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Health status of Largescale Sucker (*Catostomus macrocheilus*) collected along an organic contaminant gradient in the lower Columbia River, Oregon and Washington, USA

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HIGHLIGHTS

- Health status of male Largescale Sucker differed among study sites.
- Kidney, spleen, liver and gill histopathologies contributed to site discrimination.
- Concentrations of HCB and PBDEs in male liver contributed to site discrimination.
- Contaminant and pathologies levels were higher near urbanized sites.
- Studied histopathologies and liver contaminant concentrations were not associated.

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ABSTRACT

The health of Largescale Sucker (*Catostomus macrocheilus*) in the lower Columbia River (USA) was evaluated using morphometric and histopathological approaches, and its association with organic contaminants accumulated in liver was evaluated in males. Fish were sampled from three sites along a contaminant gradient. In 2009, body length and mass, condition factor, gonadosomatic index, and hematocrit were measured in males and females; liver and gonad tissue were collected from males for histological analyses; and organ composites were analyzed for contaminant content in males. In 2010, additional data were collected for males and females, including external fish condition assessment, histopathologies of spleen, kidney and gill and, for males, liver contaminant content. Multivariate analysis of variance indicated that biological traits in males, but not females, differed among sites in 2009 and 2010. Discriminant function analysis indicated that site-related differences among male populations were relatively small in 2009, but in 2010, when more variables were analyzed, males differed among sites in regards to kidney, spleen, and liver histopathologies and gill parasites. Kidney tubular hyperplasia, liver and spleen macrophage aggregations, and gill parasites were generally more severe in the downstream sites compared to the reference location. The contaminant content of male livers was also generally higher downstream, and the legacy pesticide hexachlorobenzene and flame retardants BDE-47 and BDE-154 were the primary drivers for site discrimination. However, bivariate correlations between biological variables and liver contaminants retained in the discriminant models failed to reveal associations between the two variable sets. In conclusion, whereas certain non-reproductive biological traits and liver contaminant contents of male Largescale Sucker differed according to an upstream–downstream gradient in the lower Columbia River, results from this study did not reveal the specific environmental factors responsible for the differences in health status among fish populations.

Abbreviations: CC, Columbia City; GiP, gill parasites; GSI, gonadosomatic index; Hct, hematocrit; *K*, Fulton condition factor; KiG, kidney glomerulus abnormalities; KiMAA, kidney macrophage aggregate area; KiMAC, kidney macrophage aggregate count; KiTD, kidney tubular degeneration; KiTH, kidney tubular hyperplasia; KiTHy, kidney tubular hyaline deposition; LiGly, liver glycogen accumulation; LiL, liver lipid accumulation; LiMAA, liver macrophage aggregate area; LiMAC, liver macrophage aggregate count; LV, Longview; MA, macrophage aggregate; SK, Skamania; SpMAA, spleen macrophage aggregate area; SpMAC, spleen macrophage aggregate count; TeIT, testicular interstitial thickness; TeMAA, testicular macrophage aggregate area; TeMAC, testicular macrophage aggregate count.

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1. Introduction

The Columbia River is the fourth largest river in the United States (US), draining an area of approximately 670,800 km^2 from Canada to the northwest of the US. The river and its tributaries provide transportation, irrigation, hydroelectric power, recreation, and food, and represent a valuable economic resource for the region (Hinck et al., 2006). Anthropogenic activities known to impact the aquatic environment that have taken place in this riverine system include mining, timber, industrial and municipal discharges, and agricultural and urban runoff. Contaminants resulting from these activities have degraded the aquatic environment and negatively affected the overall health of a number of fish and wildlife species (Henny et al., 2008; Hinck et al., 2006; Morace, 2012).

Previous studies have established the presence and concentrations of a large suite of persistent contaminants in water and biota of the Columbia River (Hinck et al., 2006; Johnson et al., 2007, 2013). Fish health may be affected by a variety of environmental contaminants including polybrominated biphenyl ethers (PBDEs; Arkoosh et al., 2010; Muirhead et al., 2006), polychlorinated biphenyls (PCBs; Gundersen et al., 2000), dichlorodiphenyltrichloroethane metabolites (DDTs; Slaninova et al., 2009), polyaromatic hydrocarbons (PAHs; Johnson et al., 2008), and heavy metals (Golovanova, 2008). However, little is known about the effects that these contaminants have on the status of fish populations, especially in regards to contaminant mixtures present in the river. Largescale Sucker (*Catostomus macrocheilus*) are native to the Columbia River and have been used in a number of field studies to monitor environmental contaminants (Hinck et al., 2006) and to assess the effects that these contaminants have on fish-eating birds (Henny et al., 2003). Largescale Sucker occur in most freshwater bodies west of the Rocky Mountains and 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 invertebrates 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 attain sexual maturity at 5 to 7 years of age and have a life span of approximately 15 years (Dauble, 1986). This species is an important food item in the diets of other fishes (Gray et al., 1984; Nigro et al., 1983) and birds (Fitzner and Hanson, 1979; Henny et al., 2003) and is a key component of the food web nutrient cycling (Schmetterling and McFee, 2006). Due to their close contact with the benthos, Largescale Sucker are continuously exposed to xenobiotics present in sediment and are good sentinels for the presence of contaminants and their dynamics in the ecosystem.

The objectives of this study were to determine the health status of Largescale Sucker collected along a contaminant gradient in the lower Columbia River based primarily on a morphometric and histopathological approach, and to examine whether the health of male fish can be associated to the concentration of selected emerging and legacy organic contaminants accumulated in their liver. Histopathology is an important tool used to evaluate tissue impairment from contaminant exposures in fish, as selected endpoint findings directly reflect the health of individuals (Bernet et al., 1999; Schwaiger et al., 1997; Stentiford et al., 2003). Also, previous studies with fishes and amphibians have demonstrated that the sex of individuals can influence their response to contaminant exposures (Lema et al., 2008; Muirhead et al., 2006; Sharma and Patiño, 2010). In fact, some studies with fishes have reported impairments in males, but not in females, after experimental exposure to certain contaminants in the laboratory (Lema et al., 2008; Muirhead et al., 2006) or natural exposures in the field (Patiño et al., 2003). This study therefore places special attention on male fish, although information for females is also presented. A concurrent analysis of the same fish showed that the concentration of organic contaminants in liver of males is higher than in other tissues such as brain, stomach, gonad, and fillet (Nilsen et al., 2014-in this issue); thus, liver contaminant content was used as index of environmental exposures in the present study. Target contaminants in this study include legacy organic contaminants and organic contaminants of emerging concern previously reported in the study area (Fuhrer et al., 1996; Henny et al., 2003; Hinck et al., 2006; Johnson et al., 2007, 2013).

2. Methods

2.1. Study sites

Three sites were sampled on the lower Columbia River Basin: Skamania, located just downstream of Bonneville Dam (45°32′41.67″ N, 122°14′55.73″ W, river mile 140); Columbia City, downstream of Portland, between St. Helens and Columbia City (45° 55′ 11.8″ N, 122° 48′44.4″ W, river mile 82); and Longview, downstream of Columbia City in the Port of Longview (46° 5′ 55″ N, 122° 56′11″ W, river mile 66) (Fig. 1). Water temperature was monitored using HOBO® water temperature data loggers (model U22-001, Onset® Computer Corporation, Bourne, MA, USA). Measurements at the study sites were done every 15 min for 30 days prior to fish sampling.

