ± 29.40 $\mu\text{g/g}$ carcass in females. These previous studies with adult fathead minnow suggest a different capacity of accumulation and sensitivity to BDE-47 between sexes. In zebrafish, Chou et al. (2010) did not report any histological effect of BDE-47 on the adult gonads after exposing zebrafish from the larval stage through adulthood; however the body burden concentrations they reported ($1,924.2 \pm 289.4$ ng/g lipid) were lower

than the ones in the mentioned studies and here. The whole body BDE-47 concentrations in our highest treatment (129 $\mu\text{g/g}$ in males and 119 $\mu\text{g/g}$ in females) are comparable with the ones reported by Lema et al. (2008) in females, but are higher than the values reported for males. We, however, did not observe significant effects of BDE-47 exposure on the rate of germ cell development from the juvenile stage through early adulthood in either males or females.

Disruption of the thyroid system in teleost fishes is known to be associated with impaired growth (Brown 1997) and reproductive development or reproductive performance (Carr and Patiño 2011). Although effects of BDE-47 on growth were observed in the present study (preceding discussion), no histological evidence supporting thyroid disruption was found. Other studies that examined the effects of BDE-47 exposure on thyroid function have reported inconsistent results. For example, Lema et al. (2008) reported a decrease in plasma T_4 (more marked in females than in males), alteration of pituitary transcripts for TSH β and changes in the mRNA levels of TH-responsive genes in fathead minnow brain after 21 days of oral exposure to BDE-47. Conversely, Chen et al. (2010) did not find effects on the gene expression of thyroid-axis components such as transthyretin (TTR), deiodinase type I (D1) and thyroid-stimulating-hormone beta-subunit (TSH- β) after orally exposing zebrafish larvae for 40 days to BDE-47. Chen et al. (2010) observations in zebrafish are consistent with the present study, where no effect in thyroid condition was observed.

An effect on body weight after BDE-47 exposure during the juvenile and pubertal period of development has not been reported before; however, reversed

effects on growth have been seen in juvenile zebrafish exposed at earlier stages of development for a shorter period of time and at lower concentrations than the ones used here (Chen et al. 2010). This finding, however, was apparently not associated to the thyroid condition, where no significant differences or response-trends in histopathological observations were found among treatments. The BDE-47 whole body concentrations of the present study are comparable to those observed in female fathead minnow by Lema et al. (2008), where reproductive detrimental effects were found in males and whole body concentrations between sexes also seemed to differ. Our findings suggest that although there were differences in growth between males and females, zebrafish does not seem to be as sensitive to PBDEs in terms of reproduction. The effect reported here might be considered mild, but worth it to report because the doses used represent the upper limit of the concentrations found in aquatic biota, as shown by several field surveys (Anderson and MacRae 2006; Goodbred et al. 2007; Hahm et al. 2009; Hale et al. 2001; Lunder and Sharp 2003; Luo et al. 2007; Mariussen et al. 2008; Montie et al. 2010; Sloan et al. 2010).

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Table 2.1. Developmental classification based on germinal cells present in males and females exposed to different concentrations of BDE-47. 1 = juvenile, 2 = pubertal, 3 = adult. (See text for classification criteria). Data expressed as mode.

Treatment	40 days		80 days		120 days	
	Male	Female	Male	Female	Male	Female
Untreated diet	3	1	3	3	3	1
Control	3	2	3	3	3	1
1 µg/g	3	2	3	3	3	1
5 µg/g	3	3	3	1	3	1
25 µg/g	3	1	3	3	3	1

Table 2.2. Thyroid histopathology measurements in males and females exposed to different concentrations of BDE-47. Follicular cell height is measured in μm ; colloid depletion units are expressed as ranks (see text); angiogenesis index corresponds to the number of blood vessels per thyroid follicle perimeter normalized to 100 μm . Mean ($\pm$ standard error).

Treatment	Follicular cell height		Colloid depletion		Angiogenesis	
	Male	Female	Male	Female	Male	Female
Untreated diet	2.8 $\pm$ 0.2	2.9 $\pm$ 0.3	0.9 $\pm$ 0.1	1.0 $\pm$ 0.1	0.006 $\pm$ 0.001	0.006 $\pm$ 0.001
Control	2.4 $\pm$ 0.03	2.6 $\pm$ 0.2	1.0 $\pm$ 0.04	0.8 $\pm$ 0.1	0.005 $\pm$ 0.001	0.002 $\pm$ 0.001
1 $\mu\text{g/g}$	2.8 $\pm$ 0.1	2.4 $\pm$ 0.1	0.9 $\pm$ 0.1	1.0 $\pm$ 0.03	0.005 $\pm$ 0.001	0.004 $\pm$ 0.001
5 $\mu\text{g/g}$	2.6 $\pm$ 0.2	2.7 $\pm$ 0.2	0.9 $\pm$ 0.04	0.8 $\pm$ 0.1	0.002 $\pm$ 0.001	0.002 $\pm$ 0.001
25 $\mu\text{g/g}$	2.4 $\pm$ 0.1	2.7 $\pm$ 0.1	0.8 $\pm$ 0.1	0.9 $\pm$ 0.1	0.003 $\pm$ 0.001	0.002 $\pm$ 0.001

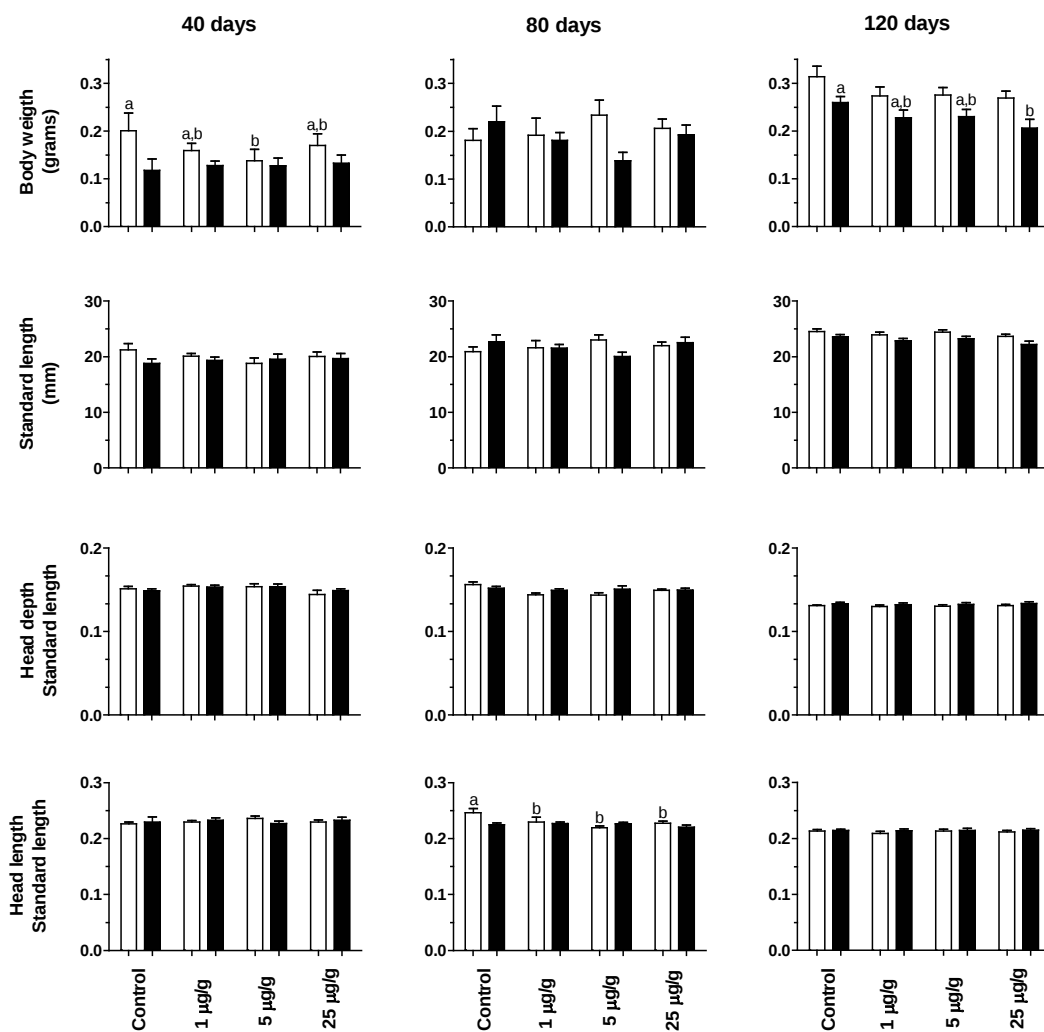


Figure 2.1. Body weight, standard length, depth and length of head of female and male zebrafish at 40, 80 and 120 days of oral exposure to 1, 5 and 25 µg/g BDE-47. Data are presented as mean±standard error. Columns associated with common letters are not significantly different, comparisons were made by sex. Empty bars correspond to females and dark bars to males.

CHAPTER III

**BIOMARKERS OF THYROID FUNCTION IN LARGESCALE
SUCKER (*CATOSTOMUS MACROCHEILUS*) COLLECTED ALONG A
CONTAMINANT GRADIENT IN THE LOWER
COLUMBIA RIVER BASIN**

Abstract

Largescale sucker is an important element of the food-web in the Columbia River ecosystem and has been previously used in assessments of contaminant exposure. The contaminants identified in the ecosystem include some that are known to disrupt the thyroid endocrine system. The objectives of this study are to (1) characterize the thyroid condition of largescale sucker collected along a contaminant gradient in the lower Columbia River, including TH and histopathological analyses ; (2) determine if the thyroid condition differs among collection sites; and, (3) determine if the thyroid condition of male fish is associated with liver contaminant content. Three sampling sites on the lower Columbia River were chosen for this study according to an anticipated contamination gradient: Columbia City and Longview (downstream), both with high levels of input from urban and industrial effluents; and Skamania (upstream), considered the reference location having a relatively less disturbed environment. Fifteen males per

site were sampled in 2009 and 2010, and 10 females per site in 2010. Liver contaminant content was also determined in males collected in 2010. The concentrations of plasma thyroxine (T_4) and triiodothyronine (T_3) were measured in addition to a number of thyroid histological endpoints. Multivariate analyses (MANOVA, DFA) were used to determine overall differences in thyroid condition of males and females and in male liver contaminant content. In addition, Canonical Correlation Analysis was used to determine if the variability of biological traits in males can be explained by the variability observed in hepatic contaminant content. All analyses were made separately for each sex. Plasma T_4 concentration was not included in the analyses because its levels in most samples fell under the detection limit; instead, chi-square contingency tables were used to determine if the proportion of values below the detection limit differs among sites. Plasma T_4 levels ranged from undetectable (LOD, 2.5 ng/ml) to 8.8 ng/ml in females and undetectable to 10.4 ng/ml in males. Plasma T_3 levels ranged from 0.9 to 8.2 ng/ml in females and from undetectable (LOD, 0.7 ng/ml) to 9.1 ng/ml in males. In 2009 males, the distribution of plasma T_4 concentrations above and below the detection limit was related to collection site, with a higher percentage of fish presenting detectable values in Longview ($p < 0.05$); this trend was not observed in 2010. Similarly, based on the other variables measured the overall thyroid condition of male largescale sucker differed among collection sites in 2009 but not 2010. Plasma T_3 concentration and follicle cell density were the main drivers for site discrimination in 2009, with Longview presenting the lowest and highest values, respectively. In 2010 females, thyroid condition not only differed among collecting sites, but also between fish from Skamania and the downstream sites ($p < 0.05$); colloid depletion and follicle cell density were the main drivers for site discrimination

with Longview presenting the highest values for both thyroid variables. The contaminant content of male livers differed significantly among sites in 2010, with higher concentrations observed in the downstream sites relative to the reference site; hexachlorobenzene and dimethyl tetrachloroterephthalate contributed the most to the discrimination ($p < 0.05$). However, clear associations between liver contaminant content and thyroid condition of male largescale sucker could not be established ($p > 0.05$), indicating that other factors not included in the analyses may be responsible for differences in thyroid condition among sites. In conclusion, thyroid condition in largescale sucker differed significantly among sites, and differences appear to be larger among females than among males, suggesting physiological differences between sexes and/or different responses to contaminant exposure.

Introduction

As consequence of industrial, agricultural and urban development, the health of aquatic ecosystems has been significantly degraded. Persistent organic pollutants (POPs) such as DDTs (dichlorodiphenyltrichloroethane), HCB (hexachlorobenzene), PCBs (polychlorinated biphenyls) and some organochlorine pesticides are found in sediments, fish and piscivorous animals, even after many were banned in the 1970s and 1980s (Beckvar and Lotufo, 2011). Additional contaminants have been cause of concern in the last few decades; environmental concentrations of flame retardants such as PBDEs (polybrominated diphenyl ethers), for example, started to increase in the 1990s and continue to be present in high concentrations in many places, despite several congeners being also currently banned (Mariussen et al. 2008; Montie et al. 2010; Sloan et al. 2010). The effects of POPs and PBDEs in mammals are relatively well known, but much less is understood about the impacts of these contaminants in fishes. Some are known to cause endocrine disruption and a subset, such as PCBs and PBDEs, can affect the thyroid endocrine axis (Brown et al. 2004; Buckman et al. 2007; Lema et al. 2008; LeRoy et al. 2006; Noyes et al. 2011).

The range of actions of thyroid hormones (TH) in vertebrates is extremely broad, including the regulation of gene expression, protein synthesis, development and growth, nervous system development, metabolic rate, homeostasis of cells and tissues; moreover, TH serves as an important permissive factor to the actions of other hormones (Franklyn 1994). In teleosts, THs are mainly involved in the regulation of growth, early development, metamorphosis, and some aspects of reproduction (Brown et al. 2004; Carr and Patiño 2011). The thyroid endocrine system can also influence the process of sex

determination in some teleosts (Mukhi et al. 2007). Several toxicological studies with teleosts have focused on the effects of contaminants under laboratory conditions, usually using concentrations above those found in the environment (Lema et al. 2007; Muirhead et al. 2006). Although laboratory studies have shown effects of various contaminants in the thyroidal axis, the study of thyroid disruption in natural populations has gotten much less attention, despite results from a number of studies suggesting that exposure to waterborne contaminants can disrupt the thyroidal axis (Carr and Patiño 2011).

The Columbia River, which is the fourth largest river in the United States, has been impacted by anthropogenic activities such as mining, timber extraction, industrial discharges, agriculture and urban runoff, which together have led to a continuing environmental degradation of the river (Henny et al. 2008a). Hinck et al. (2006) reported that the levels of DDTs and PCBs in fishes (i.e., common carp, black bass and largescale sucker) are still high in some areas of the river, but in general they exhibited a declining tendency when compared to historical data. On the other hand, Rayne et al. (2003) observed an increasing trend of PBDEs in largescale sucker (*Catostomus macrocheilus*, Catostomidae) during the 1990s, and Henny et al. (2009) observed an increasing trend of some PBDE congeners in osprey eggs between 2002 and 2006, a tendency that might be explained by the high concentrations of PBDEs in largescale sucker, the main prey of ospreys.

Largescale sucker reside mainly on the bottom of rivers and lakes. Due to their intimate contact with the sediment, they are in continuous contact with organic xenobiotics contained in the sediment and thus are good sentinels for the presence of contaminants and their dynamics in the ecosystem (Dauble 1986). In addition, this

species also constitutes a vital component of the diet of other fishes (Nigro et al. 1983; Gray et al. 1984) and birds (Fitzner and Hanson 1979; Henny et al. 2003), and consequently is an important element of the food web of this ecosystem (Schmetterling and McFee 2006).

The objectives of the present study are to (1) characterize the thyroid condition of largescale sucker collected along a contaminant gradient in the lower Columbia River, including TH and histopathological analyses ; (2) determine if the thyroid condition differs among collection sites; and, (3) determine if the thyroid condition of male fish is associated with liver contaminant content. Three sampling sites were chosen: Skamania (SK), downstream of Bonneville Dam; Columbia City (CC) downstream of Portland and Vancouver; and Longview (LV), downstream of CC. Passive sampler surveys have shown that the concentration of most contaminants increase in a downstream fashion (Alvarez et al., unpublished data), providing us with the opportunity to observe potential differences in health condition, based on differences in contaminant exposures. This study focused on organochlorine pesticides, PCBs and PBDEs and is part of a larger multidisciplinary project to characterize the level of contaminants in the lower Columbia River Basin and how these contaminants are distributed in and affect the different components of the ecosystem including water, sediment, macroinvertebrates, fish and piscivorous birds.

Methods

Study area

Three sites were sampled on the Columbia River Basin: Skamania (SK), located just downstream of Bonneville Dam (45°32'41.67" N, 122°14'55.73" W; river mile 140); Columbia City site (CC), downstream of Portland, between St. Helens and Columbia City (45° 55' 11.8" N, 122° 48'44.4" W; river mile 82); and Longview (LV), downstream of CC in the Port of Longview (46° 5' 55" N, 122° 56'11" W; river mile 66) (Figure 3.1).

SK does not have direct inputs of potential contamination, except for what may be delivered from upstream and studies have shown that the levels of contamination in the site are relatively low (Fuhrer et al. 1996; Johnson et al. 2006; Morace 2006). CC on the other hand, receives the input of the Multnomah Channel, a branch from the Willamette River and the inflow from the wastewater-treatment plant coming from St. Helens, in addition to the input from Lake River which drains Vancouver Lake. LV receives inputs from eight marine terminals, industrial property dominated by forest products and steel industries. Also, LV receives the input from the Cowlitz River and effluent from Three Rivers Regional and City of Rainier wastewater-treatment plants.

Use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Fish sampling

Protocols for the use of animals in this study were reviewed and approved by the Texas Tech University Animal Care and Use Committee (Lubbock, TX, USA). Largescale sucker were sampled by electroshocking on 4-7 May, 2009 and on 3-5 May, 2010 during

the reproductive season, when gonadal growth is complete prior to the onset of spawning. In 2009, 26 individuals (16 males and 10 females) were sampled in SK, 26 (15 males and 11 females) in CC, and 26 (15 males, 11 females) in LV; in 2010, 24 individuals (15 males, 11 females) were sampled in SK, 24 (14 males and 10 females) in CC, and 23 in LV (15 males and 8 females). Fish were euthanized using MS-222 (tricaine methane sulphonate; Cat. No. E10521, Sigma-Aldrich, St. Louis, MO, U.S.A.). Body weight, body length and gonad weight were recorded. Blood samples were collected from all males and females on both years by heart puncture, placed on ice and transported to the laboratory for plasma extraction at the end of each collection day. Plasma samples were stored at -80°C until further analyses. The lower jaw was removed and placed in 10% buffered formalin (Cat. No. 23-245-685, Fisher Diagnostics, Waltham, MA, U.S.A.) for histological analyses of thyroid follicles of all males in 2009, and from all males and females in 2010.

Histological analyses

Lower jaws were treated with a decalcifying agent (Cal-Ex, Cat. No. 6381-92-6, Fisher Scientific) and Bouin's fixative (Cat. No. 1120-16, Ricca Chemical, Arlington, TX, U.S.A.) prior to dehydration and embedding in paraffin. Sections were cut to 6 µm thickness and stained with hematoxylin and eosin.

Digital images of thyroid follicles were taken with an Olympus digital camera (DP10; Tokyo, Japan) attached to a compound microscope. All measurements were conducted digitally using Image-Pro Express Software (Media Cybernetics, Silver Spring, MD, U.S.A.). Hypertrophy was determined based on the height of the thyroid

epithelium at four specific locations (12, 3, 6, 9 o'clock around each follicle). The number of visible nuclei per 100 μm of follicular perimeter was determined as an estimate of follicle cell density. These measurements were made on five follicles per fish and their respective averages were considered the individual fish value that was used for statistical analyses. For further details of these analyses see Mukhi et al. (2005).

In a separate examination of at least 30 follicles per fish, the proportion of follicles presenting each of the following endpoints was estimated for each fish: hyperplasia (i.e., multilayered follicular epithelium), highly-columnar epithelium, base membrane separated from follicular cells, >50% colloid depletion, and presence of rodlet cells.

Radioimmunoassays

Specific immunoassays were used to measure plasma thyroxine (T_4) and triiodothyronine (T_3) in the Endocrine Diagnostic Laboratory of the Texas Veterinary Medical Diagnostic Laboratory (College Station, TX, U.S.A). Total T_4 and T_3 were measured using Coat-A-Count RIA solid phase radioimmunoassay kits (Cat. No. TKT45 and TKT35, respectively; Siemens Medical Diagnostics, Deerfield, IL, U.S.A.). Sensitivity (defined by the lowest point on the linear portion of the standard curve) of the T_4 assay was 2.5 ng/ml; inter- and intra-assay variabilities were 3.8 and 9.1% (calculated as coefficient of variation), respectively (manufacturer's data). At 1,000 ng/ml, T_3 has 2% cross reactivity in the T_4 RIA (manufacturer's data). Sensitivity of the T_3 assay was 0.7 ng/ml; inter- and intra-assay variabilities were 7.6 and 5.8%, respectively (manufacturer's data). At 100 ng/ml, T_4 has 0.38% cross reactivity in the T_3 RIA.

Plasma samples were diluted two-fold using phosphate buffered saline before T_3 values were determined.