Skamania, the upstream site, has little or no significant contaminant inputs except for Bonneville Dam and other sources considerably further upstream. Earlier studies at this site have shown contaminant levels in the aquatic environment to be relatively low (Fuhrer et al., 1996; Johnson et al., 2007; Morace, 2006). Columbia City receives contaminant inputs from the Portland/Vancouver metropolitan area, the Willamette River that flows into the Columbia River at Portland, the Multnomah Channel (part of the Willamette River), and treated wastewater effluent from St. Helens. Longview receives inputs from eight marine terminals, industrial property dominated by forest products, and steel industries. Also, Longview receives the input from the Cowlitz River and effluent from Three Rivers Regional and City of Rainier wastewater-treatment plants. Thus, the present study area provides an opportunity to observe potential differences in Largescale Sucker health status based on differences in environmental exposure to contaminants.

2.2. Fish sampling

Protocols for the use of animals in this study were reviewed and approved by Texas Tech University Animal Care and Use Committee (Lubbock, Texas, USA; approval #09021-04). Largescale Sucker were sampled by electroshocking on 4–7 May 2009 and 3–5 May 2010. These sampling times fall within or near the expected spawning seasons of the fish to facilitate diagnosis of reproductive impairments linked to contaminant exposure (R. Patiño et al., unpublished data). In 2009, 26 individuals (16 males and 10 females) were sampled in Skamania, 26 (15 males and 11 females) in Columbia City, and 27 (16 males, 11 females) in Longview. In 2010, 26 individuals (15 males, 11 females) were sampled in Skamania, 24 (14 males and 10 females) in Columbia City, and 23 (15 males and 8 females) in Longview. Fish were euthanized using 250 mg/L MS-222 (tricaine methanesulfonate; Sigma-Aldrich®, St. Louis, Missouri, USA) in river water. Body mass (g), total length (mm) and gonad mass (g) were recorded. Fulton condition factor (K, [body mass/total length^3] × 100) and gonadosomatic index (GSI, [gonad mass/total body mass] × 100) were calculated. Hematocrit (Hct, %) was determined using heparinized capillary tubes placed in a microhematocrit centrifuge and spun at 10,000 *g* for 5 min.

In 2009, gonad and liver tissues were collected from males for histopathological analyses. In 2010, gill, spleen, and kidney tissues were additionally collected from males and females at each site (except for gonadal tissue in females, which was not collected), and external characteristics and pathologies were also recorded, including the presence of nuptial tubercles, external lesions, opercular shortening, and presence of external parasites. All tissues intended for histological analyses

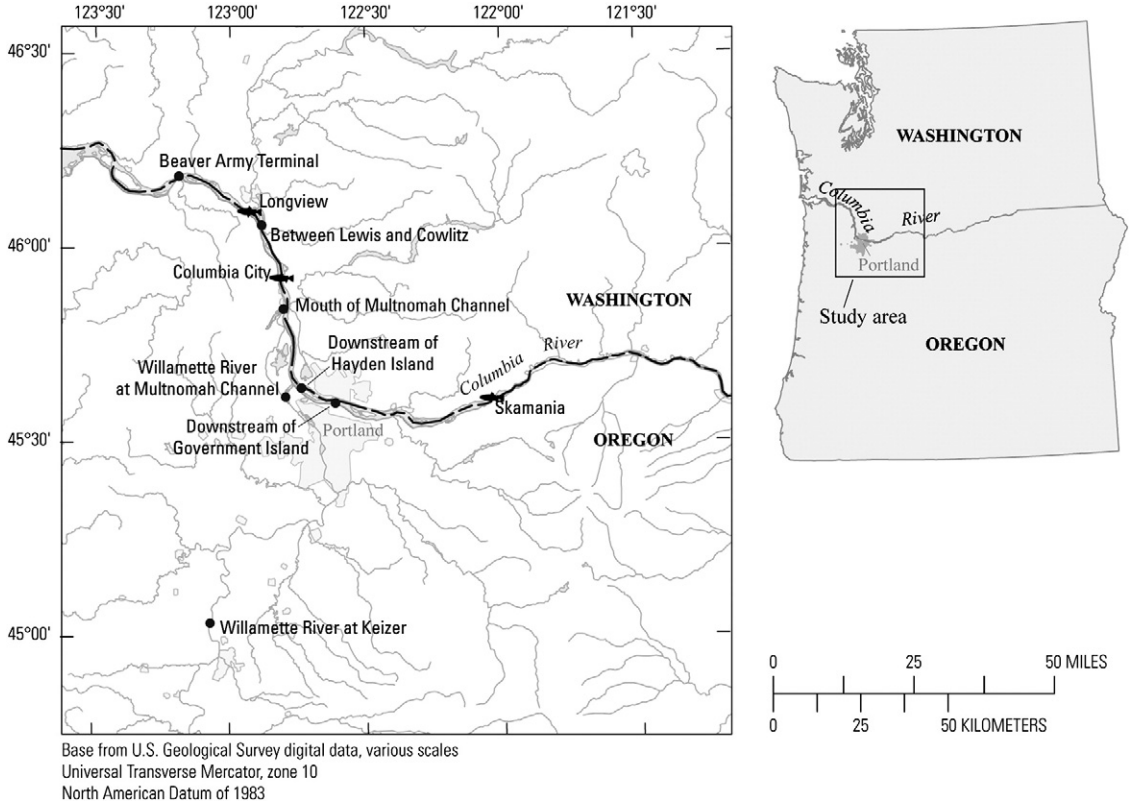

Fig. 1. Lower Columbia River study area locations: Skamania, Columbia City and Longview. See text for latitudes and longitudes for each site. (Map courtesy of USGS National Wetlands Research Center and USGS Science Publishing Network, Lafayette Publishing Service Center, Louisiana, U.S.A.).

were placed in 10% buffered formalin (Fisher Scientific®, Waltham, Massachusetts, USA).

2.3. Histological analyses

Tissues were rinsed in 70% ethanol and processed and embedded in paraffin following standard procedures (Luna, 1992). Gill arches were first decalcified (Cal-Ex, Fisher Scientific) and post-fixed in Bouin's solution (Ricca Chemical Company®, Arlington, Texas, USA) prior to dehydration and embedding in paraffin. Tissues were serially sectioned at 7 μm thickness, except for gills (5 μm). All tissues were stained with hematoxylin and eosin and sections of testis, kidney and liver were also stained with Periodic Acid Schiff (PAS) reagent (Sigma-Aldrich). Liver sections were also processed for PAS staining after α-amylase digestion (Sigma-Aldrich) to characterize glycogen accumulation. Digital images of histological specimens were taken with an Olympus digital camera (DP70; Tokyo, Japan) attached to a compound microscope. All measurements were conducted digitally using Image-Pro® Express Software (Media Cybernetics®, Silver Spring, Maryland, USA).

An increase in the number and size of macrophage aggregates (MAs) in fish tissues can be used as index of fish health and environmental degradation (Wolke, 1992). For analyses of tissue MAs, the number of MAs per unit area (MA count) and the percent area of tissue occupied by MAs (MA area) were determined in PAS-stained sections. Measurements were taken on two 50×-images of spleen and kidney, four 50×-images of liver, and two 25×-images of testes; the cumulative value of the multiple images (corrected for total area of the images) is reported as the fish value.

The thickness of the interstitial tissue in the germinal compartment of testes can increase after exposure to contaminants, a condition known as "fibrosis" (Blazer, 2002). However, spawning condition can also influence interstitial thickness; for example, it can increase as lobules become empty of sperm and their size is reduced. Thus, spawning condition and interstitial thickness were both examined in this study. Spawning condition was classified as "spawn-ready" when testicular lobules were full or almost full with sperm, "partially-spent" when lobules were only partially filled with sperm, and "spent" when lobules had only residual sperm and were 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 males were excluded from analysis.