Liver contaminant content

Liver samples were placed in certified pre-cleaned glass jars (I-CHEM® brand), frozen in the field, and analyzed at the USGS National Water Quality Laboratory in Denver, CO (U.S.A.). Thirty chemicals were analyzed in liver composites of males collected in 2009, and in the liver of individual males collected in 2010: chlorpyrifos, DDT metabolites (*p,p'*-DDE and *p,p'*-DDD), components of chlordane (cis-chlordane, trans-chlordane, cis-nonachlor and trans-nonachlor), DCPA, dieldrin, HCB, PCA, PCBs (110, 118, 138, 146, 149, 151, 170, 174, 177, 180, 183, 187, 194 and 206) and PBDEs (47, 99, 100, 153 and 154). A comprehensive list of these compounds and their complete name is in Table 3.1.

The general procedure for these analyses included solvent extraction, clean up and concentration followed by quantification using gas chromatograph-mass spectrometry (GC-MS). Specific procedures varied by compound suite as follows: semi-volatile compounds by Zaugg et al. (2006), AWI compounds by Burkhardt et al. (2006), and halogenated compounds by Zaugg et al. (unpublished data).

Data Analyses

Regardless of severity, chi-square contingency tables were used to determine whether the percentage of individuals showing a particular endpoint differed significantly among sites. The analyses were made for males and females separately. Multivariate

analysis of variance (MANOVA) was used to determine overall site differences in continuous variables for thyroid condition and whether differences occurred between SK fish (reference site) and the downstream fish. MANOVA allows the simultaneous analyses of multiple dependent variables while protecting against Type I errors. MANOVA also provides a partial correlation matrix of the sum of squares and cross products, which allows insights on the importance of the dependent variables and their strength of association while controlling for mean differences among sites (Carey, 1998).

A significant MANOVA was followed by a Discriminant Function Analysis (DFA), which allows identifying the variables that best explain group differences among the three collection sites. DFA reduces the multidimensional dataset to a smaller number of dimensions (canonical functions), and also allows an assessment of the contribution of each original variable to the canonical function by means of the structure coefficients (McGarigal et al. 2000). Only the variables whose structure coefficients were $>|0.3|$ were interpreted in the present study.

MANOVA and DFA were performed separately on male data from each year, on female data collected in 2010, and on the contaminant content of male livers collected in 2010. Plasma T_4 concentration was not included in the multivariate analyses because more than half of the samples were under the detection limit in males and females. These data were instead analyzed by chi-square contingency tables to determine whether the proportion of values below the detection limit differs among sites, for males and females separately. In 2010 males, to discard the possibility that plasma T_3 variability is related to spawning activity, rather than to contaminant exposure, a Pearson correlation analysis between plasma T_3 concentration and gonadosomatic index (GSI) was performed.

A conservative criterion suggests that a group sampling size greater or equal to three times the number of discriminating variables is appropriate for DFA (Williams and Titus 1988). In order to reduce the relatively large number of variables for the present analysis, a prior stepwise selection (forward method) was performed. Also, in the case of liver contaminant content analysis, all chemicals with more than 15% of non-detectable values (below detection limits) were eliminated, reducing the number from 30 to the following 13: DCPA, *p,p'*-DDE, PCB-138, PCB-146, PCB-170, PCB-174, PCB-180, PCB-183, PCB-187, PCB-194, BDE-47, BDE-100 and BDE-154. However, among the chemicals that were discarded, those that differed significantly among sites and whose non-detectable values were less than 25% were reincorporated into the data set; these were HCB and BDE-153. Values below the detection limits (LOD) were replaced by one-half of the LOD prior to analyses.

Canonical Correlation Analysis (CANCOR) was performed to determine if the variability of biological traits in males can be explained by the contaminants present in the liver. The contaminant content of male livers was considered the independent variable set, whereas the thyroid condition variables constituted the dependent variable set. Variables used for this analysis were those whose structure coefficients were $>|0.3|$ in the DFAs. Pearson's correlation coefficients for selected biology-contaminant pairwise associations are also reported in this study to help in the interpretation of canonical results.

Multivariate methods can tolerate slight deviations from the parametric assumptions (McGarigal et al. 2000); however, data were log-transformed to make sure that different-scale variables contribute equally to the multivariate analysis. Missing

values were estimated via expectation-maximization (EM) algorithm before the multivariate analyses were performed (8 out of 83 samples presented variables with missing values) (Strauss 2010).

SAS software, Version 9.2 (SAS Institute Inc. SAS/STAT[®], Cary, NC, USA), was used for the MANOVA and Kruskal-Wallis tests. Chi-square contingency table and Pearson's correlation analysis was done using GraphPad Prism, Version 5 (GraphPad Software, La Jolla, CA, USA). Missing values were calculated with the function MissEM (Strauss 2010) in Matlab[®] (Version 6.0.0.88). The α level of significance was ≤ 0.05 .

Results

Thyroid histological condition

Thyroid follicles of largescale sucker are distributed in the ventral region of the pharynx, forming clusters around the ventral aorta, with only few occasional isolated follicles. The clusters are not encapsulated or surrounded by connective tissue and include follicles of a wide size range. Macrophage aggregates were commonly observed in the extrafollicular space but their presence was not associated with collection site.

In 2009 males, hyperplastic follicles (Figure 3.2) were observed in all fish, and follicles with >50% colloid depletion (Figure 3.3) were present in more than 65% of fish from the three sampling sites, but the values for other histological variables were relatively low (Table 3.2) and no significant association between any endpoint incidence and collection site was found. Epithelial cell height did not differ among sites; whereas,

the average cell density appeared to be higher in LV, followed by SK and CC (Table 3.3). In 2010 males, follicular hyperplasia was also observed in all fish, and follicles >50% colloid depletion were observed in many fish from the three sites, from 50% to 73% in CC and LV, respectively. The percentage of fish presenting follicles with separated-base-membrane (Figure 3.4) was significantly associated to the collecting site (χ^2 (2, N = 44) = 10.72, p = 0.005), with 60% and 20% of the fish from LV and SK, respectively, being affected, whereas no fish from CC presented the pathology (Table 3.4). Epithelial cell height did not differ among sites (Table 3.5), whereas cell density appeared to be higher in SK (Figure 3.5).

In 2010 females, hyperplastic follicles were observed in all fish, and follicles with >50% colloid depletion were also present in more than 60% of fish in the three collecting sites. Significant associations between fish affected and collection site were not found for any of the variables observed (Table 3.3). Epithelia cell height and cell density appeared not different among sites (Table 3.5).

Thyroid hormones

Plasma T₄ levels ranged from undetectable (LOD, 2.5) to 8.8 ng/ml in females and undetectable to 10.4 ng/ml in males. Plasma T₃ levels ranged from 0.9 to 8.2 ng/ml in females and from undetectable (LOD, 0.7) to 9.1 ng/ml in males (Tables 3.3 and 3.5). Only one individual presented undetectable values for T₃.

Plasma T₄ levels were under the detection limit (2.5 ng/ml) in most male and female samples. Results of chi-square analysis showed that the distribution of plasma T₄ concentrations above and below the detection limit was related to collection site in 2009

males (X^2 (2, N = 47) = 13.79, p = 0.001) but not 2010 males ($p > 0.05$) (Table 3.2). In 2010 females, plasma T_4 concentrations above and below the detection limit were independent of collection site ($p > 0.05$) (Table 3.3).

Plasma T_3 concentration in 2009 males were higher in fish from CC, followed by SK and LV (Table 3.3). In 2010 males, this trend was reversed with CC presenting lower concentrations followed by SK and LV. The Pearson's correlation analysis between males plasma T_3 concentration and GSI was not significant ($r = -0.09$; $p > 0.05$). In females 2010, relatively higher T_3 concentrations were observed in SK followed by CC and LV (Table 3.5).

Contaminants in male liver

Fish from LV had the highest concentrations of DCPA, p,p' -DDE, HCB and some PCBs and PBDEs, and fish from CC presented higher values for some PCBs and PBDEs (Table 3.6). The geometric means of all the contaminants measured in males from SK were consistently lower than the ones observed at the other two sites. These data indicated that the liver contaminant content of largescale sucker from the Lower Columbia River Basin increase in a downstream direction. Liver composite contaminant concentration in 2009 males fell within the range of concentrations observed in 2010, and followed the same downstream tendency (Table 3.6).

MANOVA and DFA results

Males

In 2009, MANOVA yielded significant site-dependent differences in thyroid condition (Wilks' $\lambda = 0.495$; $F(16, 70) = 1.84$; $p = 0.04$); however, overall differences between fish from SK and downstream fish were not significant (Wilks' $\lambda = 0.815$; $F(8, 35) = 1.00$; $p = 0.456$). The partial correlation matrix of the sums of squares and cross products showed an association between base membrane abnormalities and presence of rodlet cells (Table 3.7).

The stepwise DFA resulted in the selection of two variables for the model: total T_3 plasma concentration and follicle cell density. The overall model was significant (Wilks' $\lambda = 0.75$; $F(4,82) = 3.09$; $p = 0.02$) and contained one significant canonical function bearing an eigenvalue of 0.32. T_3 concentration had the highest structure coefficient (0.76) followed by follicle cell density (-0.65). The overall classification of samples based on the original cases was 50% correct; in other words, the degree of group discrimination achieved by the canonical functions was relatively poor. The Mahalanobis distance between LV and CC was the largest (Table 3.8), with LV fish having lower T_3 plasma concentration and higher number of epithelial cells per follicle, fish from CC showing the opposite, and fish from SK in an intermediate position (Figure 3.6).

In 2010, thyroid condition did not differ among sites (MANOVA Wilks' $\lambda = 0.597$; $F(16, 66) = 1.21$; $p = 0.282$), or between SK and downstream sites (Wilks' $\lambda = 0.866$; $F(8,33) = 0.64$; $p = 0.74$). The partial correlation matrix of the sums of squares and cross products showed an association between follicle cell height and highly-columnar epithelia, and between highly-columnar epithelia and >50% colloid depletion

(Table 3.9). The DFA was not performed because the MANOVA was not significant; however, in order to select the variables that contributed to the CANCOR, the stepwise DFA was performed. The variables selected by the model were follicle cell density, base membrane separation and proportion of follicles with >50% colloid depletion.

In 2010, the liver contaminant concentrations differed significantly among sites (Wilks' $\lambda = 0.18$; $F(30, 50) = 2.26$; $p = 0.005$), and between fish from SK and the downstream sites (Wilks' $\lambda = 0.30$; $F(15, 25) = 3.90$; $p = 0.001$). The partial correlation matrix showed positive pairwise associations between most contaminants (Table 3.10). The stepwise DFA analysis resulted in the selection of two variables for the model, DCPA and HCB. The model was significant (Wilks' $\lambda = 0.59$; $F(4, 76) = 5.80$; $p = 0.0004$) and contained two significant canonical functions bearing eigenvalues of 0.49 and 0.14, respectively. HCB had the highest structure coefficient (0.81) on the first function; whereas in the second canonical function, DCPA had the highest coefficient (0.98). The overall classification of samples based on their contaminant composition was 64% which, although relatively low, it seems reasonable for descriptive purposes. The degree of separation was highest between SK and LV (Table 3.8), and it was mainly on the basis of HCB concentrations (function 1); whereas SK and CC were separated based on the concentration of DCPA (function 2); however a high degree of overlap is apparent (Figure 3.7).

Females

In 2010, thyroid condition differed significantly among sites (Wilks' $\lambda = 0.274$; $F(16, 38) = 2.17$; $p = 0.026$), and between fish from SK and downstream sites (Wilks' $\lambda = 0.462$; $F(8, 19) = 2.77$; $p = 0.033$). The partial correlation matrix showed a positive

association between the follicle cell height and plasma T_3 concentration; follicle cell height and the proportion of hyperplastic follicles; the proportion of hyperplastic follicles and plasma T_3 concentration; and between follicle cell height and the presence of rodlet cells (Table 3.11). The stepwise DFA resulted in the selection of four variables for the model: proportion of thyroid follicles with >50% colloid depletion, proportion of hyperplastic follicles, epithelial cells density, and follicle cell height. The model was significant (Wilks' $\lambda = 0.38$; $F(8,46) = 3.53$; $p = 0.003$) and contained one significant canonical function bearing a 0.92 eigenvalue. The proportion of follicles with >50% colloid depletion had the highest structure coefficient (0.64), followed by number of epithelial cell density (0.30) on the first function. The overall classification of samples based on the original cases was 73% correct. The degree of separation was highest between fish from SK and LV (Table 3.8). Fish from LV were separated from the other sites in terms of follicular colloid depletion (function 1), whereas fish from SK and CC differed mostly on epithelial cell density (Figure 3.8).

CANCOR results

Only contaminant or biological variables with factor structure coefficients $\geq |0.3|$ in their respective DFAs were used in CANCOR in order to limit the number of variables and reduce complications due to low sample-to-variable ratio. The selected variables thus were HCB and DCPA for contaminants, and follicle cell density, base membrane abnormalities and proportion of follicles with >50% colloid depletion for biological traits. No significant association between the two canonical variable sets was observed (Wilks' $\lambda = 0.92$; $F(6,74) = 0.53$; $p = 0.782$). In other words, variation in the thyroid

condition was not significantly associated with variation in the concentration of the selected contaminants in male liver. Pairwise correlations between thyroid condition and contaminant variables also indicated that the degree of association between DCPA and HCB with thyroid variables is rather low (Table 3.12).

Discussion

Several studies of the Lower Columbia River Basin have demonstrated the presence of a great diversity of pollutants in fish and wildlife, including largescale sucker (Henny et al. 2008b; Hinck et al. 2006; Hinck et al. 2009). This is the first study that describes the thyroid condition of a resident species in this river basin, largescale sucker, in the context of contaminant exposure. Thyroid condition of male largescale sucker differed among collecting sites in 2009, but not in 2010. Conversely in 2010 females, thyroid condition not only differed among collecting sites, but also between fish from SK and downstream fish. However, clear associations between the thyroid condition of largescale sucker and their exposure to contaminants could not be established.

To the best of my knowledge, this is the first study reporting the plasma TH concentration in largescale sucker. Plasma T₃ concentration differed among sites in 2009 males, with LV and CC presenting the lowest and highest concentrations and SK (reference site) having intermediate values; this trend was not observed in 2010. Studies with teleosts have suggested that thyroid activity varies with the reproductive cycle and that this variation is more apparent in females than in males (Cyr and Eales 1996). In the present study, all fish examined were adults, although males seemed to be at different stages of spawning activity (from ripe to spent); however, no significant correlation was found between plasma T₃ concentration and testicular gonadosomatic index ($p>0.05$),

indicating that differences in males plasma T_3 among sites are due to factors other than reproduction (spawning activity is discussed in further detail in Chapter IV).

Studies have shown that PCBs and certain pesticides may alter 5'-deiodinase activity in fish, which can lead to increased T_3 and reduced T_4 (Brar et al. 2010; Brown et al. 2004). Nevertheless, evidence of change in plasma TH concentration is not consistent among species or even among the type of contaminant causing the effect; for instance, studies have reported a decrease in TH after chronic exposure to metals (Levesque et al. 2003), whereas others found decreased T_3 levels, but not differences in T_4 , in fish living on sites affected by metals and municipal and domestic effluents compared to a reference site (Oliveira et al. 2011). In the present study, 2009 males presented lower plasma T_3 concentrations at the most contaminated site, LV, and the highest concentrations at CC, a site also affected by main sources of contamination; conversely, LV had the highest percentage of fish with detectable values of T_4 , followed by CC and SK. These results suggest that fish from the three sites are potentially exposed to a different combination of stressors that may affect the thyroid axis differently.

Changes in the structure of thyroid follicles typically reflect the level of follicular activity; therefore, they are potentially good indicators of responses to contaminant exposure (Carr and Patiño 2011). Follicle cell density was an important histopathological variable discriminating among collecting sites. Thyroid follicles with higher cell density were observed in LV in 2009 males and 2010 females. Higher density of follicular cells in the epithelium monolayer is a potential indication of cell proliferation. Factors that may cause proliferation of fish thyroid tissue include poor water quality (Nigrelli and

Ruggieri 1974) and exposure to goitrogenic substances (Hoover 1984). Severe cases of cell proliferation such as ectopic follicles, adenomas or carcinoma were not observed.

The proportion of follicles with >50% colloid depletion was also important for the separation of 2010 females by sites, with higher values in fish from LV, followed by CC and SK. The colloid contains thyroglobulin, the precursor of T_4 . A drastic depletion in follicular colloid is related to a higher demand for T_4 (Blanton and Specker 2007). Given the low proportion of follicles presenting >50% colloid depletion, a detrimental effect in the supply of T_4 may not be of concern to the health of the fish examined.

The partial correlation matrix generated by MANOVA showed that most male liver contaminants were highly associated with each other; also, because the stepwise DFA only selects the less redundant variables that explain differences among groups, only two contaminants made it into the final model, HCB and DCPA. DCPA is the herbicide active ingredient of Dacthal®. Studies in rodents have shown potential effects of this herbicide in lungs, liver and thyroid after acute exposure (EXTOXNET 1996). However DCPA does not bioconcentrate, it has very low toxicity in mammals, does not cause reproductive, teratogenic, mutagenic or carcinogenic effects, and apparently poses no hazard to aquatic organisms (EXTOXNET 1996).

The commercial production of HCB was discontinued in the 1970s, but its synthesis as a by-product or impurity of several chlorinated compounds, including DCPA and other pesticides, is reason of concern. HCB is considered an endocrine disruptor, the thyroid axis being one of its targets. Studies with mammals have found that chronic exposure to HCB may cause changes in plasma T_4 (Chiappini et al. 2009) but not in T_3 levels (Depisarev et al. 1995). The mechanism of HCB action is believed to involve

increased hepatic metabolism and excretion of T₄ (into bile) (ATSDR 2002). Other studies have reported the increased incidence of apoptosis of follicular cells but not in follicular cell proliferation after HCB exposure (Chiappini et al. 2009). However, histopathological changes have not been consistently found in rodent studies after exposure (ATSDR 2002). In the present study, concentrations of HCB were consistently higher in LV males in 2009 and 2010. However, the canonical correlation analysis showed that the variation in thyroid condition among sites cannot be explained by the variation in the liver content of HCB and DCPA. This indicates that there are other potential factors altering the thyroid condition of largescale sucker in the lower Columbia River.

This is the first histopathological assessment of thyroid condition in largescale sucker. A more detailed analysis of the distribution, shapes and sizes of the follicles is required to better understand the anatomy and physiology of the fish in order to be able to use the histopathological tools more effectively. Similarly, it is known that the thyroid activity in fish varies seasonally, reason for which a seasonal assessment is necessary in order to set a baseline of the thyroid condition of this species, in a way that its use as contaminant-monitor might provide more accurate and valuable information.

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Table 3.1. Abbreviations and complete names of the compounds analyzed in the liver of male largescale sucker collected in 2010.

Abbreviation	Complete name
Chlorpyrifos	<i>O,O</i> -Diethyl <i>O</i> -3,5,6-trichloropyridin-2-yl phosphorothioate
<i>p,p'</i> -DDE	1,1- <i>bis</i> -(4-chlorophenyl)-2,2-dichloroethene
<i>p,p'</i> -DDD	1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene
Cis-chlordane	4,7-methano-1H-indene,1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a,5,10-hexahydro-, (1 α ,2 α ,3 α ,4 β ,7 β ,7a)
Trans-chlordane	4,7-Methano-1H-indene, 1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-, (1 α ,2 β ,3 α ,4 β ,7 β ,7a α)
Cis-nonachlor	(1 α ,2 α ,3 α ,4 β ,7 β ,7a β)-1,2,3,4,5,6,7,8,8-nonachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1H-indene
Trans-nonachlor	(1S,7R)-1 α ,2 β ,3 β ,4,5,6,7,8,8-Nonachloro-2,3,3a α ,4,7,7a α -hexahydro-4,7-methano-1H-indene
DCPA	dimethyl tetrachloroterephthalate
Dieldrin	(1a <i>R</i> ,2 <i>R</i> ,2a <i>S</i> ,3 <i>S</i> ,6 <i>R</i> ,6a <i>R</i> ,7 <i>S</i> ,7a <i>S</i>)-3,4,5,6,9-hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-2,7:3,6-dimethanonaphtho[2,3- <i>b</i>]oxirene
HCB	hexachlorobenzene
PCA	1,2,3,4,5-Pentachloro-6-methoxybenzene
PCB-110	2,3,3',4',6-Pentachloro-1,1'-biphenyl
PCB-118	2,3',4,4',5-Pentachloro-1,1'-biphenyl
PCB-138	2,2',3,4,4',5'-Hexachloro-1,1'-biphenyl
PCB-146	2,2',3,4',5,5'-Hexachloro-1,1'-biphenyl
PCB-149	2,2',3,4',5',6-Hexachloro-1,1'-biphenyl
PCB-151	2,2',3,5,5',6-Hexachloro-1,1'-biphenyl
PCB-170	2,2',3,3',4,4',5-Heptachloro-1,1'-biphenyl
PCB-174	2,2',3,3',4,5,6'-Heptachloro-1,1'-biphenyl
PCB-177	2,2',3,3',4,5',6'-Heptachloro-1,1'-biphenyl,
PCB-180	2,2',3,4,4',5,5'-Heptachloro-1,1'-biphenyl
PCB-183	2,2',3,4,4',5,6-Heptachloro-1,1'-biphenyl
PCB-187	2,2',3,4',5,5',6-Heptachloro-1,1'-biphenyl
PCB-194	2,2',3,3',4,4',5,5'-Octachloro-1,1'-biphenyl
PCB-206	2,2',3,3',4,4',5,5',6-Nonachloro-1,1'-biphenyl
BDE-47	2,2',4,4'-Tetrabromodiphenyl ether
BDE-99	2,2',4,4',5-Pentabromodiphenyl ether
BDE-100	2,2',4,4',6-Pentabromodiphenyl ether
BDE- 153	2,2',4,4',5,5'-Hexabromodiphenyl ether
BDE-154	2,2',4,4',5,6'-Hexabromodiphenyl ether

Table 3.2. Incidence of thyroid endpoints in male largescale sucker collected on 2009 in the Lower Columbia River Basin. Data are presented in percentages. MA, macrophage aggregates.

Thyroid endpoints	Skamania	Columbia City	Longview
T ₄ detectable values	20	33	81
Hyperplasia	100	100	100
Columnar epithelium	19	13	0
Base membrane separation	25	13	13
>50% colloid depleted	69	73	67
Rodlet cells	25	0	13
MA presence	75	67	75

Table 3.3. Thyroid condition variables of largescale sucker collected on 2009 in the Lower Columbia River Basin. Mean $\pm$ SEM; except for T4 concentration where ranges are presented.

Thyroid variables	Skamania	Columbia City	Longview
	Males	Males	Males
GSI	3.7 $\pm$ 0.4	3.1 $\pm$ 0.5	2.0 $\pm$ 0.3
T ₄ (ng/ml)	ND-9.8	ND-10.4	ND-8.2
T ₃ (ng/ml)	2.3 $\pm$ 0.2	3.3 $\pm$ 0.4	1.7 $\pm$ 0.2
FCH (μ m)	7.6 $\pm$ 0.3	8.4 $\pm$ 0.6	6.9 $\pm$ 0.4
FCP	0.127 $\pm$ 0.003	0.121 $\pm$ 0.004	0.134 $\pm$ 0.004
PFH	0.2 $\pm$ 0.02	0.14 $\pm$ 0.02	0.2 $\pm$ 0.02
PCE	0.01 $\pm$ 0.01	0.003 $\pm$ 0.002	0
PBM	0.01 $\pm$ 0.01	0.01 $\pm$ 0.01	0.04 $\pm$ 0.03
PCD	0.1 $\pm$ 0.01	0.07 $\pm$ 0.02	0.04 $\pm$ 0.01
PRC	0.01 $\pm$ 0.01	0	0.01 $\pm$ 0.005

GSI, gonadosomatic index; FCH, follicular cell height; FCP, follicular number of cells per perimeter; PFH, proportion of follicles with hyperplasia; PCE, proportion of follicles with highly columnar epithelium; PBM, proportion of follicles with base membrane abnormalities; PCD, proportion of follicles with more than 50% colloid depletion; PRC, proportion of follicles with rodlet cells in between follicular cells.

Table 3.4. Incidence of thyroid endpoints in largescale sucker collected on 2010 in the Lower Columbia River Basin. Data are presented in percentages. MA, macrophage aggregates.

Thyroid endpoints	Skamania		Columbia City		Longview	
	Males	Females	Males	Females	Males	Females
T ₄ detectable values	33	18	25	22	40	50
Hyperplasia	100	100	100	100	100	100
Columnar epithelium	27	46	21	20	27	13
Base membrane	20	46	0	60	60	50
>50% colloid depleted	67	63	50	70	73	88
Rodlet cells	33	36	21	30	13	38
MA presence	73	46	57	80	60	25

Table 3.5. Thyroid condition variables of largescale sucker collected on 2010 in the Lower Columbia River Basin. Mean $\pm$ SEM; except for T4 concentration where ranges are presented.

Thyroid variables	Skamania		Columbia City		Longview	
	Males	Females	Males	Females	Males	Females
GSI	3.1 $\pm$ 0.6	6.4 $\pm$ 1.5	1.9 $\pm$ 0.5	6.6 $\pm$ 1.8	2.5 $\pm$ 0.4	3.1 $\pm$ 1.8
T ₄ (ng/ml)	ND-5.2	ND-8.8	ND-7.4	ND-7.3	ND-7.5	ND-6.8
T ₃ (ng/ml)	3.7 $\pm$ 0.4	4.9 $\pm$ 0.6	3.3 $\pm$ 0.4	4.3 $\pm$ 0.2	4.6 $\pm$ 0.5	3.6 $\pm$ 0.5
FCH (μ m)	8.2 $\pm$ 0.5	8.3 $\pm$ 0.6	7.7 $\pm$ 0.6	7.4 $\pm$ 0.6	7.5 $\pm$ 0.5	6.0 $\pm$ 0.8
FCP	0.138 $\pm$ 0.003	0.129 $\pm$ 0.01	0.128 $\pm$ 0.01	0.115 $\pm$ 0.003	0.128 $\pm$ 0.004	0.138 $\pm$ 0.004
PFH	0.2 $\pm$ 0.02	0.13 $\pm$ 0.02	0.2 $\pm$ 0.01	0.2 $\pm$ 0.02	0.1 $\pm$ 0.02	0.12 $\pm$ 0.02
PCE	0.01 $\pm$ 0.004	0.04 $\pm$ 0.02	0.003 $\pm$ 0.003	0.01 $\pm$ 0.01	0.01 $\pm$ 0.003	0.01 $\pm$ 0.01
PBM	0.02 $\pm$ 0.01	0.04 $\pm$ 0.02	0	0.2 $\pm$ 0.1	0.04 $\pm$ 0.01	0.2 $\pm$ 0.1
PCD	0.04 $\pm$ 0.01	0.03 $\pm$ 0.01	0.02 $\pm$ 0.01	0.04 $\pm$ 0.01	0.1 $\pm$ 0.01	0.1 $\pm$ 0.04
PRC	0.02 $\pm$ 0.01	0.01 $\pm$ 0.01	0.01 $\pm$ 0.01	0.03 $\pm$ 0.02	0.01 $\pm$ 0.01	0.5 $\pm$ 0.3

GSI, gonadosomatic index; FCH, follicular cell height; FCP, follicular number of cells per perimeter; PFH, proportion of follicles with hyperplasia; PCE, proportion of follicles with highly columnar epithelium; PBM, proportion of follicles with base membrane abnormalities; PCD, proportion of follicles with more than 50% colloid depletion; PRC, proportion of follicles with rodlet cells in between follicular cells.

Table 3.6. Geometric means (ranges) of contaminants concentrations ($\mu\text{g/kg}$, ww) male livers collected from the Lower Columbia River Basin in 2010.

Contaminants	Skamania		Columbia City		Longview	
	2009	2010	2009	2010	2009	2010
DCPA	0.9	1.8 (0.43 - 5.9)	0.7	1.0 (0.25 - 4.6)	1.4	1.9 (0.85 - 4.2)
<i>p,p'</i> -DDE	157	249.3 (4 - 1270)	221	368.8 (89 - 1340)	389	404.2 (63 - 1840)
HCB	1.4	1.1 (0.5 - 4.6)	2.9	2.2 (0.5 - 6.1)	3.9	3.8 (0.5 - 19)
PCB-138	6.4	6.3 (0.25 - 44)	9.7	10.3 (3.1 - 34)	11.8	11.7 (2.4 - 58)
PCB-146	0.9	2.7 (0.4 - 22)	3.3	5.0 (0.7 - 19)	8.5	4.5 (0.9 - 14)
PCB-170	1.3	2.9 (0.45 - 23)	6.8	6.0 (2 - 19)	8.9	5.8 (1.4 - 17)
PCB-174	0.1	1.4 (0.45 - 11)	3.1	2.7 (0.93 - 9.4)	5.5	2.9 (0.76 - 7.6)
PCB-180	2.7	5.5 (0.81 - 44)	11.8	10.3 (2.9 - 37)	18.9	9.9 (2.3 - 31)
PCB-183	0.8	1.7 (0.24 - 12)	3.3	3.0 (0.81 - 9.9)	4.8	2.9 (0.75 - 9)
PCB-187	2.3	5.3 (0.94 - 33)	7.8	8.6 (2.5 - 24)	17.2	8.2 (1.9 - 24)
PCB-194	0.4	1.0 (0.42 - 6.5)	2.3	1.7 (0.46 - 6.7)	3.4	1.5 (0.5 - 4.1)
BDE-47	16.2	43.1 (8.7 - 300)	75.8	68.3 (27 - 140)	161	96.6 (46 - 300)
BDE-100	3.9	8.0 (1.7 - 95)	26.8	13.4 (4.9 - 31)	41.6	16.8 (6.9 - 56)
BDE-153	0.2	0.6 (0.22 - 2.2)	1.5	0.8 (0.35 - 2.8)	2.7	1.3 (0.59 - 4.3)
BDE-154	0.7	1.5 (0.35 - 15)	4.6	3.3 (1.1 - 9.5)	8.7	3.2 (1.2 - 10)

Contaminants with more than 15% of non-detectable values are not included.

Table 3.7. Partial correlation matrix of the sums of squares and cross products obtained as part of the MANOVA test performed in males collected in 2009 from the Lower Columbia River Basin.

	T ₃	FCH	FCP	PFH	PCE	PBM	PCD
FCH	0.04						
FCP	0.01	0.06					
PFH	0.00	-0.10	-0.10				
PCE	0.22	0.09	0.19	0.29			
PBM	0.27	0.20	0.06	-0.25	-0.04		
PCD	0.18	0.10	0.24	0.05	-0.05	-0.01	
PRC	-0.01	0.15	-0.09	0.15	-0.17	0.43*	0.05

FCH, follicular cell height; FCP, follicular number of cells per perimeter; PFH, proportion of follicles with hyperplasia; PCE, proportion of follicles with highly columnar epithelium; PBM, proportion of follicles with base membrane abnormalities; PCD, proportion of follicles with more than 50% colloid depletion; PRC, proportion of follicles with rodlet cells in between follicular cells. *p<0.05.

Table 3.8. Squared Mahalanobis distances between group (site) means for thyroid condition and male liver-contaminant concentration in largescale sucker collected from three sites in the Lower Columbia River Basin (SK, Skamania; CC, Columbia City; LV, Longview).

Males 2009			Liver contaminant content 2010			Females 2010		
Site	SK	CC	Site	SK	CC	Site	SK	CC
CC	0.55		CC	2.13		CC	2	
LV	0.41	1.89	LV	2.27	0.82	LV	5.09	3.68

Table 3.9. Partial correlation matrix of the sums of squares and cross products obtained as part of the MANOVA test performed in males collected in 2010 from the Lower Columbia River Basin.

	T ₃	FCH	FCP	PFH	PCE	PBM	PCD
FCH	-0.22						
FCP	-0.11	0.22					
PFH	-0.06	0.09	-0.01				
PCE	-0.04	0.43*	-0.03	0.19			
PBM	0.14	0.11	0.09	-0.13	0.17		
PCD	0.05	0.08	-0.15	-0.03	0.44*	-0.16	
PRC	0.12	0.29	0.08	0.24	0.12	-0.20	0.04

FCH, follicular cell height; FCP, follicular number of cells per perimeter; PFH, proportion of follicles with hyperplasia; PCE, proportion of follicles with highly columnar epithelium; PBM, proportion of follicles with base membrane abnormalities; PCD, proportion of follicles with more than 50% colloid depletion; PRC, proportion of follicles with rodlet cells in between follicular cells. *p<0.05.

Table 3.10. Partial correlation matrix of the sums of squares and cross products obtained as part of the MANOVA test performed in liver contaminant content of males collected in 2010 from the Lower Columbia River Basin.

	DCPA	<i>p,p'</i> -DDE	HCB	PCB-138	PCB-146	PCB-170	PCB-174	PCB-180	PCB-183	PCB-187	PCB-194	BDE-47	BDE-100	BDE-153
<i>p,p'</i> -DDE	0.12													
HCB	0.40*	0.03												
PCB-138	0.21	0.30	0.24											
PCB-146	0.15	0.35*	0.35*	0.78*										
PCB-170	0.13	0.33*	0.27	0.79*	0.89*									
PCB-174	0.15	0.32*	0.38*	0.72*	0.90*	0.95*								
PCB-180	0.13	0.36*	0.30	0.80*	0.92*	0.96*	0.95*							
PCB-183	0.15	0.36*	0.31	0.78*	0.92*	0.95*	0.95*	0.99*						
PCB-187	0.18	0.38*	0.32*	0.82*	0.94*	0.95*	0.94*	0.98*	0.98*					
PCB-194	0.07	0.36*	0.24	0.70*	0.85*	0.91*	0.92*	0.94*	0.93*	0.89*				
BDE-47	0.24	0.32*	0.44*	0.66*	0.79*	0.82*	0.85*	0.80*	0.81*	0.83*	0.70*			
BDE-100	0.15	0.33*	0.40*	0.71*	0.83*	0.91*	0.90*	0.87*	0.87*	0.88*	0.79*	0.96*		
BDE-153	0.24	0.38*	0.51*	0.38*	0.59*	0.63*	0.70*	0.63*	0.65*	0.62*	0.57*	0.84*	0.78*	
BDE-154	0.13	0.29	0.32*	0.69*	0.83*	0.90*	0.89*	0.90*	0.91*	0.87*	0.84*	0.92*	0.95*	0.78*

*p<0.05.

Table 3.11. Partial correlation matrix of the sums of squares and cross products obtained as part of the MANOVA test performed in females collected in 2010 from the Lower Columbia River Basin.

	T ₃	FCH	FCP	PFH	PCE	PBM	PCD
FCH	0.39*						
FCP	-0.31	0.13					
PFH	0.46*	0.40*	0.12				
PCE	-0.24	-0.06	0.23	-0.24			
PBM	0.05	0.32	0.09	0.27	-0.09		
PCD	0.13	0.24	-0.15	-0.08	-0.03	-0.02	
PRC	0.25	0.39*	-0.22	-0.05	-0.09	-0.23	0.23

FCH, follicular cell height; FCP, follicular number of cells per perimeter; PFH, proportion of follicles with hyperplasia; PCE, proportion of follicles with highly columnar epithelium; PBM, proportion of follicles with base membrane abnormalities; PCD, proportion of follicles with more than 50% colloid depletion; PRC, proportion of follicles with rodlet cells in between follicular cells. *p<0.05.

Table 3.12. Pairwise Pearson's correlation matrix of liver contaminant content and thyroid variables of males collected in 2010 from the Lower Columbia River Basin.

	T3	FCH	FCP	PFH	PCE	PBM	PCD	PRC
DCPA	0.11	0.24	0.02	-0.05	0.10	0.14	-0.14	0.21
DDE	-0.04	0.00	-0.21	-0.14	-0.05	0.16	0.00	-0.46
HCB	0.30	-0.13	-0.19	-0.07	0.02	0.00	-0.04	0.06
PCB-138	0.15	-0.09	0.00	-0.05	-0.35	0.22	-0.05	-0.01
PCB-146	0.18	-0.03	-0.19	0.04	-0.29	-0.02	-0.04	0.00
PCB-170	0.20	-0.08	-0.22	-0.03	-0.34	0.00	-0.09	-0.07
PCB-174	0.22	-0.10	-0.20	-0.09	-0.33	-0.03	-0.08	-0.12
PCB-180	0.25	-0.06	-0.20	-0.03	-0.27	0.10	-0.10	-0.09
PCB-183	0.27	-0.04	-0.19	-0.04	-0.25	0.07	-0.10	-0.07
PCB-187	0.22	-0.02	-0.15	-0.02	-0.29	0.02	-0.07	-0.01
PCB-194	0.12	-0.11	-0.26	-0.08	-0.29	0.08	-0.10	-0.16
BDE-47	0.34	-0.07	-0.22	-0.09	-0.27	-0.10	0.02	0.02
BDE-100	0.36	-0.11	-0.22	-0.07	-0.31	-0.07	0.02	0.02
BDE-153	0.37	-0.07	-0.20	-0.08	-0.17	-0.01	0.01	-0.02
BDE-154	0.35	-0.07	-0.25	-0.06	-0.28	-0.01	-0.06	-0.02

FCH, follicular cell height; FCP, follicular number of cells per perimeter; PFH, proportion of follicles with hyperplasia; PCE, proportion of follicles with highly columnar epithelium; PBM, proportion of follicles with base membrane abnormalities; PCD, proportion of follicles with more than 50% colloid depletion; PRC, proportion of follicles with rodlet cells in between follicular cells.

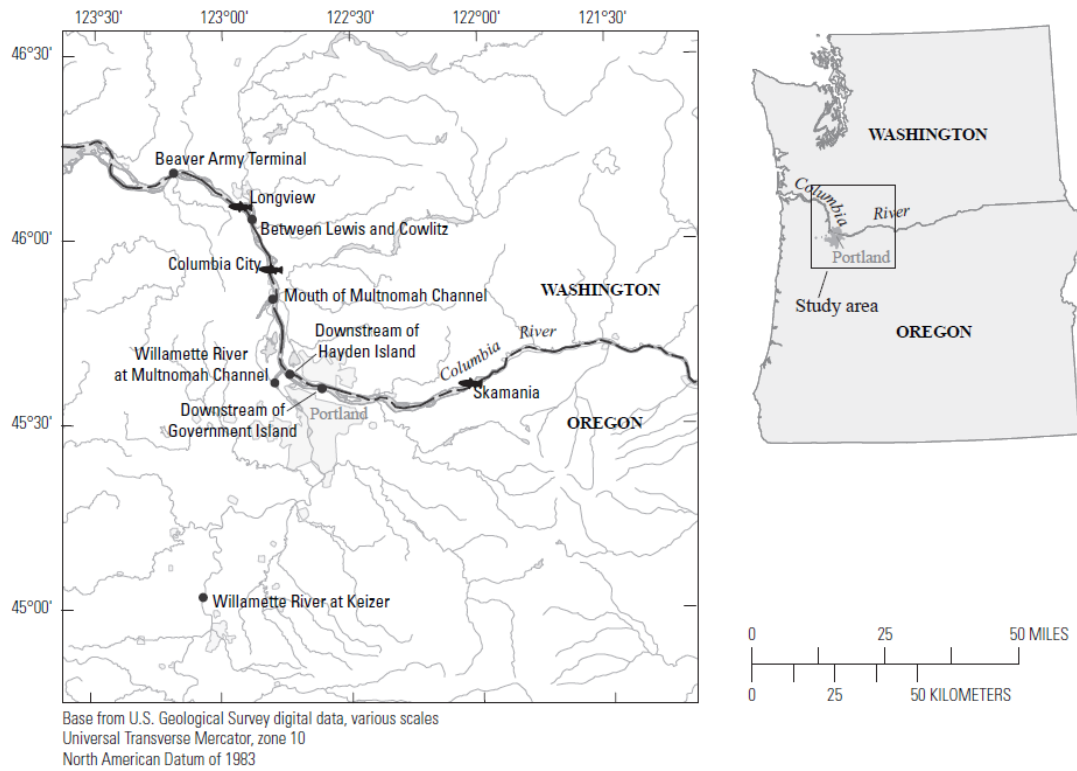


Figure 3.1. Lower Columbia River Basin Area study locations, Skamania, Columbia City and Longview. See text for latitudes and longitudes for each site. (Map courtesy of Jill Jenkins, USGS).

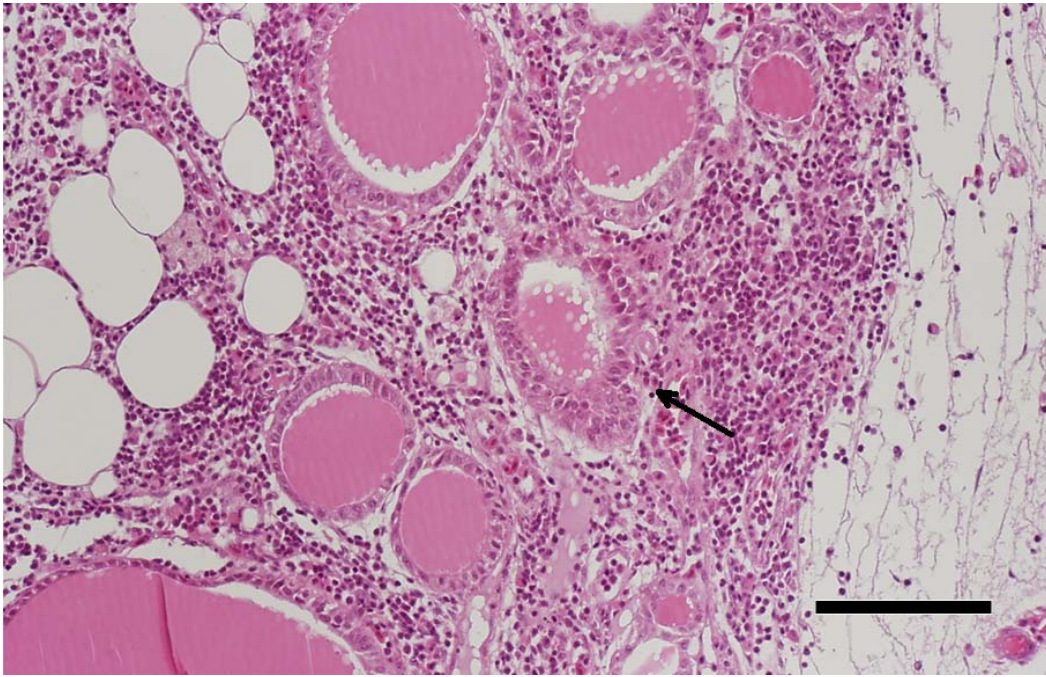


Figure 3.2. Photomicrograph of sections of thyroid follicles of largescale sucker collected in the Lower Columbia River Basin. The arrow points to a follicle presenting hyperplasia. All the fish in the three collection sites presented some proportion of hyperplastic follicles. Scale bar = 100 μ m.