To measure the thickness of the interstitium, a line was drawn diagonally from the top left corner to the bottom right corner of each of two contiguous digital images (25×) and the thickness of the tissue was measured at each point where the line intersected the interstitium. The average thickness of all intersects is reported as individual fish value (Patiño et al., 2003).

Changes in hepatic glycogen or/and lipid deposition are a common response to toxicant exposure or diet imbalance (Wolf and Wolfe, 2005). The diagnosis of glycogen deposits in hepatocytes was based on the comparison between two adjacent sections of liver tissue, one stained with PAS (positive staining) and the other digested with α-amylase prior to PAS staining (negative staining). Lipid deposits were recognized as round and well-delineated empty spaces in hepatocytes of undigested, PAS-stained sections. The accumulation of glycogen and lipid in liver was each assessed semi-quantitatively and ranked as 1 = low, 2 = moderate and 3 = high.

Renal tubular and glomerular damage may occur as a consequence of exposure to xenobiotics in fishes (Costa et al., 2010). Two 50× images (from the same section) per fish were used to measure the relative area of the trunk kidney occupied by nephron and interstitial tissue. In addition, the area occupied by respective pathological tissue conditions was determined and reported as percent of total image area. Nephron pathologies included glomerulus abnormalities (inflammation with reduced Bowman's capsule space, increased eosinophilic deposition in capillaries, and hypertrophy of capsular epithelium), and tubular

hyperplasia, degeneration (pyknotic nuclei, necrosis or edema), hyaline droplet deposition, and presence of luminal PAS-positive content. Values obtained from the two images (corrected for total area) are reported as the fish value for each endpoint.

Morphological changes of the gills are potentially related to toxicant exposure (Mallatt, 1985; Troncoso et al., 2011). Five adjacent primary lamellae per fish were haphazardly chosen for gill analysis based on preparation quality. The number of internal parasites found in each primary lamella was determined (no internal parasites were observed in secondary lamellae). Secondary-lamellae were examined for presence of fusion, bifurcation, vascular congestion, epithelial rupture, epithelial lifting, mucus cells, lamellar blood sinus dilation, leukocyte infiltration, hypertrophy, hyperplasia, and aneurism as described by Mallatt (1985).

2.4. Liver contaminant content

Organic contaminants were analyzed in liver composites of males collected in 2009, and in livers of individual males collected in 2010. A full description of sample collection, processing and analysis is provided by Nilsen et al. (2014-in this issue). Briefly, field-sampling procedures followed those typically used to collect samples for trace organic compound analyses (Lane et al., 2005; Ward and Harr, 1990). Sampling tools were cleaned with Liquinox® and methanol before each sample was collected to prevent outside contamination and cross-contamination between samples. Samples were stored in certified organics-free (I-CHEM® brand) jars, frozen in the field and shipped on wet or dry ice via overnight service to the USGS National Water Quality Laboratory (NWQL) in Denver, CO, where they were analyzed. The general laboratory processing procedure for tissue samples was high-pressure solvent extraction, clean up, and concentration. Compounds were separated in sample extracts by capillary column gas chromatography (GC) and detected by negative ion mass spectrometry (MS), with ammonia as the reaction gas, using selected ion monitoring (Agilent Technologies, Model 5975 GC/MS). Sample results are reported in micrograms per kilogram for tissues on a wet weight basis. The method and the environmental samples were validated against a comprehensive set of performance-based quality control parameters including laboratory blanks, replicate samples, matrix spike recoveries, and surrogate recoveries. Contaminants analyzed included chlorpyrifos, DDT (dichlorodiphenyltrichloroethane) metabolites (*p,p′*-DDE, *p,p′*-DDD, *p,p*-DDT), components of chlordane (*trans*-Chlordane, *cis*-Chlordane, *trans*-Nonachlor, *cis*-Nonachlor), DCPA (dimethyl tetrachloroterephthalate), dieldrin, HCB (hexachlorobenzene), PCA (pentachloroanisole), trifluralin, benfluralin, tefluthrin, pentachloronitrobenzene, desulfnyl fipronil, octachlorostyrene, fipronil sulfide, fipronil, oxychlordane, triclosan, endosulfan I, methyl-triclosan, oxyfluorfen, pentabromotoluene, cyhalothrin, tetradifon, cyfluthrin, dechlorane plus, PCBs (polychlorinated biphenyls; 101, 110, 118, 138,146, 149, 151, 170, 174, 177, 180, 183, 187, 194, 206), and PBDEs (polybrominated diphenyl ethers; 47, 66, 71, 85, 99, 100, 138, 153, 154, 183). For additional procedural descriptions and complete quality control results, refer to Nilsen et al. (2014-in this issue).

2.5. Data analyses

Multivariate Analysis of Variance (MANOVA) and Discriminant Function Analysis (DFA) were performed using SAS software, Version 9.3 (SAS Institute Inc. SAS/STAT®, Cary, NC, USA). Chi-square contingency table and bivariate correlation analyses were done using GraphPad Prism®, Version 5 (GraphPad Software, La Jolla, CA, USA). Missing histological data, related to accidental losses during tissue preparation (4 out of 149 samples had variables with missing values) were replaced with values estimated with function MissEM (Strauss, 2010) in Matlab®, Version 6.0.0.88 (MathWorks® Inc., Natick, MA, USA). For liver contaminants, data under their limit of detection (LOD) were replaced with values obtained using a distribution-based multiple imputation method (PROC MI) in SAS; the mean of six imputed values was used to replace those below the LOD and generate a complete dataset suitable for multivariate analyses. The α level of significance for all analyses was 0.05.

Contaminant data were processed for statistical analyses differently than in Nilsen et al. (2014-in this issue), especially in regards to the treatment of values below limits of analytical detection, in order to subject them to multivariate statistical analyses (see Section 2.5.1).

2.5.1. Multivariate analyses

This study is concerned with the identification of overall differences in biological traits and in liver contaminant content (in males) among fish populations from the selected sampling sites as well as with the subsets of variables that best explain those differences. This type of questions is best addressed using multivariate statistical approaches (Huberty and Morris, 1989). Therefore, MANOVA was used to determine overall site-associated differences in continuous variables for males and females in each year and in the contaminant content of male livers in 2010. A partial correlation matrix of the sum of squares and cross products was also generated during the MANOVA procedure to examine associations between the dependent variables. Based on the scale of De Muth (2006), moderate ($r \geq |0.4–0.7|$), high ($r \geq |0.7–0.9|$) and very high ($r \geq |0.9|$) correlations are interpreted in this study.

Significant MANOVA tests were followed by Discriminant Function Analysis (DFA). This procedure describes maximum differences among pre-specified sample groups (sampling sites) by reducing the multidimensionality of the complex datasets to a smaller number of new composite dimensions (canonical functions). The importance of each original variable within their respective canonical function is indicated by structure coefficients, and it is common practice to interpret only those variables with structure coefficients $\geq|0.3|$ (McGarigal et al., 2000). To minimize scale differences among variables, continuous data as well as ratios and proportions were log-transformed, while ranked data (liver lipid and glycogen accumulation) were standardized (z-score, $\mu = 0$, $\sigma = 1$). A forward-stepwise selection of variables was performed for DFA to eliminate weak or redundant variables and reduce their relatively large original number (McGarigal et al., 2000; Williams and Titus, 1988).

For liver contaminant content (2010 samples), all compounds with greater than 15% non-detectable values were excluded from the analysis; this procedure reduced the number from the original 56 contaminants 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, *p,p′*-DDE also had to be excluded because it had a broad range of LODs. Among the chemicals that were discarded, those that differed significantly among sites and had less than 25% non-detectable values were reincorporated into the data set; these were HCB and BDE-153. Values under the LOD were estimated as described earlier. The Grubbs test for outliers determined the presence of one fish from Skamania presenting an overall higher profile of contaminants than the rest of the fish sampled (see also Nilsen et al., 2014-in this issue). This individual was not included in any analysis.

Finally, Spearman correlation bivariate analyses were performed to determine potential associations between selected biological traits and liver contaminant variables. Variables used for this correlation analysis were those whose structure coefficients were $\geq|0.3|$ in the final DFA models.

2.5.2. Univariate analyses

Binary variables were analyzed separately because they are not suitable for inclusion in multivariate analyses. These variables included external morphology traits (presence/absence of nuptial tubercles, opercular shortening, external lesions, and external parasites) and most gill histopathologies (except gill parasites, which were included in the multivariate analyses). Differences in their incidence among sites were evaluated with chi-square tests of association.

3. Results

It is generally inappropriate to conduct univariate analyses following multivariate treatment of the same data (Huberty and Morris, 1989). However, knowledge of site-dependent patterns in the individual variables used for multivariate analyses can be useful to provide specific context. Therefore, general observations for variables used in the multivariate analyses are described in Sections 3.1–3.4 without implying any statistical significance of observed patterns. Results of univariate analyses of binary data and of partial correlations are also reported in Sections 3.1–3.4, and those for MANOVA and DFA in Section 3.5. Results of bivariate correlations between the primary biological and organic contaminant drivers of site discrimination are reported in Section 3.6.

Median values of water temperature during the 30-day period preceding sampling did not differ appreciably among sites in 2009 or 2010. In 2009, minimum, median, and maximum values (°C) were 6.8, 9.9, and 12.3 at Skamania; 8.1, 10.5, and 11.6 at Columbia City; and 7.1, 9.5, and 11.9 at Longview. In 2010, minimum, median, and maximum temperatures were 5.7, 10.0, and 12.2 at Skamania; 8.0, 10.9, and 12.2 at Columbia City; and 7.1, 10.2, and 11.9 at Longview.

3.1. General fish condition and external appearance

Males from Columbia City in 2009 showed overall lower total length and body mass than males from the other two sites (Table 1), a trend that was also evident in 2010 (Table 2). Females were of similar length and mass among sites in both years (Tables 1 and 2). *K* did not show any trends among sites for males or females in both years (Tables 1 and 2). In males, results of partial correlation analysis indicated that *K* was positively associated with GSI in both years of the study (Supplemental Tables A1 and A2; see also Section 3.2). In females, *K* was positively associated with GSI in 2009 ($r = 0.65$) but not in 2010 (Supplemental Table A3).

Nuptial tubercles were present in all males except two individuals from Longview, one classified as undeveloped (not used in statistical analyses) and the other as spent based on gonadal histology. Nuptial tubercles normally do not develop in females; however, two females, one from Skamania and one from Longview, also presented nuptial tubercles (Table 2). Chi-square analysis did not show significant differences in the incidence of nuptial tubercles in males or females from the various sites ($p > 0.05$). Opercular shortening was observed only in individuals from Longview; in males, chi-square analysis showed that its presence was significantly associated with collection site [χ^2 (2, N = 44) = 6.22, $p = 0.04$], but in females this relationship was not significant ($p > 0.05$) (Table 2). External lesions (hemorrhaging, ulcers, scale loss) were commonly found in individuals of both sexes, and their presence was not significantly associated with collection site ($p > 0.05$) (Table 2). The presence of external parasites (*Lernaea* sp., Copepoda) was not significantly associated with collection site in males or females ($p > 0.05$); however, fish with external parasites were not observed in Skamania while 7–38% of individuals from the two downstream sites had parasites (Table 2).

3.2. General gonadal condition

In 2009, male GSI was highest in Skamania and decreased in a downstream fashion with the lowest value observed in Longview (Table 1). The thickness of the interstitial tissue generally showed the opposite trend, with the highest values observed at Longview (Table 1). Most males from Skamania and Columbia City were spawn-ready, while Longview presented a higher proportion of spent males. These data suggest that the lower GSI in Longview was due to a relatively earlier onset of spawning at this site and not to any impairment in male reproductive condition (Table 1). In 2010, most males from Longview and Skamania were spawn-ready and had relatively high GSI, whereas most males from Columbia City were spent and had lower GSI (Table 2). The thickness of the interstitial tissue of the germinal epithelium also varied inversely with spawning condition, indicating the same relationship among these variables that was observed in 2009. These general patterns in testicular condition derived from visual examination of descriptive statistics (Tables 1 and 2) were confirmed by the results of partial correlation analysis; namely, GSI was negatively correlated with the thickness of the interstitium in 2009 and 2010 (Supplemental Tables A1 and A2). In females, trends in GSI among sites were not apparent (Tables 1 and 2).

Spawn-ready testes showed little if any active spermatogenesis in 2009 and 2010 but the majority of partially spent or spent testes in 2009 (7 of 8 fish) and a few in 2010 (3 of 18 fish) were spermatogenic. These observations suggest that the process of spermatogenesis for the next breeding cycle begins soon after completion of spawning.

Table 1
Biological condition of Largescale Sucker collected from the lower Columbia River in 2009. Values shown are mean ± SEM; *n* = sample size; NM, not measured. *K*, Fulton condition factor; Hct, hematocrit; GSI, gonadosomatic index; MA, macrophage aggregate.

		Skamania		Columbia City		Longview	
Biological traits		Male	Female	Male	Female	Male	Female
(*n*)	Units	(*16*)	(*10*)	(*15*)	(*11*)	(*16*)	(*11*)
General features							
Length	mm	431 ± 8	472 ± 9	406 ± 7	469 ± 8	436 ± 5	488 ± 11
Mass	g	779 ± 44	950 ± 48	645 ± 34	948 ± 63	765 ± 31	1080 ± 81
K	(g/cm^3)100	0.96 ± 0.01	0.90 ± 0.02	0.95 ± 0.02	0.91 ± 0.03	0.92 ± 0.02	0.91 ± 0.02
Hct	%	31 ± 2	29 ± 3	29 ± 1	32 ± 1	33 ± 1	31 ± 1
Gonads							
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
Spawn-ready	% of fish	100	NM	85	NM	63	
Partially-spent	% of fish	0	NM	0	NM	6	
Spent	% of fish	0	NM	15	NM	31	
Interstitial thickness	μm	9.0 ± 1.3	NM	7.8 ± 0.8	NM	13.3 ± 1.8	NM
MA area	%	0.011 ± 0.004	NM	0.02 ± 0.01	NM	0.03 ± 0.01	NM
MA count	# of MA/mm^2	1.6^{-6} ± 1.4	NM	2.7 ± 2.5	NM	3.7 ± 5.2	NM
Liver							
MA area	%	0.4 ± 0.1	NM	0.9 ± 0.3	NM	3.1 ± 1.6	NM
MA count	# of MA/mm^2	23 ± 12	NM	32 ± 25	NM	52 ± 39	NM
Glycogen accumulation	Score	1.8 ± 0.2	NM	2.2 ± 0.2	NM	1.6 ± 0.2	NM
Lipid accumulation	Score	2.5 ± 0.2	NM	1.8 ± 0.2	NM	1.8 ± 0.2	NM

Table 2
Biological condition of Largescale Sucker collected from the lower Columbia River in 2010. Values shown are mean ± SEM; *n* = sample size; NM, not measured. *K*, Fulton condition factor; Hct, hematocrit; GSI, gonadosomatic index; MA, macrophage aggregate.