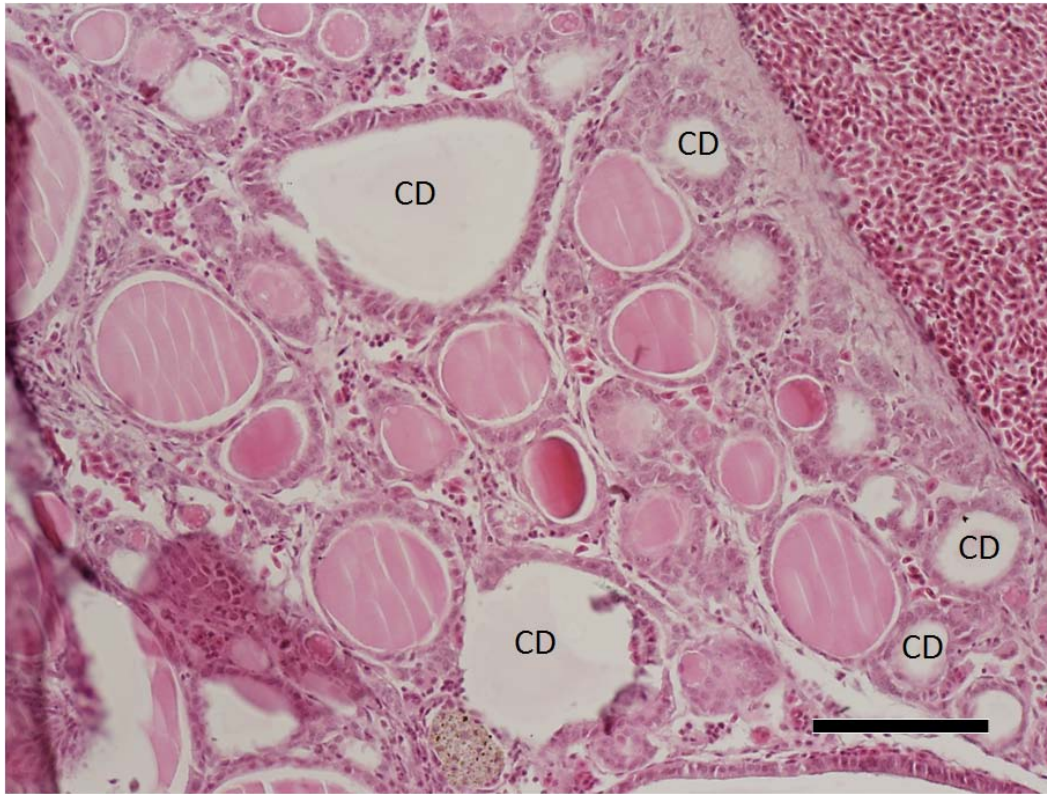


Figure 3.3. Photomicrograph of sections of thyroid follicles of 2010 male largescale sucker collected in the Longview site of the Lower Columbia River Basin. Several follicles present >50% colloid depletion (CD). The incidence of fish presenting follicles with >50% colloid depletion was high in the three collection sites. Scale bar = 100 μ m.

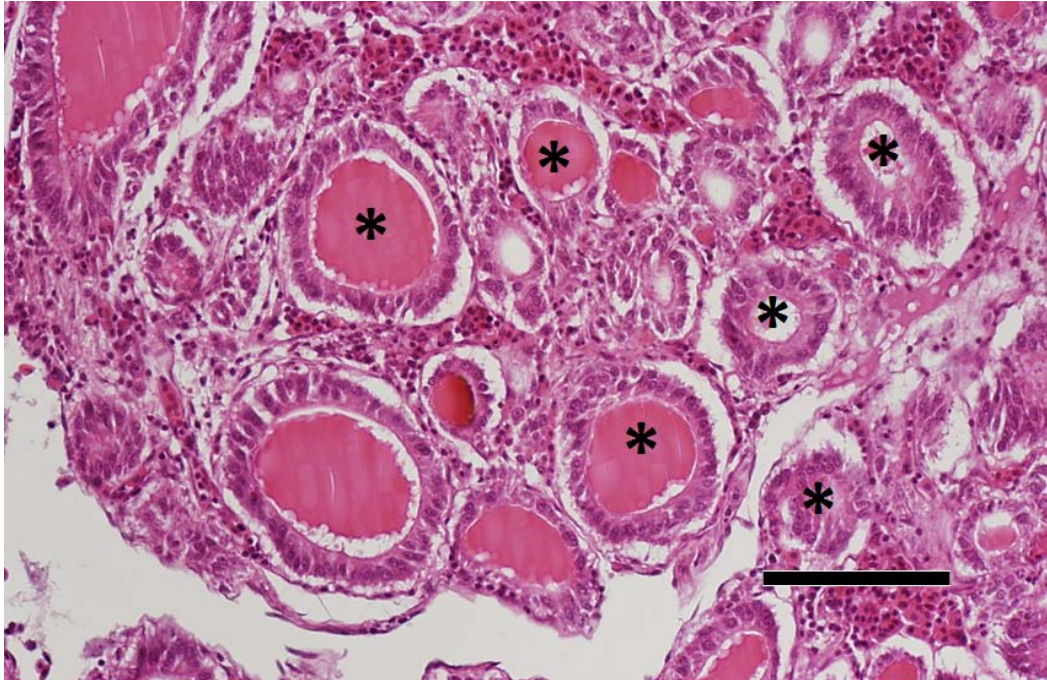


Figure 3.4. Photomicrograph of sections of thyroid follicles of a 2010 male largescale sucker collected in the Longview site in the Lower Columbia River Basin. The asterisks show follicles with the base membrane separated from the follicular epithelia. Scale bar = 100 μ m.



Figure 3.5. Photomicrograph of sections of thyroid follicles of a 2010 male largescale sucker collected in the Skamania site on the Lower Columbia River Basin. The arrows point to follicles presenting higher cell density. Scale bar = 100 μ m.

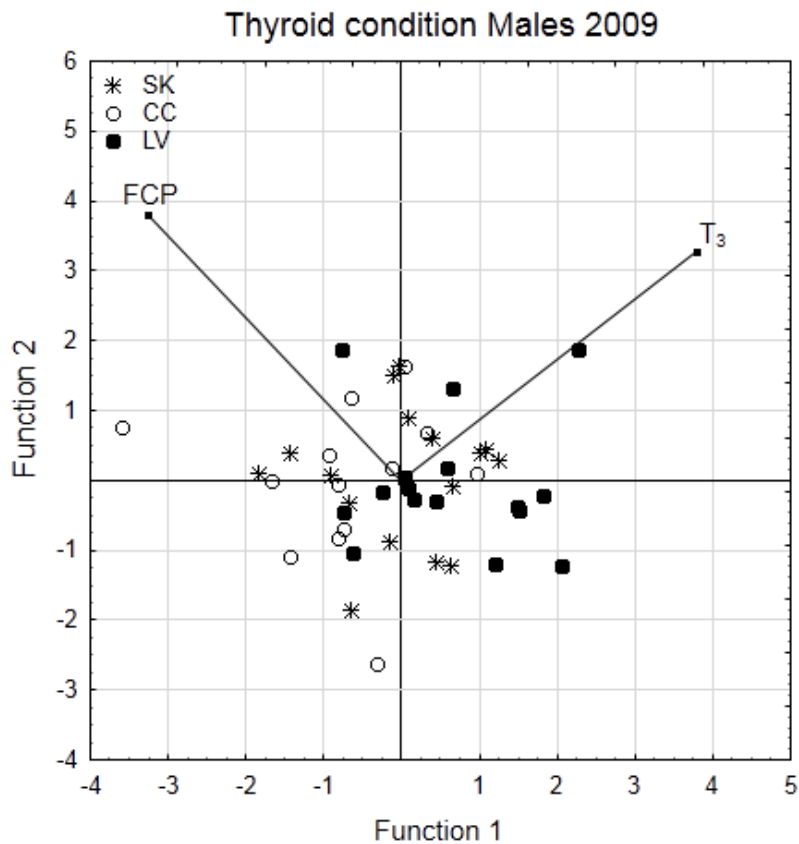


Figure 3.6. Discriminant function plot of thyroid condition data for male largescale sucker collected from three different sites in Low Columbia River Basin (Skamania, SK; Columbia City, CC; Longview, LV) in 2009. Vectors representing the standardized coefficients for each thyroid trait are superimposed on the plot to indicate each trait's contribution to the discrimination between sites (vector lengths are relative to each other and not according to the axes scales). Only those traits with coefficients $\geq |0.30|$ on the first two discriminant functions are shown on the plot. FCP, follicular number of cells per perimeter; T3, total T3 plasma concentration.

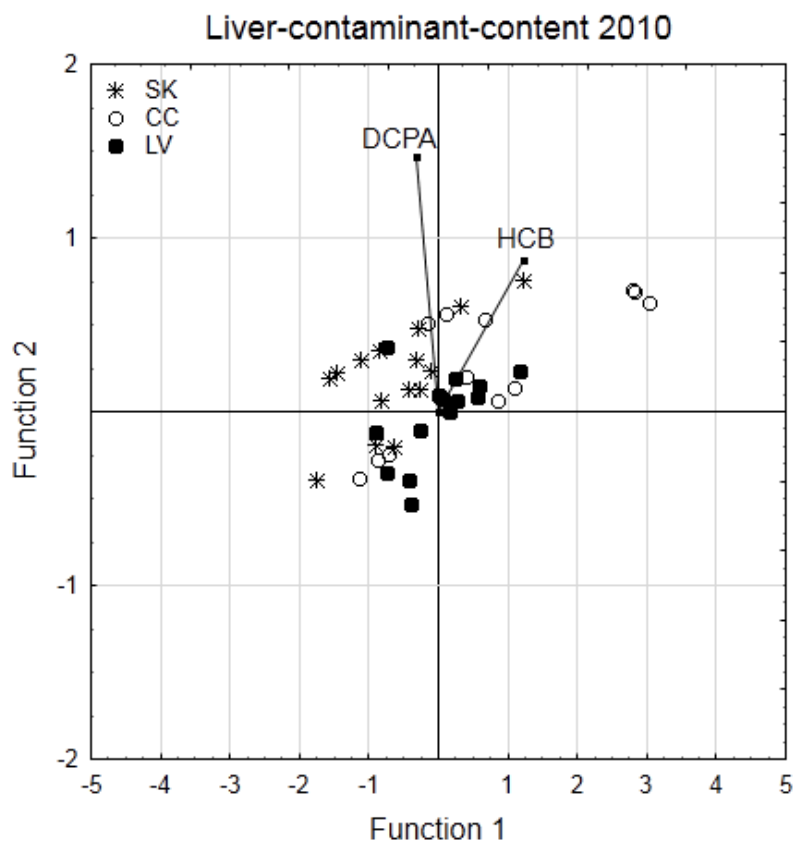


Figure 3.7. Discriminant function plot of liver contaminant concentration data for male largescale sucker collected from three different sites in Low Columbia River Basin (Skamania, SK; Columbia City, CC; Longview, LV) in 2010. Vectors representing the standardized coefficients for each biological trait are superimposed on the plot to indicate each trait's contribution to the discrimination between sites (vector lengths are relative to each other and not according to the axes scales). Only those traits with coefficients $\geq |0.30|$ on the first two discriminant functions are shown on the plot.

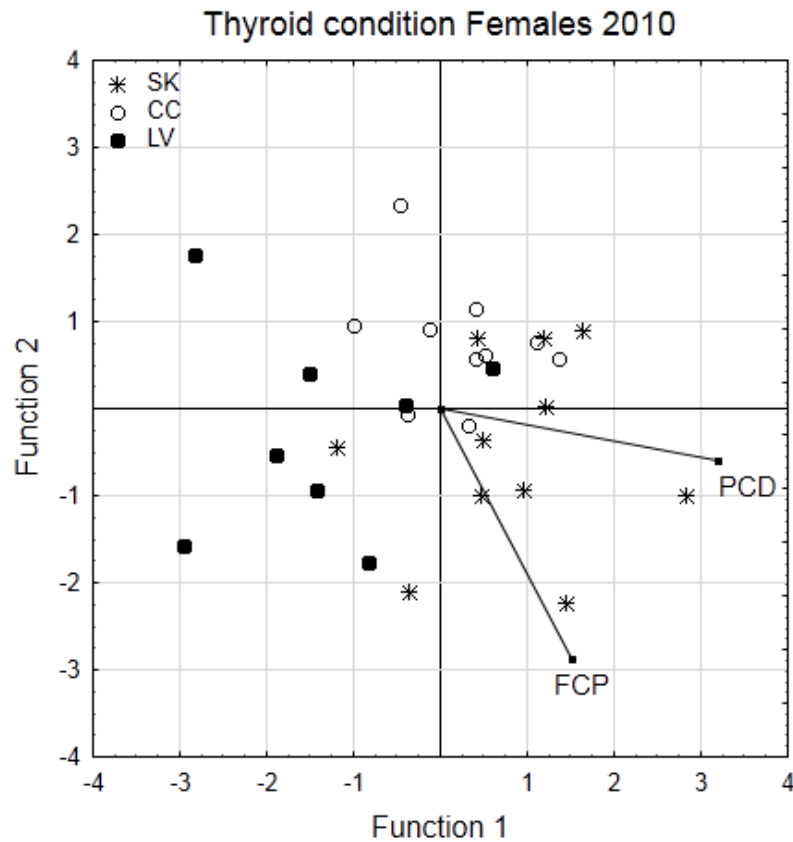


Figure 3.8. Discriminant function plot of thyroid condition data for female largescale sucker collected from three different sites in Low Columbia River Basin (Skamania, SK; Columbia City, CC; Longview, LV) in 2010. Vectors representing the standardized coefficients for each thyroid trait are superimposed on the plot to indicate each trait's contribution to the discrimination between sites (vector lengths are relative to each other and not according to the axes scales). Only those traits with coefficients $\geq |0.30|$ on the first two discriminant functions are shown on the plot. FCP, follicular number of cells per perimeter; PCD, proportion of follicles presenting colloid depletion greater than 50%.

CHAPTER IV

HEALTH CONDITION OF LARGESCALE SUCKER
(*CATOSTOMUS MACROCHEILUS*) COLLECTED ALONG A
CONTAMINANT GRADIENT IN THE LOWER COLUMBIA
RIVER BASIN

Abstract

The general health of largescale sucker from the lower Columbia River was evaluated at three sites along a contamination gradient: Columbia City and Longview (downstream), both with high levels of input from urban and industrial effluents; and Skamania (upstream), considered the reference location having a relatively less disturbed environment. The objectives of this study were to (1) characterize the health condition of largescale sucker collected along a contaminant gradient in the lower Columbia River based primarily on a histopathological approach and with emphasis on males; (2) determine if the health condition differs among collection sites; and, (3) determine if the health condition of male fish is associated with liver contaminant content. Fifteen males and 10 females per site were sampled in 2009 and 2010, and liver contaminant content was determined in 2010 in males. Fish length, weight, condition factor, gonadosomatic index and hematocrit were measured for males and females in 2009, and liver and gonad tissue were collected from males for histological analyses. In 2010, additional information collected included a general assessment of

the external condition and histopathological data for spleen, kidney and gills. Multivariate analyses (MANOVA, DFA) were used to determine overall differences in health condition of males and females, and in male liver contaminant content. In addition, Canonical Correlation Analysis was used to determine if the variability of biological traits in males can be explained by the variability observed in hepatic contaminant content. Presence of external lesions and parasites were evaluated using chi-square analysis. In a separate analysis, the severity of gill pathologies was ranked and differences among collecting sites were estimated using Kruskal-Wallis test and the incidence of the pathologies and their dependence from sampling site was determined with chi-square analysis. Potential associations between gill pathologies and the contaminants discriminating among sites were evaluated using pairwise Spearman correlation analysis. The results of MANOVA showed no significant differences in biological traits in 2009 males or females. However, in 2010, when more variables were analyzed, males differed among sites mostly in regards to kidney and spleen histopathologies and females could be separated by site primarily on the basis of kidney and liver histopathologies. Kidney pathologies were generally more severe in the downstream sites. The liver contaminant content of males also differed significantly among sites, with higher concentrations observed downstream; hexachlorobenzene (HCB) and *dimethyl tetrachloroterephthalate* (DCPA) contributed the most to the site discrimination ($p < 0.05$). However, despite the site discrimination of males by health condition and by liver contaminant profiles, results of the canonical correlation analysis suggested that these two variable sets are not associated with each other. Also, pairwise Spearman correlation analyses between gill histopathologies and

HCB and DCPA were not significant. However, some of the health condition variables seemed to differ among sampling sites according to the up-to-downstream contaminant gradient. For example, the incidence of external (skin) and gill parasites –a possible indication of impaired health– was higher in downstream sites, suggesting that fish on these sites are experiencing a higher level of stress possibly due to the higher loads of contaminants. Overall, the results of this study indicate that although the health of largescale sucker populations differ along the lower Columbia River Basin, specific factors other than those examined in the present study may be responsible for these differences.

Introduction

The Columbia River is the fourth largest river in the United States (US), draining an area of approximately 670,800 km², from Canada to the northwest of the US. The river and its tributaries provide transportation, irrigation, hydroelectric power, recreation, and food, representing a valuable resource for the economy of the area (Hinck et al. 2006). Unfortunately, many activities that are harmful to the environment have also taken place in this river system, such as mining, timber, industrial discharges, agriculture and urban runoff. These various activities have led to a continuing environmental degradation, threatening several species and jeopardizing the health of human populations (Henny et al. 2008a).

A number of studies have been conducted to determine the presence of contaminants in water and biota of the Columbia River (Hinck et al. 2006), but how mixtures of contaminants present in the river might impact the health of fish

populations is still poorly understood. One teleost species that has been used in a number of monitoring studies (Hinck et al. 2006), and also in studies to assess the exposure of fish-eating birds to contaminants (Henny et al. 2003) is largescale sucker (*Catostomus macrocheilus*, Catostomidae). Largescale sucker occur in most freshwater bodies on the west of the Rocky Mountains, from British Columbia (Canada) to Oregon (US) (Scott and Crossman 1973). They reside mainly on the bottom of rivers and lakes, feeding on zooplankton and periphyton as juveniles and becoming opportunistic omnivores as adults, when their diet shifts to aquatic insects, gastropods, crayfish and small fish. Sexual dimorphism is apparent during the breeding season as males develop nuptial tubercles on the rays of the anal and caudal fins. Largescale sucker reach sexual maturation at 5 to 7 years and have a life span of approximately 15 years. This species also constitutes a vital element on the diet of several fish species (Gray et al. 1984; Nigro et al. 1983) and birds (Fitzner and Hanson 1979; Henny et al. 2003), being a key component of the nutrient cycling (Schmetterling and McFee 2006). In addition, due to their intimate contact with the sediment, largescale sucker are in continuous contact with organic xenobiotics contained in the sediment and thus are good sentinels for the presence of contaminants and their dynamics in the ecosystem.

The objectives of the present study are to (1) characterize the health condition of largescale sucker collected along a contaminant gradient in the lower Columbia River based primarily on a histopathological approach and with emphasis on males; (2) determine if the health condition differs among collection sites; and, (3) determine if the health condition of male fish is associated with liver contaminant content. Three

sampling sites were chosen: Skamania (SK), downstream of Bonneville Dam; Columbia City (CC) downstream of Portland and Vancouver; and Longview (LV), downstream of CC. Passive sampler surveys have shown that the concentration of most contaminants increase in a downstream fashion (Alvarez 2010, unpublished data), providing us with the opportunity to observe potential differences in health condition, based on differences in contaminant exposures. This study focused on organochlorine pesticides, PCBs and PBDEs, contaminants persistent in the environment that have been reported in sediment and fish from the area, representing agricultural, industrial and urban inputs.

Histopathology was used because it is considered a strong tool to evaluate toxicity in fish, since it directly reflects the health status of the individuals (Stentiford et al. 2003). Previous studies have demonstrated that gender plays a significant role in the response to certain contaminants (Sharma and Patiño 2010); particularly, studies in fish have reported impairments in males, but not in females, after exposure to PBDEs (Lema et al. 2008; Muirhead et al. 2006; Torres, see Chapter II), reason for which our attention was centered in males.

This study is part of a larger multidisciplinary project to characterize the level of contaminants in the lower Columbia River Basin and how these contaminants are distributed in and affect the different components of the ecosystem, including water, sediment, macroinvertebrates, fish and piscivorous birds.

Use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Methods

Study area

Three sites were sampled on the Columbia River Basin: Skamania (SK), located just downstream of Bonneville Dam (45°32'41.67" N, 122°14'55.73" W; river mile 140); Columbia City site (CC), downstream of Portland, between St. Helens and Columbia City (45° 55' 11.8" N, 122° 48'44.4" W; river mile 82); and Longview (LV), downstream of CC in the Port of Longview (46° 5' 55" N, 122° 56'11" W; river mile 66) (Figure 3.1). See Chapter III.

Fish sampling

Protocols for the use of animals in this study were reviewed and approved by the Texas Tech University Animal Care and Use Committee (Lubbock, TX, USA). Largescale sucker were sampled by electroshocking on 4-7 May, 2009, and on 3-5 May, 2010. In 2009, 26 individuals (16 males and 10 females) were sampled in SK, 26 (15 males and 11 females) in CC, and 26 (15 males, 11 females) in LV; in 2010, 24 individuals (15 males, 11 females) were sampled in SK, 24 (14 males and 10 females) in CC, and 23 in LV (15 males and 8 females). Fish were euthanized using MS-222 (tricaine methane sulphonate; Sigma-Aldrich, Cat. No. E10521, St. Louis, MO, US). Body weight, body length and gonad weight were recorded. Fulton's condition factor ($\text{body weight}/\text{total length}^3$) and gonadosomatic index (GSI, $[\text{gonad weight}/\text{total body weight}] \times 100$) were calculated. The hematocrit (Hct, %) was determined using heparinized capillary tubes in a microhematocrit centrifuge.

In 2009, gonad and liver tissues were collected from all males and only few females at each site. In 2010, gonad, liver, gill, spleen and kidney tissues were collected from all males and females at each site. In addition, external characteristics and pathologies were also recorded in 2010, including nuptial tubercles, external lesions, opercula shortening and presence of external parasites. All tissues intended for histological analyses were placed in 10% buffered formalin (Fisher Diagnostics, Cat. No. 23-245-685, Waltham, MA, US).

Histological analyses

All tissues were rinsed in 70% ethanol, processed and embedded in paraffin following standard procedures (Luna, 1992). Gill arches were treated differently, as they were additionally placed in a decalcifying agent (Cal-Ex, Fisher Scientific, Cat. No. 6381-92-6, Waltham, MA, US) and in Bouin's fixative (Ricca Chemical, Cat. No. 1120-16, Arlington, TX, US) prior to dehydration and embedding in paraffin. Tissues were sectioned to 7 μm thickness, except for gills (5 μm). All tissues were stained with hematoxylin and eosin; additionally, sections of testis, kidney and liver were stained with Periodic Acid Schiff (PAS) (Sigma-Aldrich, Cat. No. 395B). Liver was also processed using PAS with α -amylase digestion (Sigma-Aldrich, Cat. No. A3176) for characterization of glycogen accumulation.

Digital images of the different tissues were taken with an Olympus digital camera (DP10; Tokyo, Japan) attached to a compound microscope. All measurements were conducted digitally using Image-Pro Express Software (Media Cybernetics, Silver Spring, MD, USA). The number and total area of macrophage aggregates (MA)

in PAS-stained sections were quantified using digital images, and the percentage of tissue in an image occupied by MA was calculated based on these values. Two 50X-images of spleen and kidney, four 50X-images of liver, and two 25X-images of testes tissues, per sample, were used for MA analyses.

The accumulation of glycogen and lipid in liver were assessed semi-quantitatively and ranked as: 1 = minimal, 2 = moderate, and 3 = high. The diagnostic of glycogen deposits in hepatocytes was based on the comparison between two consecutive sections of liver tissue, one stained with PAS and the other treated with α -amylase prior to PAS staining. Lipid deposits were identified as round and well-delineated empty spaces in hepatocytes of PAS-stained sections. In addition, liver tissues were analyzed for other pathologies including necrosis, pyknotic nuclei and neoplasm.

In kidney, two 50X images per sample were used to measure the total nephron and interstitial areas, then the areas that presented any abnormalities were measured, and the percentage of the affected area was calculated per sample. Nephron pathologies included, glomerulus abnormalities (i.e., inflammation with reduced Bowman's space, increased eosinophilic deposition on the glomerular capillaries, and hypertrophy of the epithelium of the Bowman's capsule); and for tubules: hyperplasia, degeneration (i.e., pyknotic nuclei, necrosis and or edema), hyaline droplet deposition, and tubule lumen filled with PAS-positive content. Interstitial inflammation was defined by the presence of PAS-positive neutrophil gatherings.

In gills, five consecutive primary-lamellae per fish were randomly chosen for analysis. The number of parasites found in each primary lamellae was transformed

into a numerical score, which was added to the gill health index. The parasite score ranged from 0 to 3, being 0 = none, 1 = minimal (1 to 2 parasites), 2 = moderate (3 to 5 parasites), 3 = high (> 5 parasites). Secondary-lamellae were also examined for the incidence of fusion, bifurcation, vascular congestion, epithelial rupture, epithelial lifting, presence of mucus cells, lamellar blood sinus dilation, leukocyte infiltration, hypertrophy, hyperplasia and lamellar aneurism (as described in Mallatt 1985, Figure 4.1). Each pathology was scored according to its severity on a scale of 0 to 3; being 0 = no incidence, 1 = mild, 2 = moderate, and 3 = high. The scores for each pathology were added to form a gill health index, which was used for statistical analyses. The overall health index ranged from 0 to 36, being 0 = normal, 1 – 16 = mild, 17 – 26 = moderate, and 27 to 36 = high.

In testes, in addition to MA analyses, the thickness of the germinal epithelium and the spawning status were evaluated. Spawning status was classified into three stages: *ripe*, lobules full or almost full with sperm; *partially-spent*, lobules partially filled with sperm; and *spent*, lobules only with residual sperm, partially collapsed. A few *undeveloped* testes were observed, which contained only spermatogonia, potentially some spermatocytes, and undefined lobules. Because this study is focused on adult fish, *undeveloped* individuals were not included in any statistical analysis. Ovaries were not analyzed.

Liver contaminant content

Please refer to Chapter III.

Data analyses

Multivariate analysis of variance (MANOVA) was used to determine overall site differences in continuous variables for thyroid condition and whether differences occurred between SK fish (reference site) and the downstream fish. A significant MANOVA was followed by a Discriminant Function Analysis (DFA), which allows identifying the variables that best explain group differences among the three collection sites. Canonical Correlation Analysis (CANCOR) was performed to determine if the variability of biological traits in males is associated with the contaminants present in the liver. Detailed information about these analyses is provided in the *Data Analyses* section of Chapter III.

Multivariate methods have shown to be quite robust to slight deviations from the parametric assumptions (McGarigal et al. 2000); however, in order to make sure that different-scale variables contribute equally to the multivariate analysis, measurement values, ratios and proportions were log-transformed; whereas non-parametric variables (ranks) were standardized (z-score, $\mu=0$, $\sigma=1$). The only two non-parametric variables, included along with other liver parametric variables, were liver lipid and glycogen deposition. Additional 12 non-parametric (ranks) variables describing gill histopathology were not included in the multivariate analyses and were, rather, analyzed independently using Kruskal-Wallis test to determine if significant differences occurred among sites, and pairwise Spearman correlation analyses to evaluate potential associations between gill variables and the contaminants selected in the DFA. We used false discovery rate (FDR) to control error rates in these multiple comparison and correlation analyses. The FDR is the proportion of false positives out

of all rejected null hypotheses, as opposed to the probability of committing a single Type I error (Benjamini and Hochberg 1995; Storey 2002; Storey et al. 2004). This analysis yields “*q*-values” that are considered the minimum FDR at which the statistic can be called significant and are interpreted similarly to the analogous *p*-value (Storey 2003).

The prevalence of binary and rank variables was analyzed using contingency tables Chi-square test; these variables included presence/absence of nuptial tubercles, shortening of the opercula, external lesions, external parasites and gill histopathological variables. Missing values were calculated via expectation-maximization (EM) algorithm before analyses were performed (4 out of 149 samples presented variables with missing values) (Strauss 2010). Because of known physiological differences, males and females were analyzed separately.

SAS software, Version 9.2 (SAS Institute Inc. SAS/STAT[®], Cary, NC, USA), was used for the MANOVA, DFA and Kruskal-Wallis tests. Chi-square contingency table and Spearman’s correlation analysis was done using GraphPad Prism, Version 5 (GraphPad Software, La Jolla, CA, USA). Missing values were calculated with the function MissEM (Strauss 2010) in Matlab[®] (Version 6.0.0.88). The α level of significance was ≤ 0.05 .

Results

External appearance and general fish condition

Nuptial tubercles were present in all males except for two individuals in LV, one defined as undeveloped (and thus not used in statistical analyses) and the other as

spent after histological diagnostic of the gonads. In females, nuptial tubercles normally do not develop; however, two females, one from SK and one from LV presented these structures. Shortening of the opercula was only observed in individuals from LV, and in males its presence was associated with collection site (X^2 (2, N = 44) = 6.22, $p = 0.04$), but not in females ($p > 0.05$). External lesions were common in individuals of both sexes at all sites. Presence of external parasites was not associated with collection site in either males or females ($p > 0.05$), however, parasites were observed in CC and LV, but not in SK (Table 4.1).

Males collected at CC in 2009 had lower standard length and weight than males from the other two sites (Table 4.2), a trend that was not repeated in 2010 (Table 4.1). Females were of similar length among sites on both years. Fulton's condition factor was similar among sites for males and females.

Gonads

In 2009, male GSI showed a trend to decrease in a downstream order, with higher values observed at SK. The thickness of the testicular germinal epithelium showed the opposite trend, with the highest values observed at LV. In addition, most males from SK and CC were ripe, while LV presented several spent males. These data suggest that the lower GSI in LV was primarily due to a more advanced stage of spawning activity and not to differences in male reproductive development (Table 4.2). In 2010, most of the males at LV and SK were ripe and had relatively high GSI, whereas most of the males in CC were spent and had lower GSI. The thickness of germinal epithelium also varied according to spawning activity status, indicating the same relationship among these variables that was observed in 2009 (Table 4.1). No

unusual histopathological abnormalities were found in testes. The percentage of tissue occupied by MA and MA density (#MA/area) did not differ among sites in any year. Large MA ($> 2,000 \mu\text{m}^2$) were only observed in fish from CC in 2010. In females, differences in GSI among sites were not observed (Table 4.1).

Liver

Glycogen accumulation in male liver was mostly moderate and did not differ among sites in any year. Lipid accumulation was higher in males from SK collected in 2009 but no differences among sites were observed in 2010. Female livers collected on 2009 were not included in any analyses due to the small sampling size. Liver lipid accumulation in females collected in 2010 varied from low (LV) to moderate; whereas glycogen accumulation was primarily moderate at all sites. The percentage of liver tissue occupied by MA and MA density did not differ among sites in any year for males or in 2010 for females. Large individual MAs ($> 5,000 \mu\text{m}^2$) were observed in males from CC and LV in 2009, but not in 2010 at any site. In females, large MAs were observed only at LV in 2010 (Tables 4.1 and 4.2). Other pathologies observed in liver included the presence of necrosis, pyknotic nuclei and foci of basophilic cellular alterations. These were observed in few fish at each site, except for pyknotic nuclei which were more frequent in males from SK (7 out of 15 males) in 2009 (data not shown).

Kidney and spleen

Males from CC presented a higher percentage of tubular-area affected by hyperplasia (Figure 4.2), but also a lower percentage of tubular-area undergoing degeneration (Figure 4.3). In females, a higher percentage of tubular-area affected by

hyaline depositions was observed in LV (Figure 4.4), but the percentage of tubular-area presenting degeneration was higher in SK. No differences were observed in the percentage of glomerulus abnormalities among sites, however, they seemed to be more frequent in males than in females (Table 4.2); the most common glomerular pathology observed was a reduction of the Bowman's space (Figure 4.5). The percent area of the kidney tissue occupied by MA and MA density did not differ among sites in males or females. In the spleen, males from CC had higher density of MA but no site differences were observed in females. The percent area occupied of splenic tissue occupied by MAs did not differ among sites in any gender (Figure 4.6) (Table 4.1). Large splenic MAs ($>40,000 \mu\text{m}^2$) were found only in few males from SK.

Gills

In males, vascular congestion and blood sinus dilation of the secondary lamellae were observed in most of the fish from all three sites (Figure 4.7); however, blood sinus dilation was significantly higher in males from SK compared to fish from CC ($q<0.05$) (Table 4.3). Significant differences among sites were not observed in any other variable. Most of the pathologies were common in all sites (Table 4.4), except for lamellar aneurism that was present in only 20% of the fish from LV, 40% in SK and 64% of the fish from CC, however there was not a significant association between these values and collection site ($p>0.05$). In females, gill pathologies were common in most fish from the three sites, except for epithelial rupture and lamellar aneurism that had a highest incidence of 40% in fish from CC (Table 4.4). No significant differences in any gill pathology were observed among sites in females.

None of the pathologies described were associated to collection site in either males or females.

Parasites on primary lamellae corresponded to cysts - metacercaria- of digeneans (Trematode) (Figure 4.8). Significant differences among sites were not observed; however, the severity of infestation appeared to be higher in LV for males and females, with the lowest values found in SK (Table 4.3). The incidence of parasites, however, was significantly associated to collection site in both, males (X^2 (2, N = 44) = 8.66, $p = 0.01$) and females (X^2 (2, N = 29) = 6.13, $p = 0.04$), being higher the number of affected males in LV and affected females in CC (Table 4.4). A second type of parasite was observed on the surface of secondary lamellae, a suckorian ciliate (*Capriniana* sp., protozoan), they were observed only in two male individuals, one from CC (120 individuals) and one from LV (one individual), these data were not included in the statistical analysis.

Spearman's correlation analyses between male gill variables and the contaminants analyzed in liver were not significant for any pairwise combination ($q > 0.05$) (data not shown).

Contaminants in male liver

Fish from LV presented the highest concentrations of DCPA, *p,p'*-DDE, HCB and some PCBs and PBDEs. Similarly, CC presented mean higher values for some PCBs and PBDEs. The geometric means of all the contaminants measured in males from SK were consistently lower than the ones observed at the other two sites. These data indicated that the contaminant content of fish liver in the Lower Columbia River Basin increased in a downstream manner (Table 4.5). Liver composite contaminant

concentration in males collected in 2009 fell within the range of concentrations observed in 2010, and followed the same downstream tendency (Table 4.5).

MANOVA and DFA results

Males

In 2009, biological traits did not differ among sites (MANOVA Wilks' $\lambda = 0.438$; $F(20, 66) = 1.69$; $p = 0.06$), or between SK and downstream sites (Wilks' $\lambda = 0.656$; $F(10,33) = 1.73$; $p = 0.11$). The partial correlation matrix of the sums of squares and cross products confirmed the negative association between GSI and the thickness of the testicular germinal epithelium mentioned previously. GSI was also negatively associated with percentage of testicular area occupied by MA. In addition, the area occupied by MA and MA density were positively associated in testes and liver. Liver lipid deposition showed positive relationships with condition factor, GSI, area occupied by MA in liver and liver glycogen deposition (Table 4.6).

In 2010, biological traits differed significantly among sites (MANOVA Wilks' $\lambda = 0.11$; $F(38, 44) = 2.28$; $p = 0.046$), but not among fish from SK and downstream fish (Wilks' $\lambda = 0.511$; $F(19,22) = 1.11$; $p = 0.41$). The partial correlation matrix of the sums of squares and cross products showed the same type of association between GSI and thickness of the testicular germinal epithelium observed in 2009. Similarly, associations among liver and testes MA, and between liver lipid deposition with liver glycogen and GSI were observed again in 2010. Interestingly, the area occupied by MA and their density were positively associated among the four different tissues where they were measured: testes, liver, gonads and kidney. Glomerular

abnormalities were associated to condition factor, GSI, thickness of the testicular epithelia and liver MA densities (Table 4.7)

The stepwise DFA resulted in the selection of five variables: liver MA density, tubular degeneration, tubular hyperplasia, glomerulus abnormality and spleen MA density. The DFA led to a significant model (Wilks' $\lambda = 0.30$; $F(10,72) = 5.87$; $p < 0.0001$) with one significant canonical function bearing a 1.69 eigenvalue. Tubular hyperplasia had the highest structure coefficient (-0.55), followed by tubular degeneration (0.54) and spleen MA density (-0.41). The degree of group discrimination achieved by the canonical functions was 79%. Mahalanobis distances between SK and LV are small, whereas LV and CC seemed to be more separated (Table 4.8). This is clear in the biplot, where exist some overlap between LV and SK, whereas LV and CC are clearly separated mainly by renal variables (function 1) (Figure 4.9).

In 2010, the liver contaminant concentrations differed significantly among sites (Wilks' $\lambda = 0.18$; $F(30, 50) = 2.26$; $p = 0.005$), and between fish from SK and the downstream sites (Wilks' $\lambda = 0.30$; $F(15, 25) = 3.90$; $p = 0.001$). The partial correlation matrix showed positive pairwise associations between most contaminants (Table 4.9). The stepwise DFA analysis resulted in the selection of two variables for the model, DCPA and HCB. The model was significant (Wilks' $\lambda = 0.59$; $F(4,76) = 5.80$; $p = 0.0004$) and contained two significant canonical functions bearing eigenvalues of 0.49 and 0.14, respectively. HCB had the highest structure coefficient (0.81) on the first function; whereas in the second canonical function, DCPA had the highest coefficient (0.98). The overall classification of samples based on their

contaminant composition was 64% which, although relatively low, it seems reasonable for descriptive purposes. The biplot shows a high degree of overlap of the three sites (Figure 4.10), Mahalanobis distances, however, help elucidate that the larger distances occurred between SK and LV, whereas LV and CC were much closer (Table 4.8).

Females

In 2009, biological traits did not differ among sites (MANOVA Wilks' $\lambda = 0.90$; $F(6, 54) = 0.49$; $p = 0.81$), or between SK and downstream sites (Wilks' $\lambda = 0.91$; $F(3,27) = 0.93$; $p = 0.44$). The partial correlation matrix of the sums of squares and cross products showed that, as expected, condition factor and GSI were positively associated (Table 4.10).

In 2010, biological traits differed significantly among sites (MANOVA Wilks' $\lambda = 0.05$; $F(32, 22) = 2.35$; $p = 0.02$), and between fish from SK and downstream fish (Wilks' $\lambda = 0.18$; $F(16,11) = 3.12$; $p = 0.03$). The partial correlation matrix of the sums of squares and cross products showed that GSI was negatively associated with the degree of deposition of lipid and glycogen in the liver. A positive association among the area occupied by MA and MA density of liver and spleen, but not kidney was observed (Table 4.11).

The stepwise analysis resulted in the selection of six variables: liver glycogen accumulation, liver MA density, tubular degeneration, tubular hyaline deposition, spleen MA density and spleen MA area. The DFA led to a significant model (Wilks' $\lambda = 0.16$; $F(12,42) = 5.38$; $p < 0.0001$) with two significant canonical functions bearing an eigenvalues of 2.59 and 0.79, respectively. Tubular degeneration had the highest structure coefficient (0.47) on the first function; whereas tubular hyaline

deposition had the highest structure coefficient (-0.39), followed by liver MA density (-0.37) on the second function. The degree of group discrimination achieved by the canonical functions was of 85%. SK is clearly separated from the downstream sites (Table 4.8) mainly on the basis of tubular degeneration (function 1), whereas CC and LV are separated by tubular hyaline deposition and liver MA density (function 2) (Figure 4.11).

CANCOR results

Only contaminant or biological variables with factor structure coefficients $\geq |0.3|$ in their respective DFAs were used in CANCOR in order to limit the number of variables and reduce complications due to low sample-to-variable ratio. The selected variables thus were HCB and DCPA for contaminants, and tubular degeneration, tubular hyperplasia and spleen MA density. No significant association between the two canonical variable sets was observed (Wilks' $\lambda = 0.72$; $F(6,74) = 2.18$; $p = 0.0547$). In other words, the variation in the biological traits was not significantly associated with the concentration of the selected contaminants in male livers. Pairwise Pearson's correlation coefficients show a lack of association between HCB and any of the selected biological variables (Table 4.12).

Discussion

The health condition of largescale sucker from the Lower Columbia River Basin could be generally separated by collection site by DFA. In 2009, health condition differences were not observed among sites in males or females. In 2010,

when a higher number of biological variables were included in the analysis, males differed among sites mostly in terms of kidney pathologies followed by spleen MA density; whereas females could be separated primarily on the basis of kidney histopathology, followed by liver MA density. Liver contaminant content of male largescale sucker collected in 2010 was also statistically separated by collecting site, being the organochlorines HCB and DCPA, the factors better explaining the separation. However, despite the site discrimination of males by health condition and by liver contaminant profiles results of the CANCOR analyses suggested that these two variable sets are not associated with each other.

Variation in GSI among males was attributed to differences in spawning activity; namely, GSI seem to be associated with the “spent” condition of the testes – the post-spawning content of testicular sperm combined with the thickness of lobular walls. In 2009, LV males had lower testicular GSI and higher levels of spent testes than SK and CC males; whereas in 2010, CC males had the lower GSI and higher incidence of spent condition. No clear trends were apparent for female fish. Largescale sucker for this study were sampled during their spawning season, which can last several months (Dauble, 1986). However, little is known about spawning strategies of this species such as the number of spawning events at the level of individual fish in a single season. Thus, GSI measurements must be interpreted with caution. Given that the larger number of variables measured in 2010 led to a significant site separation, data interpretation will be mainly focused from this point forward on the results obtained in 2010, unless specified otherwise.

A general assessment of the external fish condition showed that, even though external lesions were common in individuals of both genders at all sites, external (skin) parasites and shortening of the opercula were restricted to individuals from the downstream sites. A similar trend was observed in gill parasites, which were more frequently found in fish (male and female) from CC and LV. The presence of parasites is generally an indication of reduced health of the host (Noga 2000); whereas shortening of the opercula might be a consequence of diet unbalance and/or exposure to contaminants during early developmental stages (Beraldo 2003).