		Skamania		Columbia City		Longview	
Biological traits		Male	Female	Male	Female	Male	Female
(*n*)	Units	(*15*)	(*11*)	(*14*)	(*10*)	(*15*)	(*8*)
General features							
Length	mm	428 ± 11	476 ± 6	405 ± 8	457 ± 12	420 ± 10	461 ± 16
Mass	g	780 ± 61	1046 ± 45	661 ± 41	994 ± 76	722 ± 48	910 ± 131
K	(g/cm^3)100	0.96 ± 0.02	0.96 ± 0.02	0.98 ± 0.02	1.02 ± 0.03	0.95 ± 0.02	0.9 ± 0.09
Hct	%	38 ± 1	38 ± 1	37 ± 2	35 ± 3	42 ± 2	37 ± 3
Nuptial tubercles	% of fish	100	9	100	0	87	13
Opercular shortening	% of fish	0	0	0	0	20	13
External lesions	% of fish	73	91	79	80	80	88
External parasites	% of fish	0	0	7	10	20	38
Gonads							
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
Spawn-ready	% of fish	60	NM	36	NM	79	NM
Partially-spent	% of fish	7	NM	14	NM	0	NM
Spent	% of fish	33	NM	50	NM	21	NM
Interstitial thickness	μm	12.7 ± 2.0	NM	13.9 ± 1.8	NM	9.9 ± 2.0	NM
MA area	%	0.02 ± 0.01	NM	0.1 ± 0.1	NM	0.02 ± 0.01	NM
MA count	# of MA/mm^2	0.6 ± 1	NM	2.2 ± 3.5	NM	0.74 ± 2.1	NM
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 count	# of MA/mm^2	38 ± 33	34 ± 14	25 ± 22	71 ± 86	34 ± 13	32 ± 22
Glycogen accumulation	Score	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 accumulation	Score	2.1 ± 0.2	1.5 ± 0.2	2.2 ± 0.2	1.5 ± 0.2	2.1 ± 0.2	1.4 ± 0.2
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 count	# of MA/mm^2	22 ± 10	29 ± 17	35 ± 18	25 ± 9	20 ± 7	32 ± 12
Kidney							
Tubular hyperplasia	% area	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	% area	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	% area	69.4 ± 5.2	81.6 ± 4.3	35.2 ± 8.3	34.1 ± 4.2	67.8 ± 5.7	39.2 ± 12.2
Glomerular abnormalities	% area	6.1 ± 2.9	4.8 ± 1.6	7.4 ± 3.5	7.3 ± 2.7	8.8 ± 1.7	3.2 ± 0.5
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 count	# of MA/mm^2	11 ± 6	6 ± 4	10 ± 4	8 ± 5	8 ± 3	10 ± 5
Gill parasites	# of cysts/fish	1.5 ± 2.5	0.6 ± 2	5.4 ± 9.3	5.6 ± 11.1	11.1 ± 14.1	3.0 ± 5.8

3.3. Tissue histopathologies

3.3.1. Testes

No major histopathological abnormalities were observed in testes. Similarly, no patterns in testicular MA count or MA area were apparent among sites in any year (Tables 1 and 2). Individual fish values for MA count and area were positively correlated in both years of the study (Supplemental Table A1 and A2).

3.3.2. Liver

Glycogen accumulation in male livers was mostly moderate in all sites and no trends were apparent in both years (Tables 1 and 2).

Table 3
Contaminant concentrations (μg/kg, wet mass) in liver of male Largescale Sucker collected from the lower Columbia River. Only those contaminants included in the initial statistical analysis are shown. Values for 2009 were measured in a single composite sample; those for 2010 are mean (standard error) of individual fish values. *n* = sample size.

Contaminants	Skamania		Columbia City		Longview	
	2009	2010	2009	2010	2009	2010
n	16	15	15	14	16	15
DCPA	0.9	2.1 (0.43–5.9)	0.7	1.5 (0.25–4.6)	1.4	2.1 (0.85–4.2)
p,p′-DDE	157	425.7 (4–1270)	221	522.2 (89–1340)	389	596.1 (63–1840)
HCB	1.4	1.5 (0.5–4.6)	2.9	3.2 (0.5–6.1)	3.9	5.0 (0.5–19)
PCB-138	6.4	11.3 (0.25–44)	9.7	13.0 (3.1–34)	11.8	17.8 (2.4–58)
PCB-146	0.9	4.9 (0.4–22)	3.3	6.6 (0.7–19)	8.5	5.7 (0.9–14)
PCB-170	1.3	5.3 (0.45–23)	6.8	7.6 (2–19)	8.9	7.0 (1.4–17)
PCB-174	0.1	2.4 (0.45–11)	3.1	3.5 (0.93–9.4)	5.5	3.4 (0.76–7.6)
PCB-180	2.7	10.0 (0.81–44)	11.8	13.6 (2.9–37)	18.9	12.4 (2.3–31)
PCB-183	0.8	2.9 (0.24–12)	3.3	3.9 (0.81–9.9)	4.8	3.5 (0.75–9)
PCB-187	2.3	8.4 (0.94–33)	7.8	10.4 (2.5–24)	17.2	9.8 (1.9–24)
PCB-194	0.4	1.5 (0.42–6.5)	2.3	2.3 (0.46–6.7)	3.4	1.8 (0.5–4.1)
BDE-47	16.2	69.8 (8.7–300)	75.8	76.5 (27–140)	161	109.7 (46–300)
BDE-100	3.9	16.0 (1.7–95)	26.8	15.6 (4.9–31)	41.6	19.8 (6.9–56)
BDE-153	0.2	0.8 (0.22–2.2)	1.5	0.9 (0.35–2.8)	2.7	1.7 (0.59–4.3)
BDE-154	0.7	2.9 (0.35–15)	4.6	4.1 (1.1–9.5)	8.7	3.8 (1.2–10)

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

Lipid accumulation appeared to be more pronounced in males from Skamania collected in 2009 (Table 1) but this trend was not apparent in 2010 (Table 2). Liver lipid accumulation in female livers collected in 2010 varied from low to moderate, whereas glycogen accumulation was primarily moderate at all sites (Table 2). Curiously, results of partial correlation analyses showed that liver lipid was positively associated with GSI in males and negatively in females in 2010, and liver glycogen was also negatively correlated with GSI in females (Supplemental Tables A2 and A3). Also, liver lipid and glycogen were negatively correlated with each other in males (Supplemental Tables A1 and A2) but positively in females (Supplemental Table A3).

In 2009, liver MA area and count both seemed to be lowest in males from Skamania and highest in Longview (Table 1); in 2010, liver MA area also seemed to be highest in males from Longview but liver MA count was lowest in Columbia City (Table 2). Liver MA area and count values were positively correlated in both males and females (Supplemental Tables A1, A2 and A3). Other liver pathologies observed included the presence of necrosis, pyknotic nuclei, and foci of basophilic cellular alterations, which were present in few fish at all sites.

3.3.3. Kidney and spleen

Males and females collected from Columbia City seemed to be consistently affected by renal tubular hyperplasia to a greater degree than fish from the other two sites (Table 2). In females, tubular hyaline deposition seemed lowest in Skamania but tubular degeneration was highest at this site, whereas in males, kidney hyaline deposition was highest in Columbia City and kidney tubular degeneration was lowest at this site (Table 2). Aggregated occurrence of the various glomerular abnormalities examined did not seem to differ among sites (Table 2). In general, the most common glomerular abnormality observed in males and females was a reduction of the Bowman's space (data not shown).