In 2010, overall renal condition contributed the most to the strength of the discrimination of male and female fish by site, followed by spleen MA density in males, and liver MA density in females. Tubular hyperplasia and tubular degeneration were the primary renal pathologies in males, and tubular degeneration and tubular hyaline deposition in females. Kidneys from CC males presented tubules with higher hyperplasia but lower degeneration percentage than the fish from SK and LV; the latter two, on the other hand, differed mainly in terms of hyperplasia, with SK presenting the lowest values. Females from SK presented a higher degree of tubular degeneration, and females from CC and LV were more affected by tubular hyaline deposition. Exposure to toxicants, particularly organochlorine pesticides, has been associated with the appearance of tubular degeneration and other kidney pathologies (Ayas et al. 2007), for instance, Costa et al. (2010) reported renal and gill lesions in flatfish (*Solea senegalensis*) after exposure to sediments containing organic xenobiotics, attributing the damage mainly to PCBs and PAHs. The higher degree of tubular degeneration observed in fish from SK (the reference site), indicates that there

are other potential stressors, not measured in the present study, that are affecting the SK population. Such stressors are more likely to come from upstream SK, since the sampled area does not apparently present point sources of pollution (Morace 2011, personal communication).

A second factor contributing to the separation of males and females by collecting sites was the density of MA in spleen (males) and in liver (females). Increased MA size and density have been commonly used as robust indicators of fish exposure to degraded environments (Fournie et al. 2005; Micale and Perdicchizzi 1990). In the present study, the density of MA in spleen and liver of largescale sucker was higher in CC, followed by LV; in addition, it was shown that the area and size of MA were positively associated among tissues (testes, liver, spleen and kidney), suggesting that the fish from downstream sites might be under potentially more stressful conditions than the fish from SK.

The gill epithelium is the main route of exposure to soluble xenobiotics; therefore, histopathological changes indicate potential exposure to toxicants and other stressors and can potentially affect gill function (Mallat 1985). In the present study, all fish presented certain degree of gill pathology; however, significant differences were observed in the degree of blood sinus dilation, higher in males from SK. Blood sinus dilation is considered part of inflammatory responses against environmental stressors (Mallat 1985). These data concur with the previously mentioned higher degree of tubular degeneration observed in kidney at SK, suggesting that other stressors - potentially targeting the osmotic balance of fish- are present in SK.

Male largescale sucker were statistically separated by collecting site on the basis of liver concentrations of the organochlorines HCB and DCPA. Organochlorine pesticides and PCBs have been decreasing in the Pacific Northwest since the 1970's (Rayne et al., 2003; Henny et al. 2008, 2009); however, they are still found in wildlife, fish and sediments sometimes at high concentrations (Henny et al. 2008b; Hinck et al. 2006; Johnson et al. 2007). HCB concentration increased downstream, with the highest mean concentration found in fish from LV, whereas mean concentrations of DCPA did not differ among sites (Table 4.5). The production of HCB, mainly used as pesticide, ended in the late 1970's; however, today it can be indirectly produced as by product of other chlorinated compounds such as DCPA.

Hinck et al. (2006) analyzed contaminants in largescale sucker whole body composites along the Columbia River, in 1997 and 1998. They focused in elemental contaminants, PCBs and *p,p'*-DDE. HCB was not analyzed. They observed an increasing trend in total PCBs concentrations in a downstream direction. The congeners they measured, however, were not mentioned in their study. Hinck et al. (2006) also assessed the fish condition, focusing in the histological analyses of suspicious lesions, nodules or discolorations in liver, kidney and spleen, concluding that those were inflammatory responses associated with parasites. Splenic MA were also analyzed, MA area and density differed among sites, although a downstream trend was not observed (Hinck et al., 2006).

With the goal of analyzing bioaccumulation patterns in ospreys nesting along the Willamette River, a major tributary of the lower Columbia River, Henny et al. (2009) measured the concentration of several contaminants in three fish species—

including largescale sucker. The analyses were conducted with whole-body composites and included several contaminants in common with the present study, such as *p,p'*-DDE, HCB and several PCBs. They compared data from 1993 and 2001, and their results showed a decreasing trend in contaminants concentration during that period of time. Health condition of the fish was not assessed in this study.

PBDEs concentrations have followed a different trend than PCBs and other banned organochlorines. Whereas organochlorines have been declining for the last four decades, PBDEs have until recently an increasing, particularly in biota of the Columbia River (Rayne et al. 2003). After the banning of the penta-brominated congeners, the levels of PBDE are apparently starting to go down (Henny et al. 2011). In the present study, BDE-47 (tetra-congener) and BDE-153 (hexa-congener) increased in a downstream fashion, with the highest values observed in LV (See Chapter III).

The higher contaminant concentrations in CC and LV are a consequence of these sites being downstream of the cities of Portland and Vancouver, important sources of persistent organic pollutants (Johnson et al. 2007). In addition, CC receives the input from the Multnomah Channel, which branches off the Willamette River. The Willamette River not only flows through the three largest cities in Oregon (Eugene, Salem and Portland), but it also is bordered by 89% of the agricultural land of the lower Columbia River Basin; in addition, most of the industrial and irrigation withdrawals in the area occur in the Willamette River Basin (Fuhrer et al. 1996). Consequently, the contribution of the Willamette River in terms of nutrients, pesticides and urban contaminants is significant. Similarly, LV have several inputs of

contamination, such as a considerable effluent discharge from the local wastewater-treatment plants (Three River Regional and City of Rainer), and the Reynolds Aluminum Smelter (now Alcoa). In addition, CC and LV are also affected by effluents coming from the Chevron Chemical Company which produces fertilizers in the Saint Helens facility (Fuhrer et al. 1996).

Although significant differences among collection sites were observed in the liver contaminant content of males, those differences did not reflect what it would be expected to find given the various sources of contamination downstream; thus a further characterization of the different contaminants present in those areas is necessary. Also, despite SK being apparently not affected by considerable point sources of contamination, the fish presented similar pathologies than the fish downstream, although the degree of severity was generally lower, suggesting that important point sources of contamination need to be evaluated upstream SK. Largescale sucker is a good monitoring tool for contaminants in the area. The present study offers important reference information regarding the physiology of this species.

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Table 4.1. Biological variables measured in males and females collected in 2010 along the Lower Columbia River Basin

Biological traits	Skamania		Columbia City		Longview	
	Male	Female	Male	Female	Male	Female
(N)	(15)	(11)	(14)	(10)	(15)	(8)
Presence of tubercles	15	1	14	0	13	1
Shortening of opercula	0*	0	0*	0	3*	1
External lesions	11	10	11	8	12	7
External parasites	0	0	1	1	3	3
Length	428.3±11.4	476.4±6.1	404.6±8.0	457.0±12.3	420.0±10.4	460.6±16.0
Weight	779.7±61.3	1045.5±45.2	660.7±41.2	994.1±76.4	722.4±47.5	909.6±130.9
Condition Factor	0.96±0.02	0.96±0.02	0.98±0.02	1.02±0.03	0.95±0.02	0.9±0.09
Hematocrit	38.0±1.3	37.7±1.0	36.7±1.9	34.8±2.6	41.9±1.7	37.1±2.5
GSI	3.1±0.6	6.4±1.5	1.9±0.5	6.6±1.8	2.5±0.4	3.1±1.8
Testes						
Spawning status	1.4±0.1	NM	1.6±0.1	NM	1.1±0.1	NM
Thickness of germinal epithelium	12.7±2.0	.	13.9±1.8	.	9.9±2.0	.
MA area	0.02±0.01	.	0.1±0.1	.	0.02±0.01	.
MA density	6.2x10 ⁻⁷ ±0.3x10 ⁻⁸	.	2.2x10 ⁻⁶ ±9.2x10 ⁻⁷	.	7.4x10 ⁻⁷ ±5.4x10 ⁻⁷	.
Liver						
MA area	0.8±0.4	0.6±0.2	0.6±0.2	0.8±0.3	1.1±0.4	0.7±0.2
MA density	3.8x10 ⁻⁵ ±8.6x10 ⁻⁶	3.4x10 ⁻⁵ ±4.4x10 ⁻⁶	2.5x10 ⁻⁵ ±5.9x10 ⁻⁶	7.1x10 ⁻⁵ ±2.7x10 ⁻⁵	3.4x10 ⁻⁵ ±3.7x10 ⁻⁶	3.2x10 ⁻⁵ ±7.6x10 ⁻⁶
Glycogen	2.3±0.2	2.4±0.2	2.3±0.1	1.8±0.2	1.9±0.2	2.1±0.2
Lipid	2.1±0.2	1.5±0.2	2.2±0.2	1.5±0.2	2.1±0.2	1.4±0.2

Mean ± SEM. NM not measured.

Table 4.1. (Continued) Biological variables measured in males and females collected in 2010 along the Lower Columbia River Basin

Biological traits	Skamania		Columbia City		Longview	
	Male	Female	Male	Female	Male	Female
Spleen						
MA area	2.3±0.5	5.2±1.1	2.8±0.5	2.2±0.7	2.4±0.5	4.3±1.5
MA density	$2.2 \times 10^{-5} \pm 2.6 \times 10^{-5}$	$2.9 \times 10^{-5} \pm 5.2 \times 10^{-6}$	$3.5 \times 10^{-5} \pm 4.7 \times 10^{-6}$	$2.5 \times 10^{-5} \pm 3.0 \times 10^{-6}$	$2.0 \times 10^{-5} \pm 1.8 \times 10^{-6}$	$3.2 \times 10^{-5} \pm 4.2 \times 10^{-6}$
Kidney						
% Kidney nephron affected	36.0±4.1	48.1±5.3	36.9±3.2	11.2±2.9	40.8±3.9	40.1±5.1
% Tubular hyperplasia	1.2±0.6	4.5±2.0	19.0±4.8	29.4±8.6	3.2±1.1	7.2±4.0
% Tubular hyaline deposition	22.8±5.7	9.0±4.3	36.4±8.1	22.5±8.2	20.3±5.4	27.7±8.9
% Tubular degeneration	69.4±5.2	81.6±4.3	35.2±8.3	34.1±4.2	67.8±5.7	39.2±12.2
% Glomerulus abnormalities	6.1±2.9	4.8±1.6	7.4±3.5	7.3±2.7	8.8±1.7	3.2±0.5
% Kidney interstitium affected	7.9±1.8	17.5±1.5	10.1±2.2	13.2±3.4	8.2±1.4	12.9±2.8
MA area	1.6±0.3	1.3±0.2	2.1±0.4	1.4±0.4	1.6±0.3	1.4±0.3
MA density	$1.1 \times 10^{-5} \pm 1.4 \times 10^{-6}$	$6.1 \times 10^{-6} \pm 1.1 \times 10^{-6}$	$1.0 \times 10^{-5} \pm 1.1 \times 10^{-6}$	$7.7 \times 10^{-6} \pm 1.5 \times 10^{-6}$	$7.9 \times 10^{-6} \pm 9.0 \times 10^{-7}$	$9.8 \times 10^{-6} \pm 1.9 \times 10^{-6}$

Mean ± SEM. NM not measured.

Table 4.2. Biological variables measured in males and females collected in 2009 along the Lower Columbia River Basin.

Biological traits	Skamania		Columbia City		Longview	
	Male	Female	Male	Female	Male	Female
(N)	(16)	(10)	(15)	(11)	(16)	(11)
Length	430.9±8.0	472.0±9.2	406.3±6.7	468.6±7.9	435.6±5.0	487.73±10.7
Weight	778.5±43.8	950.1±48.2	644.9±34.3	947.73±62.5	764.6±30.8	1079.5±80.7
Condition Factor	0.96±0.01	0.90±0.02	0.95±0.02	0.91±0.03	0.92±0.02	0.91±0.02
Hematocrit	31±1.9	28.8±2.5	29±1.4	31.8±1.2	32.7±1.4	30.9±1.4
GSI	3.7±0.4	3.9±1.4	3.1±0.5	5.1±1.4	2.0±0.3	6.2±1.8
Testes						
Spawning status	1±0.0	NM	1.1±0.1	NM	1.3±0.1	NM
Thickness of germinal epithelium	9.0±1.3	*	7.8±0.8	*	13.3±1.8	*
MA area	0.011±0.004	*	0.02±0.01	*	0.03±0.01	*
MA density	1.6x10 ⁻⁶ ±0.3x10 ⁻⁶	*	2.7x10 ⁻⁶ ±0.6x10 ⁻⁶	*	3.7x10 ⁻⁶ ±0.1x10 ⁻⁶	*
Liver						
MA area	0.4±0.1	NM	0.9±0.3	NM	3.1±1.6	NM
MA density	2.3x10 ⁻⁵ ±0.3x10 ⁻⁵	NM	3.0x10 ⁻⁵ ±0.6x10 ⁻⁵	NM	5.2x10 ⁻⁵ ±0.9x10 ⁻⁵	NM
Glycogen accumulation	1.8±0.2	NM	2.2±0.2	NM	1.6±0.2	NM
Lipid accumulation	2.5±0.2	NM	1.8±0.2	NM	1.8±0.2	NM

Mean ± SEM. NM not measured.

Table 4.3. Gill histopathological variables measured in males and females collected in 2010 along the Lower Columbia River Basin. Columns sharing the same letter are not significantly different, comparisons were made by gender ($\alpha = 0.05$).

Gill Histopathology	Skamania		Columbia City		Longview	
	Male	Female	Male	Female	Male	Female
Fused lamellae	0.35±0.41a	0.8±0.5a	0.6±0.6a	0.9±0.9a	0.4±0.4a	0.8±0.6a
Bifurcated lamellae	0.29±0.27a	0.7±0.5a	0.5±0.6a	0.9±0.9a	0.3±0.3a	0.6±0.6a
Vascular congestion	1.8±0.7a	2.0±0.5a	1.2±0.7a	1.9±0.8a	1.1±0.5a	1.6±0.8a
Epithelial rupture	0.4±0.7a	0.5±0.9a	0.4±0.4a	0.3±0.5a	0.2±0.3a	0.1±0.2a
Epithelial lifting	1.9±0.4a	1.7±0.5a	1.7±0.5a	1.9±0.4a	2.0±0.5a	1.8±0.8a
Mucus cell proliferation	2.4±0.4a	2.5±0.8a	2.0±0.8a	2.8±0.2a	2.0±0.8a	1.9±1.1a
Blood sinus dilated	1.9±0.6a	1.6±0.5a	1.1±0.7b	1.6±0.8a	1.4±0.5ab	1.5±0.8a
Leukocyte infiltration	1.4±0.9a	1.4±0.9a	1.2±0.8a	1.4±1.0a	1.4±1.0a	1.4±1.1a
Hypertrophy	1.7±0.7a	1.5±0.6a	1.2±0.5a	1.2±0.5a	1.7±0.8a	1.1±1.1a
Hyperplasia	1.8±0.5ab	2.3±0.8a	1.9±0.8a	2.1±0.7a	1.2±1.0b	1.5±0.7a
Aneurism	0.1±0.1ab	0.1±0.1a	0.2±0.3a	0.1±0.2a	0.1±0.1b	0a
Parasite cysts	0.3±0.5a	0.1±0.3a	0.7±1.0ab	0.7±0.9a	1.2±1.1b	0.8±0.7a

Table 4.4. Incidence of gill histopathological variables in largescale sucker collected in 2010 at the Lower Columbia River Basin. Data are presented in percentages.

	Skamania		Columbia City		Longview	
	Males	Females	Males	Females	Males	Females
Fusionated lamellae	67	100	86	80	60	100
Forked lamellae	73	100	86	90	67	75
Vascular congestion	100	100	93	100	93	100
Epithelial rupture	53	36	79	40	47	25
Epithelial lifting	100	100	100	100	100	100
Mucus cell proliferation	100	100	93	100	100	100
Blood sinus dilated	100	100	100	100	100	100
Leukocyte infiltration	93	100	93	90	93	88
Hypertrophy	100	91	100	100	100	75
Hyperplasia	100	100	100	100	93	100
Aneurism	40	27	64	40	20	0
Total parasites	27	27	57	80	80	63

Table 4.5. Geometric means (ranges) of contaminants concentrations ($\mu\text{g/kg}$, ww) male livers collected from the Lower Columbia River Basin in 2010.

Contaminants	Skamania		Columbia City		Longview	
	2009	2010	2009	2010	2009	2010
DCPA	0.9	1.8 (0.43 - 5.9)	0.7	1.0 (0.25 - 4.6)	1.4	1.9 (0.85 - 4.2)
<i>p,p'</i> -DDE	157	249.3 (4 - 1270)	221	368.8 (89 - 1340)	389	404.2 (63 - 1840)
HCB	1.4	1.1 (0.5 - 4.6)	2.9	2.2 (0.5 - 6.1)	3.9	3.8 (0.5 - 19)
PCB-138	6.4	6.3 (0.25 - 44)	9.7	10.3 (3.1 - 34)	11.8	11.7 (2.4 - 58)
PCB-146	0.9	2.7 (0.4 - 22)	3.3	5.0 (0.7 - 19)	8.5	4.5 (0.9 - 14)
PCB-170	1.3	2.9 (0.45 - 23)	6.8	6.0 (2 - 19)	8.9	5.8 (1.4 - 17)
PCB-174	0.1	1.4 (0.45 - 11)	3.1	2.7 (0.93 - 9.4)	5.5	2.9 (0.76 - 7.6)
PCB-180	2.7	5.5 (0.81 - 44)	11.8	10.3 (2.9 - 37)	18.9	9.9 (2.3 - 31)
PCB-183	0.8	1.7 (0.24 - 12)	3.3	3.0 (0.81 - 9.9)	4.8	2.9 (0.75 - 9)
PCB-187	2.3	5.3 (0.94 - 33)	7.8	8.6 (2.5 - 24)	17.2	8.2 (1.9 - 24)
PCB-194	0.4	1.0 (0.42 - 6.5)	2.3	1.7 (0.46 - 6.7)	3.4	1.5 (0.5 - 4.1)
BDE-47	16.2	43.1 (8.7 - 300)	75.8	68.3 (27 - 140)	161	96.6 (46 - 300)
BDE-100	3.9	8.0 (1.7 - 95)	26.8	13.4 (4.9 - 31)	41.6	16.8 (6.9 - 56)
BDE-153	0.2	0.6 (0.22 - 2.2)	1.5	0.8 (0.35 - 2.8)	2.7	1.3 (0.59 - 4.3)
BDE-154	0.7	1.5 (0.35 - 15)	4.6	3.3 (1.1 - 9.5)	8.7	3.2 (1.2 - 10)

Contaminants with more than 15% of non-detectable values are not included.

Table 4.6. Partial correlation matrix of the sums of squares and cross products obtained as part of the MANOVA test performed in males collected in 2009 from the Lower Columbia River Basin. * $p < 0.05$

Biological traits	Condition Factor	Hct	GSI	Testes epithelia	Testes MA area	Testes MA density	Liver MA area	Liver MA density	Liver glycogen
Hematocrit (Htc)	0.12								
GSI	0.65*	0.10							
Testes epithelia	-0.20	-0.19	-0.43*						
Testes MA area	-0.24	0.08	-0.35*	0.22					
Testes MA density	-0.24	-0.03	-0.26	0.29	0.78				
Liver MA area	-0.26	-0.27	-0.25	0.05	0.26	0.11			
Liver MA density	-0.22	-0.24	-0.28	0.08	0.24	0.20	0.74*		
Liver glycogen	0.04	-0.02	0.06	-0.03	-0.06	-0.09	-0.16	-0.17	
Liver lipid	0.31*	0.08	0.36*	0.02	-0.21	0.06	-0.37*	-0.30	-0.46*

Table 4.7. Partial correlation matrix of the sums of squares and cross products obtained as part of the MANOVA test performed in males collected in 2010 from the Lower Columbia River Basin. *p<0.05

	Condition factor	Hct	GSI	TE	TMAA	TMAD	LMAA	LMAD	LG	LL	SMAA	SMAD	TH	TD	KI	KMAA	KMAD	GA
Hematocrit (Hct)	-0.39*																	
GSI	0.49*	-0.15																
Testes epithelia (TE)	-0.55*	0.34*	-0.78*															
Testes MA area (TMAA)	-0.05	0.11	-0.38*	0.33*														
Testes MA density (TMAD)	-0.10	0.02	-0.43*	0.32*	0.79*													
Liver MA area (LMAA)	0.09	-0.01	-0.15	0.22	0.25	0.15												
Liver MA density (LMAD)	-0.22	0.22	-0.31*	0.39*	0.30	0.23	0.74*											
Liver glycogen (LG)	-0.09	-0.08	-0.24	0.16	0.10	0.14	0.12	0.08										
Liver lipid (LL)	0.40*	0.00	0.54*	-0.55*	-0.18	-0.18	0.12	-0.09	-0.38*									
Spleen MA area (SMAA)	0.10	-0.03	-0.15	0.17	0.45*	0.40*	0.24	0.21	0.05	-0.16								
Spleen MA density (SMAD)	-0.03	0.09	-0.10	0.23	0.48*	0.31*	0.33*	0.39*	0.11	-0.16	0.56*							
Tubular hyaline (TH)	-0.11	-0.02	-0.38*	0.24	0.09	0.25	0.02	0.06	0.29	-0.08	0.03	-0.21						
Tubular degeneration (TD)	-0.11	0.14	0.08	-0.06	0.03	0.00	0.03	0.01	0.01	0.07	-0.06	0.17	-0.60*					
Kidney interstitia (KI)	0.10	-0.01	-0.02	-0.01	-0.32*	-0.31*	0.05	-0.01	0.01	0.08	0.05	-0.07	0.19	-0.24				
Kidney MA area (KMAA)	0.26	-0.23	-0.11	0.06	0.31*	0.17	0.17	-0.07	0.21	0.03	0.32*	0.10	-0.08	0.05	-0.05			
Kidney MA density (KMAD)	0.26	-0.27	0.11	-0.17	0.06	0.13	0.10	-0.03	0.03	0.21	0.14	-0.11	-0.12	0.05	-0.05	0.74*		
Glomerular abnormality (GA)	0.43*	-0.19	0.33*	-0.34*	-0.04	-0.08	0.10	-0.31*	-0.07	0.11	0.00	-0.29	0.06	-0.24	0.00	0.10	0.11	
Tubular hyperplasia	-0.07	0.22	0.00	-0.02	0.30	0.21	0.26	0.31*	0.14	0.06	0.22	0.26	0.13	-0.15	-0.06	0.02	-0.12	-0.10

Table 4.8. Squared Mahalanobis distances between group (site) means for biological traits and male liver-contaminant concentration in largescale sucker collected from three sites in the Lower Columbia River Basin (SK, Skamania; CC, Columbia City; LV, Longview).

Biological traits males 2010			Biological traits females 2010			Liver contaminant content 2010		
Site	SK	CC	Site	SK	CC	Site	SK	CC
CC	6.6		CC	10.8		CC	2.1	
LV	1.4	8.2	LV	11.2	4.6	LV	2.3	0.8

Table 4.9. Partial correlation matrix of the sums of squares and cross products obtained as part of the MANOVA test performed for contaminants in the liver of male largescale sucker collected in 2009 from the Lower Columbia River Basin.

*p<0.05partial correlation contaminants

	DCPA	DDE	HCB	PCB-138	PCB-146	PCB-170	PCB-174	PCB-180	PCB-183	PCB-187	PCB-194	BDE-47	BDE-100	BDE-153
DDE	0.12													
HCB	0.39*	0.03												
PCB-138	0.21	0.30	0.24											
PCB-146	0.15	0.34*	0.34*	0.78*										
PCB-170	0.13	0.33*	0.27	0.78*	0.88*									
PCB-174	0.15	0.32*	0.38*	0.72*	0.89*	0.95*								
PCB-180	0.13	0.35*	0.29*	0.79*	0.92*	0.96*	0.94*							
PCB-183	0.15	0.36	0.31	0.78	0.92	0.95	0.95	0.99						
PCB-187	0.18	0.38	0.32	0.82	0.94	0.95	0.94	0.98	0.98					
PCB-194	0.07	0.35*	0.24	0.70*	0.85*	0.91*	0.92*	0.93*	0.92*	0.89*				
BDE-47	0.24	0.32*	0.43*	0.65*	0.79*	0.82*	0.84*	0.80*	0.81*	0.82*	0.70*			
BDE-100	0.15	0.32*	0.40*	0.70*	0.82*	0.90*	0.90*	0.86*	0.87*	0.88*	0.78*	0.95*		
BDE-153	0.24	0.37*	0.50*	0.38*	0.58*	0.63*	0.70*	0.62*	0.64*	0.61*	0.56*	0.84*	0.78*	
BDE-154	0.13	0.29	0.32*	0.69*	0.83*	0.89*	0.89*	0.90*	0.90*	0.87*	0.84*	0.91*	0.94*	0.77*

Tables 4.10. Partial correlation matrix of the sums of squares and cross products obtained as part of the MANOVA test performed in female largescale sucker collected in 2009 from the Lower Columbia River Basin. * $p < 0.05$

	Condition Factor	Hematocrit
Condition Factor	-0.19	
Hematocrit	0.65*	-0.01

Table 4.11. Partial correlation matrix of the sums of squares and cross products obtained as part of the MANOVA test performed in females collected in 2010 from the Lower Columbia River Basin. *p<0.05

	Condition factor	Hct	GSI	LMAA	LMAD	LG	LL	SMAA	SMAD	THp	TH	TD	KI	KMAA	KMAD
Hematocrit (Hct)	-0.40*														
GSI	0.18	-0.34													
Liver MA area (LMAA)	0.30	-0.30	-0.08												
Liver MA density (LMAD)	0.16	-0.21	0.07	0.86*											
Liver glycogen (LG)	-0.07	0.22	-0.43*	0.19	0.08										
Liver lipid (LL)	-0.30	0.23	-0.48*	-0.29	-0.33	0.53*									
Spleen MA area (SMAA)	0.12	-0.25	0.28	0.57*	0.57*	0.07	-0.31								
Spleen MA density (SMAD)	0.09	-0.18	0.15	0.37	0.62*	0.05	-0.38*	0.67*							
Tubular hyperplasia	0.27	0.14	0.15	0.16	0.10	0.23	-0.40*	0.21	0.23						
Tubular hyaline (TH)	-0.15	-0.22	-0.14	0.19	0.10	0.37	0.34	0.08	-0.21	-0.45*					
Tubular degeneration (TD)	0.10	-0.05	0.10	-0.20	-0.08	-0.45*	0.00	-0.35	-0.16	-0.48*	-0.19				
Kidney interstitia (KI)	-0.30	0.15	-0.32	-0.25	-0.17	0.07	0.40*	-0.38	-0.14	-0.29	-0.18	0.20			
Kidney MA area (KMAA)	0.19	-0.40*	0.51*	0.01	0.00	-0.36	-0.20	0.25	0.01	0.08	-0.02	-0.06	-0.40*		
Kidney MA density (KMAD)	0.30	-0.32	0.28	0.06	0.02	-0.20	-0.13	0.38	0.15	0.14	0.03	-0.28	-0.52*	0.70*	
Glomerular abnormality (GA)	-0.10	0.00	-0.17	-0.17	-0.14	-0.11	-0.18	-0.10	0.10	0.12	-0.27	-0.12	0.21	-0.18	-0.05

Table 4.12. Pairwise Pearson's correlation coefficients between biological variables and contaminants used in the CANCELL. *p<0.05

	Spleen MA	TD	TH	DCPA
Spleen MA				
TD	-0.14			
TH	0.44*	-0.44*		
DCPA	-0.43*	0.14	-0.33*	
HCB	-0.19	-0.12	0.11	0.31*

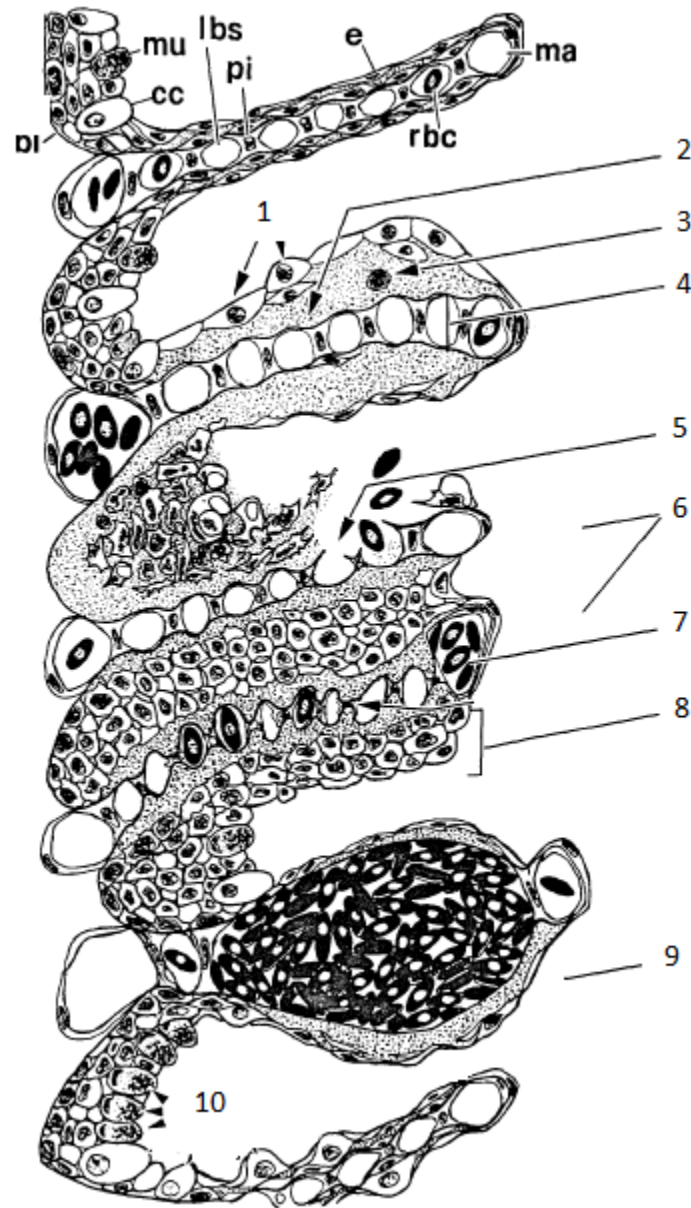


Figure 4.1. Composite diagram of common gill lesions. The lesions are numbered as: 1, hypertrophy; 2, epithelial lifting; 3, lymphocyte infiltration; 4, lamellar blood sinus dilation; 5, epithelial rupture; 6, lamellar fusion; 7, vascular congestion; 8, hyperplasia; 9, aneurism; 10, proliferation of mucus cells. Abbreviations: bl, basal lamina; cc, chloride cell; e, typical lamellar epithelial cells; lbs, lamellar blood sinus; ma, marginal blood channel; mu, mucus cell; pi, pillar cell; rbc, erythrocyte. (Reproduced with permission. Mallatt 1985).

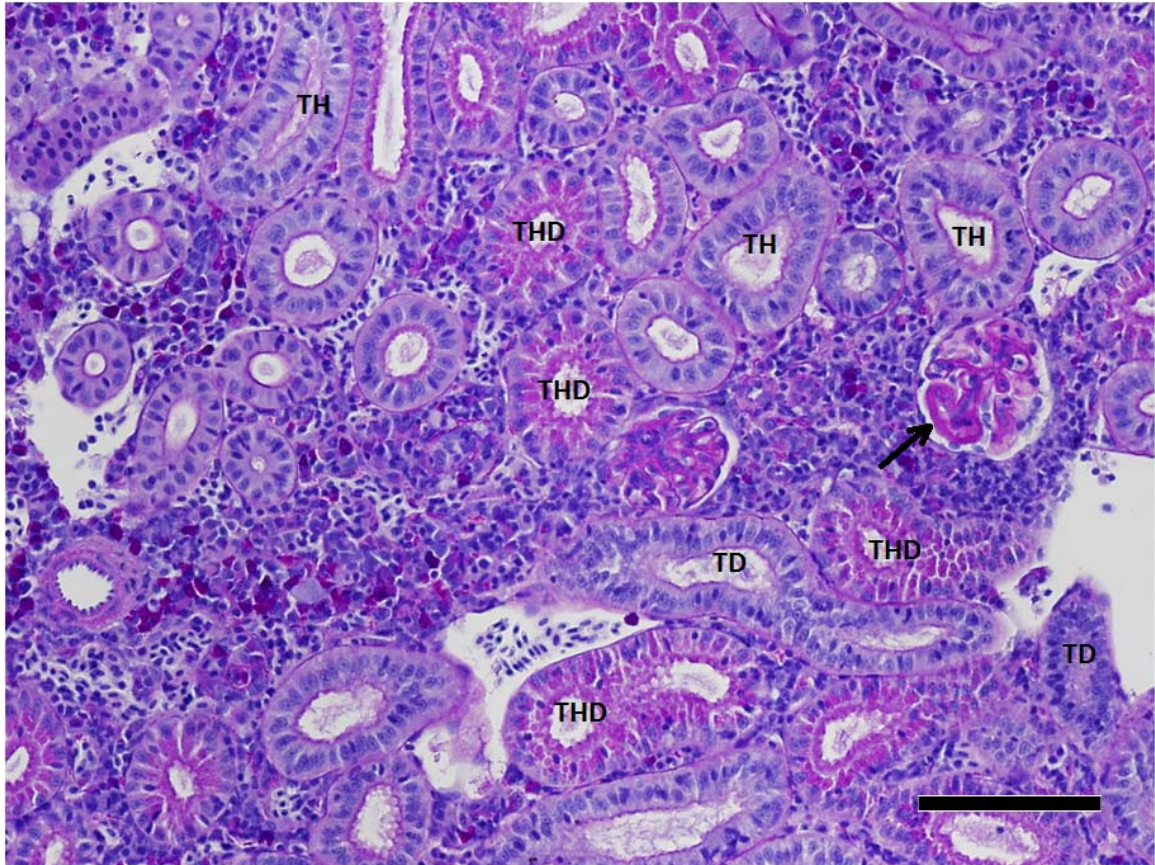


Figure 4.2. Photomicrograph of sections of kidney of largescale sucker collected in the Lower Columbia River Basin. TH, tubular hyperplasia, THD, tubular hyaline deposition; TD, tubular degeneration. Arrow points to a glomerulus with increased eosinophilic deposition in the capillaries. Scale bar = 100 μm .

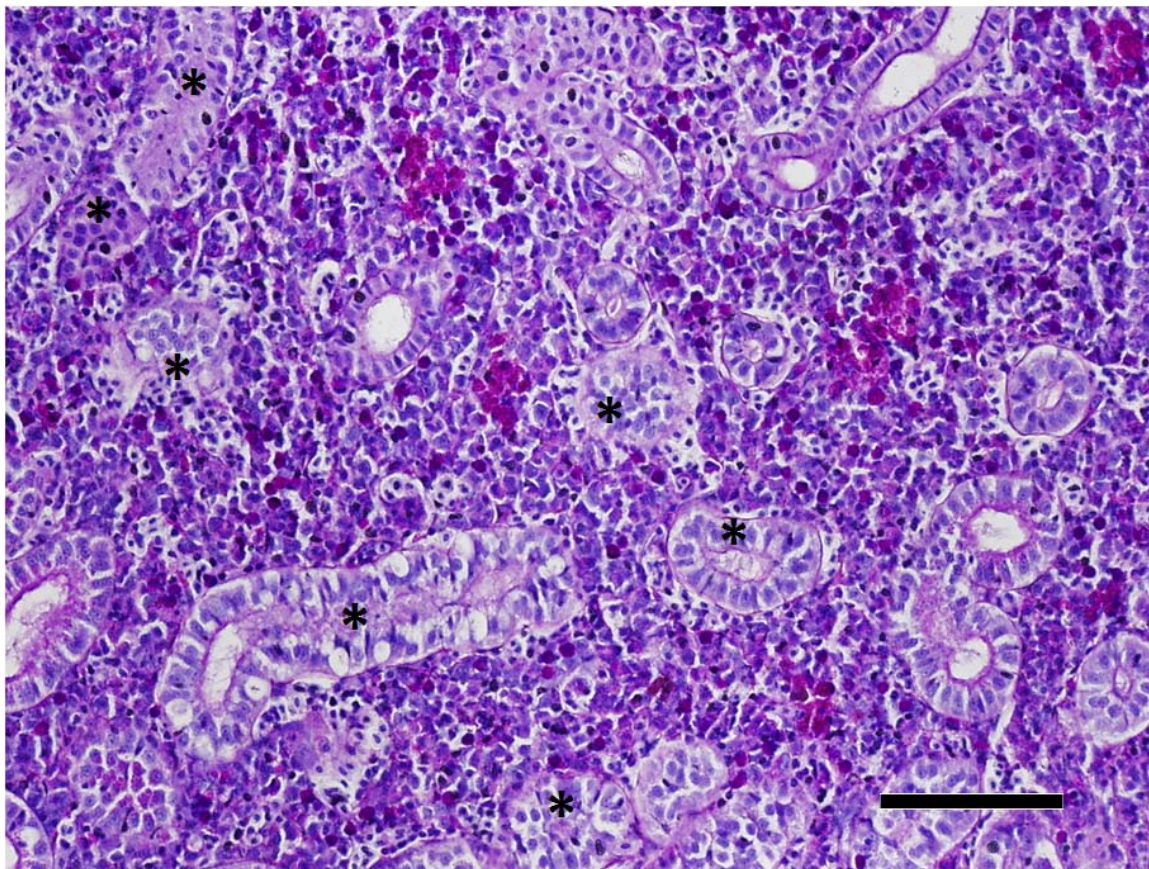


Figure 4.3. Photomicrograph of sections of kidney of largescale sucker collected in the Lower Columbia River Basin. Asterisks show renal tubules undergoing degeneration. Scale bar = 100 μ m.

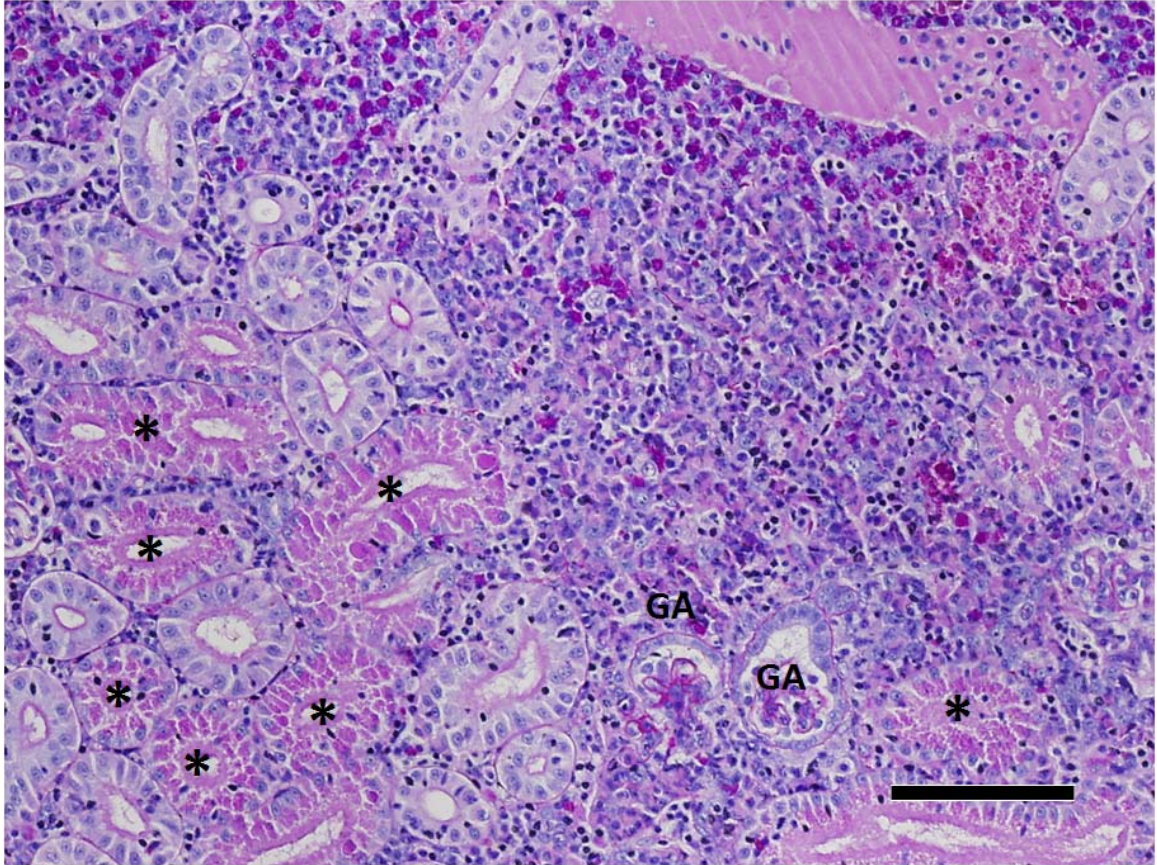


Figure 4.4. Photomicrograph of sections of kidney of largescale sucker collected in the Lower Columbia River Basin. Asterisks show renal tubules with hyaline deposition. GA, abnormal glomeruli with hypertrophic Bowman's epithelia. Scale bar = 100 μ m.