Renal MA count and area did not show apparent trends among sites in fish of either sex (Table 2). Spleen MA count seemed higher in males from Columbia City than the other sites, but this trend was not observed in females. Spleen MA area appeared to be similar among sites for males and females (Table 2). MA area and count values were positively correlated in spleen and kidney in both males and females (Supplemental Tables A2 and A3).

3.3.4. Gills

In males, vascular congestion and blood sinus dilation of the secondary lamellae were observed in most individuals collected from all three sites. In females, the degree of gill histopathologies was mostly moderate at all three sites. The results of chi-square analyses showed that none of these pathologies were associated with collection site in males or females ($p > 0.05$).

Parasites in primary lamellae included cysts (metacercariae) of digenean trematodes. The number of cysts per fish suggested that, on average, male and female fish from the downstream sites were more severely affected (Table 2). A suctorian ciliate species (*Capriniana* sp.) was observed on the surface of secondary lamellae of two males, one from Columbia City and one from Longview; these data were not included in the statistical analyses because of the very low rates.

3.4. Organic contaminants in male liver

Most organic contaminants measured in male liver composites in 2009 showed increasing concentrations in a downstream direction. Likewise, individual contaminants in male livers in 2010 showed the lowest concentrations in Skamania compared to the downstream sites (Table 3; Nilsen et al., 2014-in this issue). Longview had the highest concentrations of HCB as well as some PCBs and PBDEs. The partial correlation matrix generally showed moderate to very high ($r \geq 0.51$–0.92) positive pairwise associations between PCBs and PBDEs, except PCB-138, which was not significantly correlated with any PBDE. HCB showed moderate-to-high, positive associations with PBDEs ($r \geq 0.54$–0.74) and low-to-moderate with PCBs ($r = 0.40$–0.46), except PCB-138. See Nilsen et al. (2014-in this issue) for a comprehensive description of organic contaminant concentrations in male livers.

3.5. Multivariate analyses of biological traits and liver organic contaminant profiles

3.5.1. Males

Biological traits differed significantly among sites in 2009 (MANOVA Wilks' $\lambda = 0.393$; $F_{(22,64)} = 1.73$; $p = 0.046$). Significant differences among sites were also observed in 2010 (MANOVA Wilks' $\lambda = 0.095$; $F_{(40,42)} = 2.36$; $p = 0.0034$).

In 2009, the stepwise DFA selected the following biological variables for inclusion in the model: GSI, liver lipid accumulation and total length. Results of DFA yielded a significant model (Wilks' $\lambda = 0.572$; $F_{(6,80)} = 4.30$; $p = 0.0008$), with two significant canonical functions bearing eigenvalues of 0.35 and 0.30, respectively. GSI had the highest structure coefficient on the first function (0.89), and liver lipid accumulation (0.79) and total length (0.73) on the second function. A biplot (with 95% confidence ellipses) shows that the fish from Longview differed from the other two sites mainly in terms of GSI along function 1, whereas fish from Skamania and Columbia City were generally separated along function 2, primarily due to differences in liver lipid accumulation (Fig. 2).

In 2010 there were more variables available for analysis than in 2009. The following biological variables were selected for inclusion in the 2010 DFA model: total length, renal tubular degeneration, tubular hyperplasia, spleen MA count, liver MA area and count, and gill parasites. Results of DFA yielded a significant model (Wilks' $\lambda = 0.227$; $F_{(14,68)} = 5.33$; $p < 0.0001$) with two significant canonical functions bearing eigenvalues of 2.04 and 0.45, respectively. Variables with structure coefficients $\geq|0.3|$ on the first function were renal tubular hyperplasia (-0.53), renal tubular degeneration (0.49) and spleen MA count (-0.36); and on the second function were liver MA area (0.50) and count (0.30) and gill parasites (0.64) (Fig. 2). The biplot indicates a relatively clear separation of Columbia City from Skamania and Longview along function 1, primarily due to differences in kidney histopathologies (Fig. 2), and a less distinct but still considerable separation between Skamania and Longview along function 2 (most Skamania data fall below axis 1 and most Longview data, above it) that is primarily due to differences in the occurrence of gill parasites and liver MAs (Fig. 2). Therefore, group separations between the reference site (Skamania) and Columbia City and between Skamania and Longview were not based on the same biological traits.

Liver contaminant concentrations differed significantly among sites in 2010 (MANOVA Wilks' $\lambda = 0.19$; $F_{(28,50)} = 2.33$; $p = 0.004$). The stepwise DFA selected two variables, DCPA and HCB for inclusion in the model. Results of the DFA yielded a significant model (Wilks' $\lambda = 0.56$; $F_{(4,74)} = 6.29$; $p = 0.0002$), with one significant canonical function bearing an eigenvalue of 0.66. HCB was the only variable with a structure coefficient $>|0.3|$ on this function ($= 0.78$). Although the second function, and therefore DCPA (structure coefficient, 0.99), did not contribute significantly to group separation, the DCPA vector is included in the biplot as reference. Despite some degree of overlap, group separation on the first function (HCB) was evident. Overall levels of HCB were lowest in Skamania (reference site) followed by Columbia City and Longview (Fig. 3; Table 3).

Stepwise DFA eliminates not only variables that are poor group discriminators but also those that may be correlated with other, strong discriminatory variables (McGarigal et al., 2000). Results of partial correlation analysis suggested the presence of moderate-to-high correlations between liver content of HCB and PCBs/PBDEs (see Section 3.4 and Supplemental Table A4). Thus, a second stepwise DFA was conducted where HCB was removed from the analysis to allow identification of additional discriminatory variables that may have been obscured by their association with HCB in the first DFA. This second analysis led to the selection of

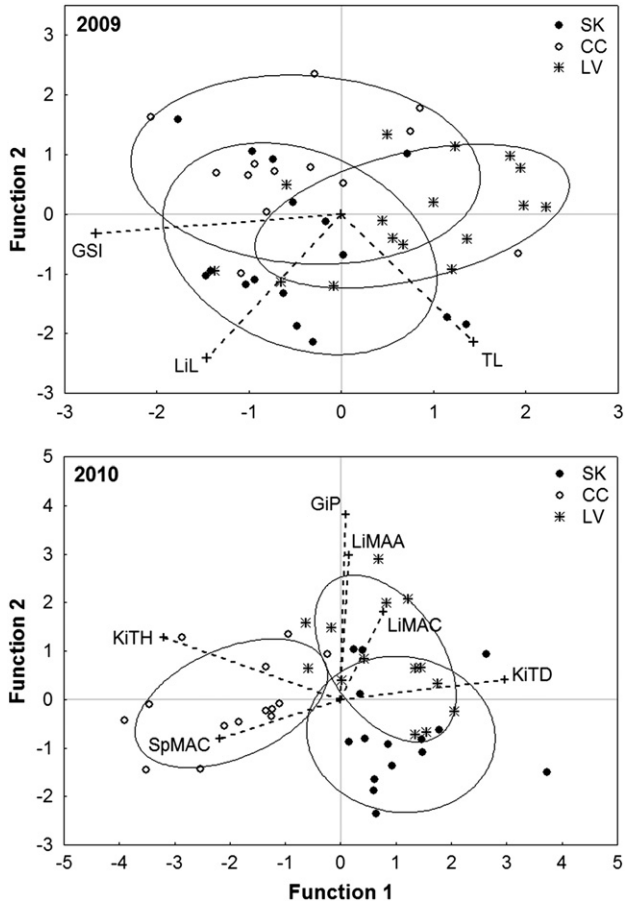

Fig. 2. Discriminant function plot of morphometric data of male Largescale Sucker collected from three different sites in lower Columbia River (Skamania, SK; Columbia City, CC; Longview, LV) in 2009 (upper plot) and 2010 (lower plot). Vectors representing structure coefficients (≥ 0.30) of the primary discrimination drivers are superimposed on the plot to indicate the variable's contribution to the discrimination between sites. Vector lengths are relative to each other and not according to axes scales. GiP, gill parasites; GSI, gonadosomatic index; KiTD, kidney tubular degeneration; KiTH, kidney tubular hyperplasia; LiL, liver lipid accumulation; LiMAA, liver macrophage aggregate area; LiMAC, liver macrophage aggregate count; SpMAC, spleen macrophage aggregate count; TL, total length.

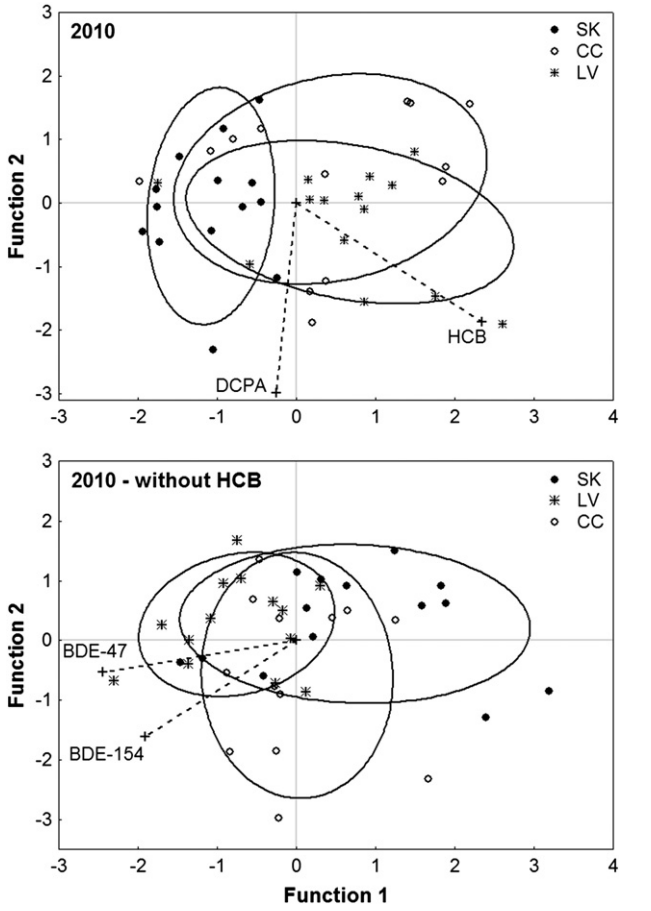

Fig. 3. Discriminant function plot of liver organic-contaminant concentration data of male Largescale Sucker collected from three different sites in lower Columbia River in 2010 (Skamania, SK; Columbia City, CC; Longview, LV). The upper plot was generated when all variables were included in the analysis and the lower plot when hexachlorobenzene (HCB) was excluded (see text for explanation). Vectors representing structure coefficients (≥ 0.30) of the primary discrimination drivers are superimposed on the plot to indicate the variable's contribution to the discrimination between sites. Vector lengths are relative to each other and not according to axes scales. BDE-47, 2,2′,4,4′-tetrabromodiphenyl ether; BDE-154, 2,2′,4,4′,5,6′-hexa-bromodiphenyl ether; DCPA, dimethyl tetrachloroterephthalate.

two variables, BDE-47 and BDE-154, which yielded a significant DFA model (Wilks' $\lambda = 0.60$; $F_{(4,74)} = 5.29$; $p = 0.0008$) with two significant canonical functions bearing eigenvalues of 0.41 and 0.17, respectively. The highest structure coefficient on the first function was for BDE-47 (0.98) followed by BDE-154 (0.76). The structure coefficient for BDE-47 on the second function was also relatively high (−0.65) but lower than on the first function; thus, the second function is not interpreted in this study. Data variability was higher in the reference site, Skamania, than in the other two sites (Fig. 3). However, compared to Skamania, fish from Columbia City and Longview generally had higher levels of BDE-47 and BDE-154 (Fig. 3; Table 3).

Note that the concentration of all three organic contaminants identified by the DFA models as the main drivers of site discrimination (HCB, BDE-47, and BDE-154) generally increased in a downstream direction in both years (Table 3); this observation is consistent with the general trend in liver organic contaminant concentrations described by Nilsen et al. (2014-in this issue) elsewhere in this issue.

3.5.2. Females

Female biological traits did not differ among sites in 2009 (MANOVA Wilks' $\lambda = 0.835$; $F_{(8,52)} = 0.61$; $p = 0.762$) or in 2010 (MANOVA Wilks' $\lambda = 0.055$; $F_{(34,20)} = 1.92$; $p = 0.0632$).

3.6. Association between liver organic-contaminant content and fish health status

As mentioned earlier, some of the biological variables as well as the content of most liver organic contaminants showed an upstream–downstream trend in males. [Note: because Longview, the site farthest downstream, has some unique inputs of contaminants (see Section 2.1), in this study an upstream–downstream "trend" for organic contaminants or presumed biological impacts is considered to be one in which Skamania has the lowest values irrespective of the relative values

Table 4
General trends in biological traits and liver contaminants of male Largescale Sucker collected in 2010 that were retained in the DFA models. Based on average values, ranks are designated as lowest (L), intermediate (I) or highest (H). SK, Skamania; CC, Columbia City; LV, Longview; LiMAA, liver macrophage aggregate area; LiMAC, liver macrophage aggregate count; SpMAC, spleen macrophage aggregate count; KiTD, kidney tubular degeneration; KiTH, kidney tubular hyperplasia; GiP, gill parasites.

	LiMAA	LiMAC	SpMAC	KiTD	KiTH	GiP	BDE-47	BDE-154	HCB
SK	I	H	I	H	L	L	L	L	L
CC	L	L	H	L	H	I	I	H	I
LV	H	I	L	I	I	H	H	I	H

between Columbia City and Longview.] The clearest trends were for renal tubular hyperplasia and gill parasites among the biological traits, and HCB, BDE-47 and BDE-154 among the liver organic contaminants (Table 4). However, bivariate correlation analyses between biological variables (renal tubular hyperplasia, tubular degeneration, spleen MA count, liver MA area and count, and gill parasites) and liver contaminants (HCB, BDE-47, and BDE-154) with the highest structure coefficients in the DFAs failed to reveal any significant associations between the two variable sets (Table 5).

4. Discussion

The objectives of this study were to determine the health status of Largescale Sucker collected along a contaminant gradient in the lower Columbia River based primarily on a histopathological approach, and whether the health of male fish is associated with the concentration of selected emerging and legacy contaminants accumulated in their liver. These objectives were met. Results indicated that the overall health status of male Largescale Sucker as well as the organic contaminant concentration in their liver generally differed between the upstream, reference site and the two downstream sites. Bivariate correlation analyses between biological traits and liver organic contaminants responsible for site separation in males, however, did not reveal significant associations between these two variable sets at the level of individual fish. Associations between collection site and the health status of female fish were not as clear as they were for males.