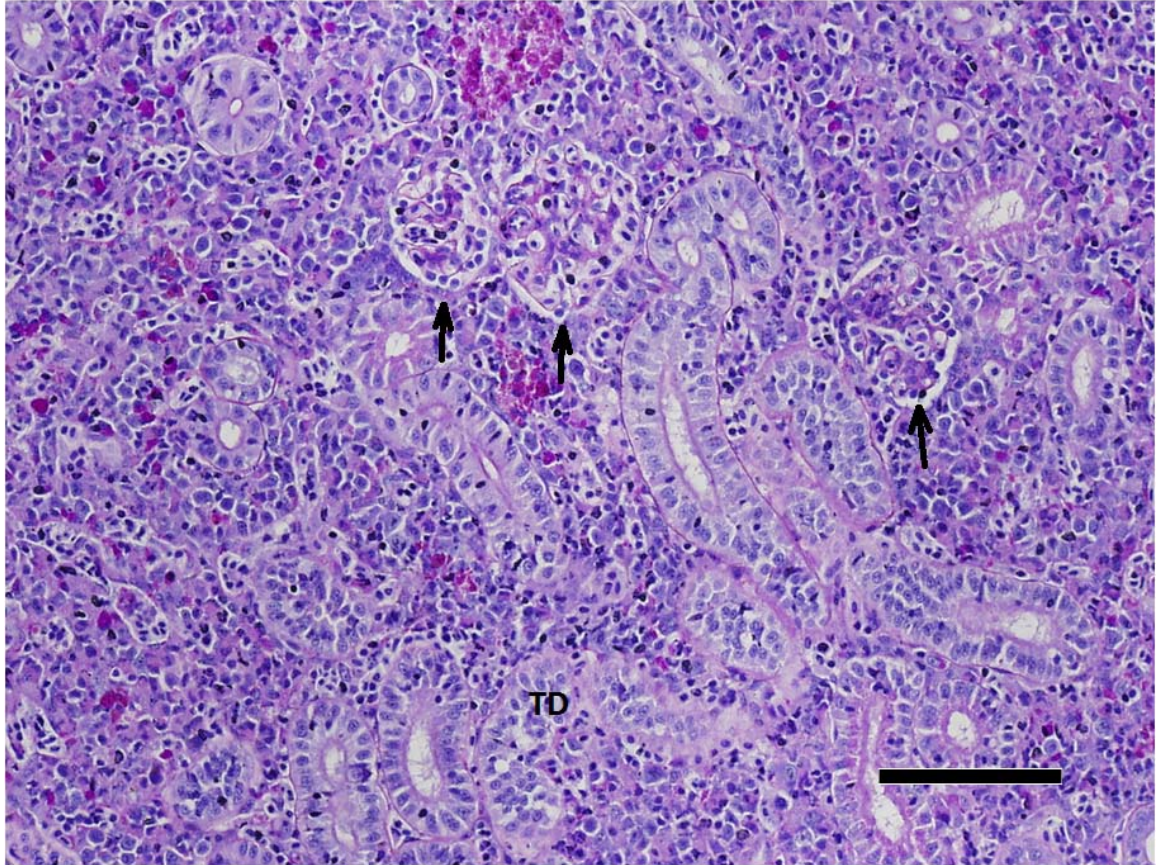


Figure 4.5. Photomicrograph of sections of kidney of largescale sucker collected in the Lower Columbia River Basin. Arrows point to glomeruli with reduced Bowman's space, the most common glomerular abnormality observed in the collected fish. TD, tubular degeneration. Scale bar = 100 μ m.

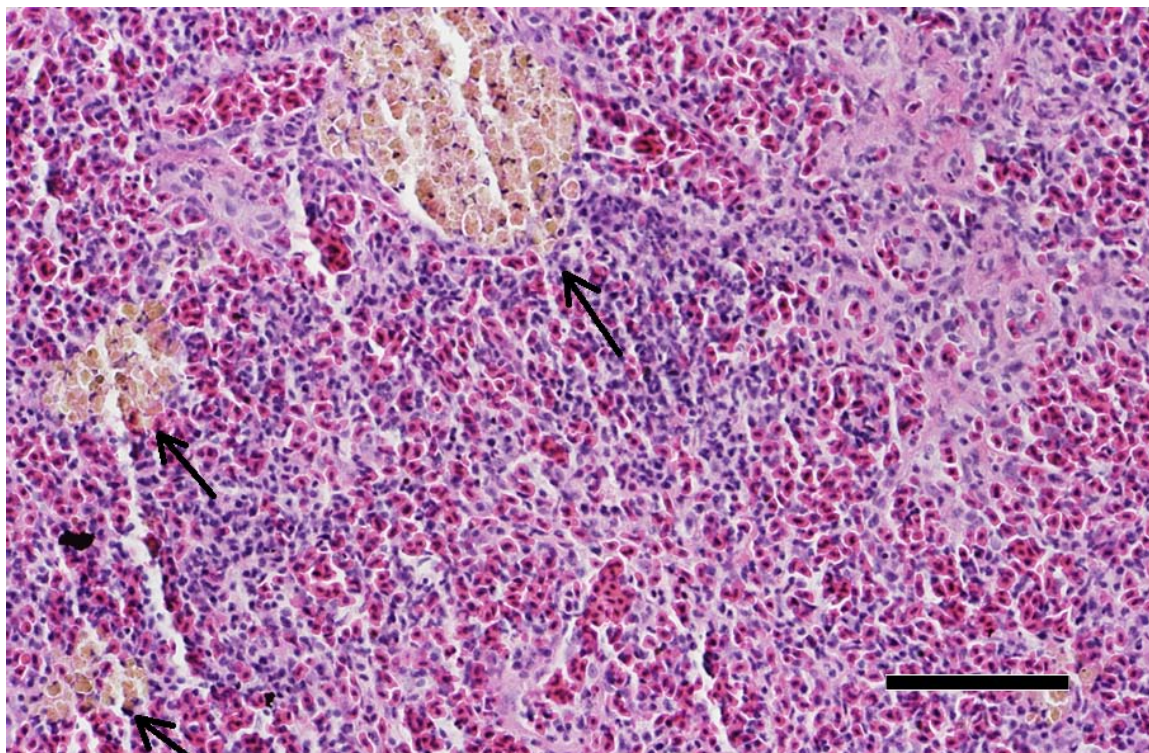


Figure 4.6. Photomicrograph of sections of spleen of largescale sucker collected in the Lower Columbia River Basin. Arrows point to macrophage aggregates. Scale bar = 100 μ m.

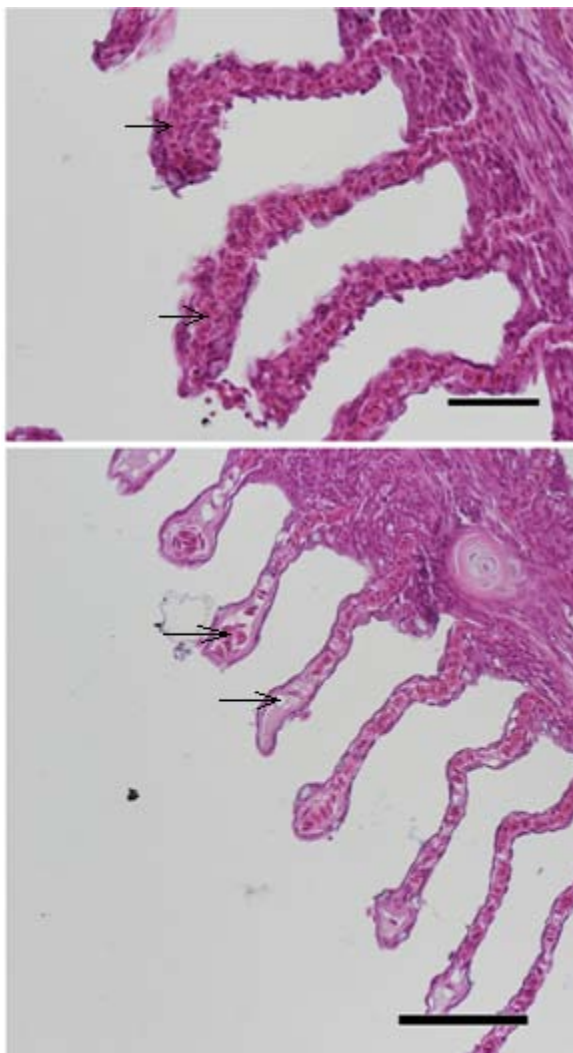


Figure 4.7. Photomicrograph of sections of gill showing blood sinus dilation in the secondary lamellae of largescale sucker collected in the Lower Columbia River Basin. Scale bar = 100 μ m.

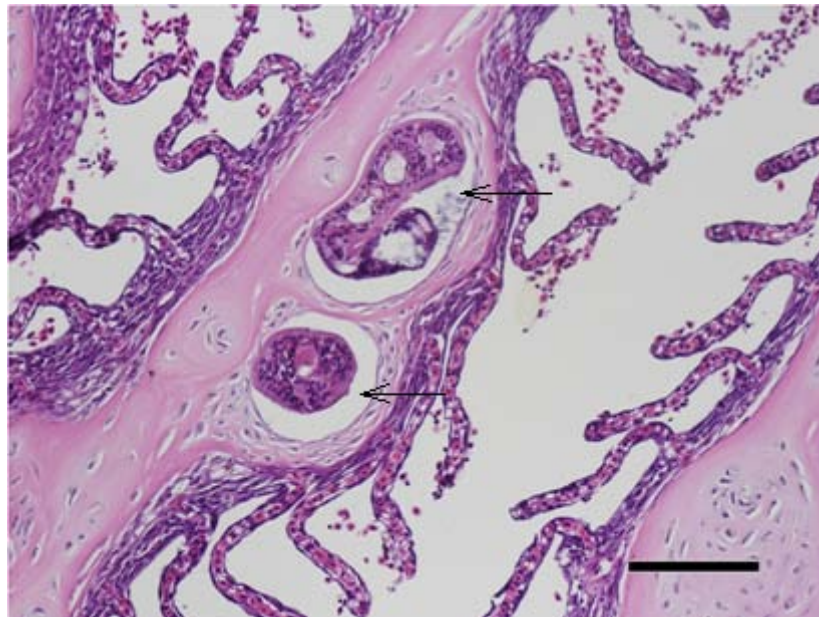


Figure 4.8. Photomicrograph of sections of gill showing cysts of metacercaria in the primary lamellae of largescale sucker collected in the Lower Columbia River Basin. Scale bar = 100 μ m.

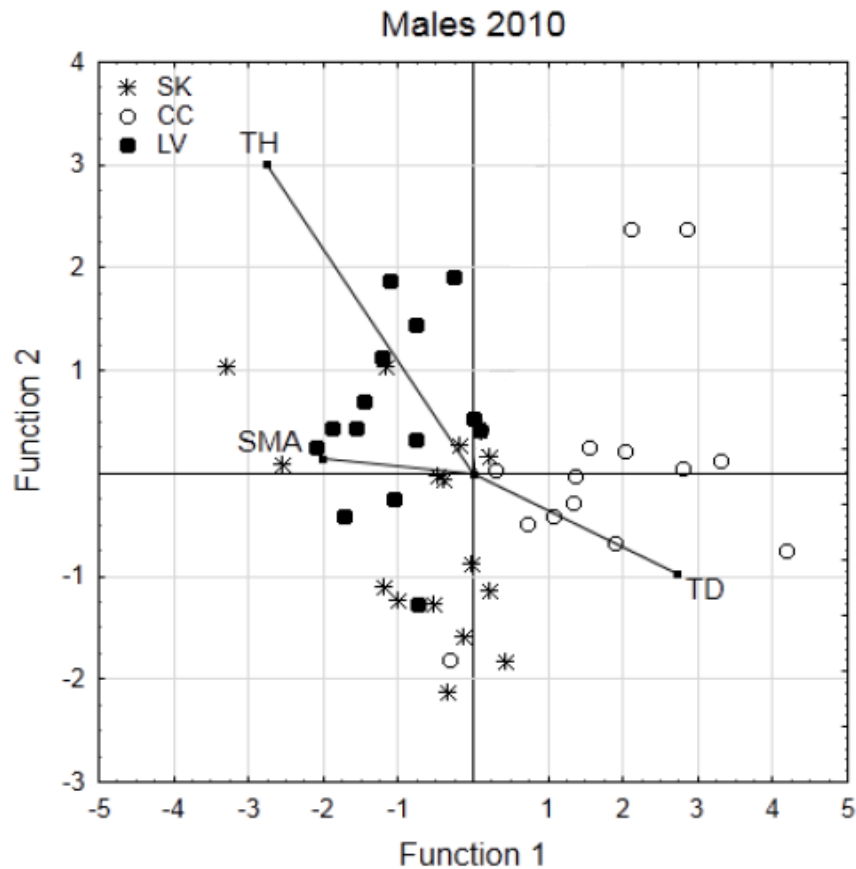


Figure 4.9. Discriminant function plot of morpho-physiological data for male largescale sucker collected from three different sites in Low Columbia River Basin (Skamania, SK; Columbia City, CC; Longview, LV) in 2010. Vectors representing the standardized coefficients for each biological trait are superimposed on the plot to indicate each trait's contribution to the discrimination between sites (vector lengths are relative to each other and not according to the axes scales). Only those traits with coefficients $\geq |0.30|$ on the first two discriminant functions are shown on the plot. SMA, spleen MA density; TD, tubular degeneration; TH, tubular hyperplasia.

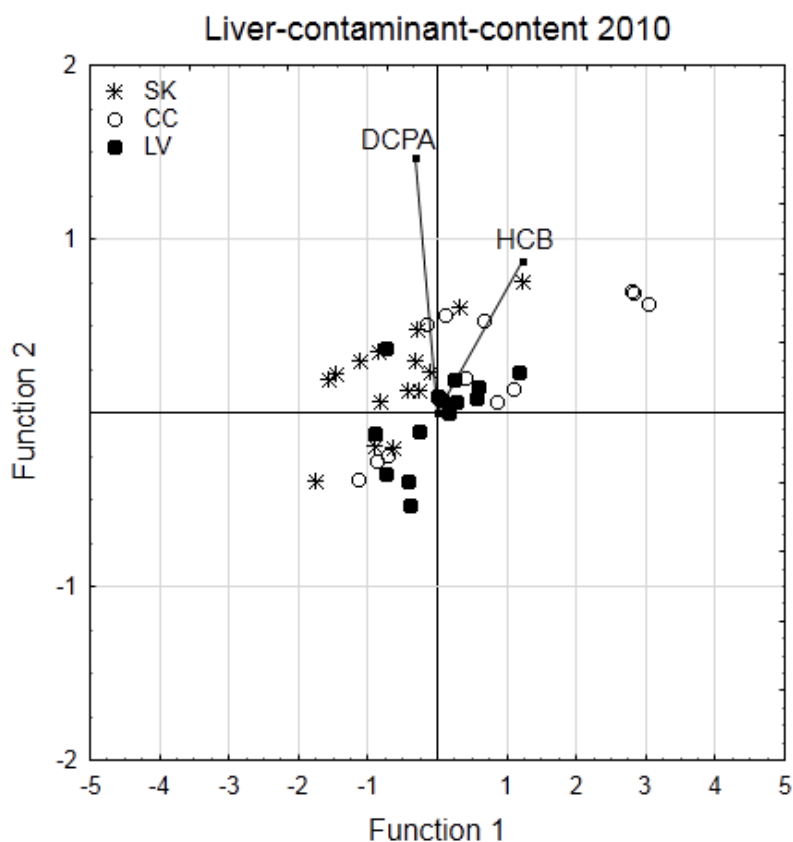


Figure 4.10. Discriminant function plot of liver contaminant concentration data for male largescale sucker collected from three different sites in Low Columbia River Basin (Skamania, SK; Columbia City, CC; Longview, LV) in 2010. Vectors representing the standardized coefficients for each biological trait are superimposed on the plot to indicate each trait's contribution to the discrimination between sites (vector lengths are relative to each other and not according to the axes scales). Only those traits with coefficients $\geq |0.30|$ on the first two discriminant functions are shown on the plot.

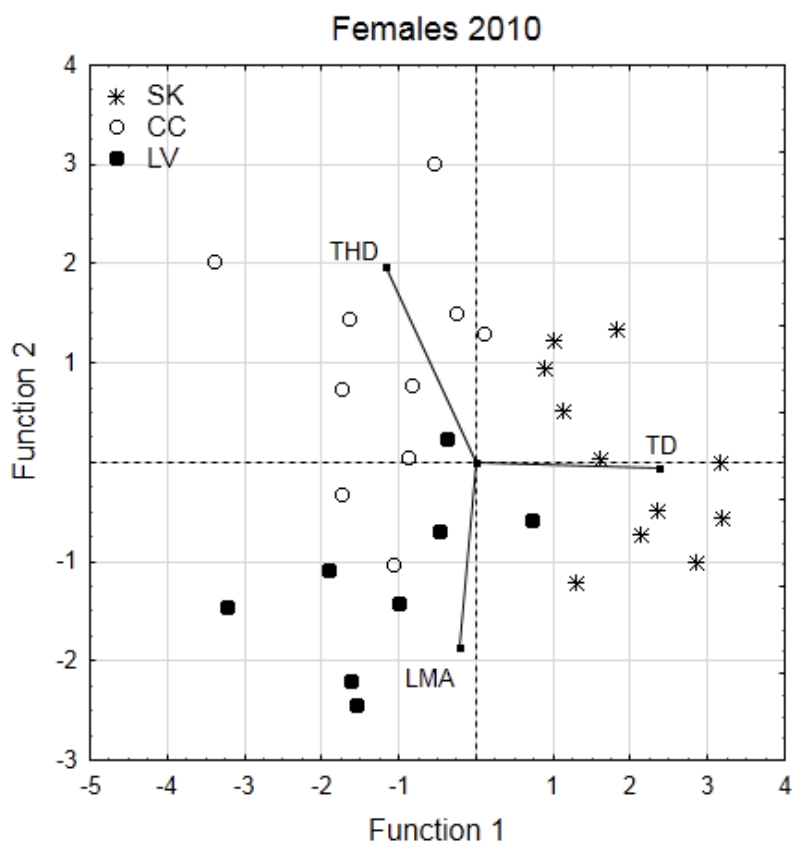


Figure 4.11. Discriminant function plot of morpho-physiological data for female largescale sucker collected from three different sites in Low Columbia River Basin (Skamania, SK; Columbia City, CC; Longview, LV) in 2010. Vectors representing the standardized coefficients for each biological trait are superimposed on the plot to indicate each trait's contribution to the discrimination between sites (vector lengths are relative to each other and not according to the axes scales). Only those traits with coefficients $\geq |0.30|$ on the first two discriminant functions are shown on the plot. LMA, liver MA density; TD, tubular degeneration; THD, tubular hyaline deposition.

CHAPTER V

CONCLUSION

The present study assessed the effects of PBDEs and other contaminants on fishes using laboratory and field approaches. In the laboratory, I found that dietary exposure to the brominated flame retardant BDE-47 led to a significant negative effect on zebrafish growth when the exposure began during the juvenile stage and continued through adulthood. This effect was temporal in females and occurred earlier during exposure (at 40 and 80 days of exposure); whereas in males, the effect was not observed until after the end of the exposure (120 days). The difference in growth responses between females and males was not related to the amount of BDE-47 concentrated in the fish because whole-body levels were similar in both sexes. Also, the effect of BDE-47 on somatic growth seemed to be independent from the status of the thyroid axis, which did not differ (histologically) among treatments in either males or females at the conclusion of the study. Additionally, I found that the stage of male and female gamete development was similar among all treatment groups at all sampling times (40, 80 and 120 days), an observation indicating that reproductive development was not affected by BDE-47 exposure. Lastly, I found that whole-body burdens of BDE-47 were associated with dietary concentrations and bracketed whole-body levels reported in wild fishes; consequently, my laboratory study and its results can be interpreted as representing environmentally relevant conditions and biological effects.

My laboratory findings support the results of other studies with zebrafish, where temporal decreases in growth and no effects on thyroid or reproductive status were observed (Chen et al. 2010). On the other hand, studies in other species, fathead minnow particularly (Lema et al. 2008), have shown detrimental thyroid and reproductive effects but mainly in males. Why fathead minnow males are more sensitive to PBDEs than females, or growth of zebrafish males but not females is impacted over the long term (present study) is still not well understood. Further studies are needed to clarify the role of sex as a factor influencing the biological impacts of PBDEs.

The Columbia River ecosystem has shown a significant increase in the concentration of PBDEs in the last decades. In my field study of the lower Columbia River, I focused on the largescale sucker, a teleost species important for the food web of the system that has been previously used in contaminant monitoring surveys. Fish were collected along an anticipated contaminant gradient from just downstream of Bonneville Dam (reference site) to downstream of Portland (Oregon) and Vancouver (Washington). Thyroid condition was examined histologically and by measuring plasma thyroid hormone levels; liver PBDE, PCB and organochlorinated compound content was determined in male livers; and fish health was evaluated by recording external and internal (tissue histology) conditions. The overall thyroid condition of largescale sucker differed significantly among collection sites in both males and females. Whereas health condition differed significantly among sites in males and females, no significant associations between health condition and male liver contaminant content were observed. Some of the health condition variables, however, seemed to differ among sampling sites according to the up-to-downstream contaminant gradient. For example, the incidence of

external (skin) and gill parasites -an indication of detrimental health- was higher in downstream sites, suggesting that fish on those sites are under more stressful conditions than the fish upstream. This indicates the need for further examination of other factors that may be responsible for the observed physiological differences. A broader group of contaminants should be studied in the area, including personal care and pharmaceutical products, polycyclic aromatic hydrocarbons and metals, all frequently found in urban and industrial areas.

Studies of the biological impacts of environmental toxicants in the field are relatively uncommon given the number of caveats in their design and difficulty in data interpretation as well as their intensive labor requirements and high cost. However, field studies are necessary not only to confirm results obtained from simplistic laboratory studies, but also to determine which contaminants and their mixtures deserve attention and to generate hypotheses on the links between contaminant exposures and real-life apical endpoints of concern. Largescale sucker are a good environmental monitoring tool in the Columbia River basin given their close contact with the sediment and role in the food web. However, in order to use this tool effectively, additional information regarding its physiology, reproduction, migration ranges and other aspects of its life history are vital for interpretation purposes. My study has contributed to the development of largescale sucker as environmental assessment tool by providing the first information on its thyroid endocrine axis, histopathological condition of several organs, and a preliminary approach on the relationship of these variables to contaminants.

Literature Cited

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