The present finding of an upstream–downstream gradient in the organic contaminant concentration of male fish livers is consistent with results of earlier studies of the lower Columbia River that reported increased levels of contaminants in water and biota downstream of the Portland (OR)/Vancouver (WA) area (Fuhrer et al., 1996; Johnson et al., 2007, 2013; Morace, 2006, 2012). In 2009, organic contaminant concentrations in composites of liver and other tissues were generally higher in the downstream sites (Columbia City and Longview) compared to the upstream reference site (Skamania; Nilsen et al., 2014-in this issue) and, in 2010, univariate (Nilsen et al., 2014-in this issue) and multivariate analyses (present study) of liver organic contaminants in individual males confirmed this trend; compounds with significantly higher downstream concentrations in 2010 included HCB, PCB-170, PCB-174, BDE-47, BDE-100, BDE-153 and BDE-154. In addition, the present study identified the legacy organochlorine pesticide, HCB, and the lower brominated flame retardants, BDE-47 and BDE-154, as the primary drivers for the separation of fish populations according to sampling site. HCB is no longer produced commercially but can be a byproduct of the production of other chlorinated compounds. BDE-47 (tetra-BDE), and BDE-154 (hexa-BDE) are lower brominated flame retardants found in commercial mixtures (Konstantinov et al., 2008) or produced as metabolites of higher brominated PBDEs (Isosaari et al., 2005; Nyholm et al., 2009). The male liver concentrations of HCB, BDE-47 and BDE-154 generally increased from Skamania to Longview. Overall, these observations validate the sampling design of the present study for the purpose of exploring associations between the biological condition of Largescale Sucker and different levels of contaminant exposures in the lower Columbia River.

Table 5
Pairwise Spearman correlation coefficients between biological variables and liver contaminants in males collected in 2010. Significant associations were not observed. LiMAA, liver macrophage aggregate area; LiMAC, liver macrophage aggregate count; SpMAC, spleen macrophage aggregate count; KiTD, kidney tubular degeneration; KiTH, kidney tubular hyperplasia; GiP, gill parasites.

	Biological variable					
Liver contaminant	LiMAA	LiMAC	SpMAC	KiTD	KiTH	GiP
BDE-47	0.28	0.03	−0.03	−0.05	0.04	−0.02
BDE-154	0.29	0.11	0.2	−0.1	0.15	−0.1
HCB	0.17	−0.1	−0.2	−0.1	0.16	0.14

Largescale Sucker for this study were collected near the beginning of their expected spawning period, in early May of 2009 and 2010. The spawning period of Largescale Sucker may span several months (Dauble, 1986) and, based on histological analysis of spermatogenesis, males in the lower Columbia River seem to complete testicular development in the fall but spawning does not begin until an undetermined period of time after March (Hinck et al, 2006). Some of the males collected for the present study had spent testes in early May, a condition indicating that spawning activity had already started by this time. The pattern of associations between GSI, relative sperm content, and testicular interstitial thickness strongly suggests that these variables were all influenced by normal spawning activity and, consequently, that the site-dependent variation in testicular condition observed in this study is primarily due to temporal differences in the initiation and progress of spawning. Namely, as male fish begin to spawn, their testicular lobules release sperm and contract resulting in thicker interlobular (interstitial) spaces, and GSI decreases. In fact, males from Longview had lower testicular GSI, higher levels of spent testes, and thicker interstitium than males from Skamania and Columbia City in 2009, and males from Columbia City had lower GSI, higher rates of spent testes, and thicker interstitium in 2010. Partial correlation analysis confirmed the negative relationship between GSI and testicular interstitial thickness at the individual fish level in both years. Thus, despite the usefulness of GSI and interstitial thickness as biomarkers of male fish reproductive health in some situations (Blazer, 2002; Patiño et al., 2003, 2012), they must be interpreted with caution. In females, the pattern of GSI variation among sites was also inconsistent between years; mean values were lowest in Skamania in 2009 and lowest in Longview in 2010. In addition, an association of GSI with liver energy stores (lipid and glycogen) was observed in males and females, suggesting that liver energy stores may be influenced by gonadal development or spawning condition; curiously, however, the association was negative for females and positive (lipid) for males. Water temperature did not differ appreciably among sites (differences in median values were ≤ 1 °C), suggesting that differences in the onset of spawning among the three populations occurred independently of this environmental factor. Overall, the present study did not reveal morphometric differences in gonadal condition of Largescale Sucker that could be associated with gradients of exposure to organic contaminants or other environmental factors (i.e., temperature) in the lower Columbia River (see also later discussion). The lack of association between morphometric indices of gonadal condition and the downstream increase in liver organic contaminant concentrations of Largescale Sucker is noteworthy because other field studies with species such as White Sturgeon (*Acipenser transmontanus*) (Feist et al., 2005), European Chub (*Leuciscus cephalus*) (Randak et al., 2009) and English Sole (*Parophrys vetulus*) (Sol et al., 2008) have reported negative correlations between tissue (in some cases including liver) organic contaminants, and morphometric gonadal indices such as GSI. As noted already, GSI measurements must be interpreted with caution, especially if collected near or during spawning periods. However, functional analyses of gamete (sperm) condition indicate that sperm quality in fish from the various sampling sites of the present study may have differed in a manner consistent with differences in contaminant exposures (Jenkins et al., 2014-in this issue).

Multivariate analyses of 2009–2010 biological data indicated that the (non-reproductive) health status of male but not female Largescale Sucker differed among sampling sites. In 2009, GSI was the strongest discrimination driver but site discrimination was weak relative to 2010. Because changes in GSI are the result of spawning activity (see preceding discussion) and the number of variables available for analysis in 2009 was much lower than in 2010, results for 2009 are of relatively limited value to specifically understand the patterns and causes of site discrimination among male populations; therefore, the present discussion will henceforth emphasize observations recorded for males in 2010. These observations indicated that the primary biological drivers for site discrimination in 2010 were renal tubular hyperplasia and

degeneration, spleen MA count, liver MA area and count, and internal gill parasites.

The average number of internal gill parasites was generally lower in male and female fish from Skamania than the two downstream sites, and results of DFA with males indicated that this variable was a major driver for discrimination between Skamania and Longview. The presence of parasites and pathogens is a general index of reduced health (Noga, 2000), and infection severity can be significantly increased by exposure to contaminants. For example, studies in salmonids have shown that exposure to PBDEs can impair immune function (Birchmeier et al., 2005) and may lead to a higher risk of disease (Arkoosh et al., 2010). Although mean liver PBDE concentrations in male fish of the present study were also generally lowest in Skamania relative to both downstream sites, at the individual fish level, concentrations of PBDEs as well as other contaminants did not correlate with internal gill parasites. Therefore, although a role for increased levels of contaminant exposure in the downstream sites of this study is still possible, if not likely, the specific factors responsible for the higher occurrence of gill parasites in fish from Columbia City and Longview cannot be ascertained from the results of the present study.

Kidney histopathologies also contributed significantly to site discrimination among male populations in 2010. Tubular hyperplasia and degeneration were the two kidney variables that contributed the most to site discrimination in males. Consistent with the relative levels of liver contaminants, the highest level of tubular hyperplasia in males was seen in Columbia City and the lowest in Skamania, the reference site (this pattern was also observed in females; see Table 2). Curiously, however, the lowest level of tubular degeneration was seen in males from Columbia City and no overall difference was observed between Skamania and Longview. These mixed results make their interpretation difficult; in addition, results of bivariate correlations between kidney histopathologies and the primary drivers of site discrimination for liver organic contaminants (HCB, BDE-47, and BDE-154) did not yield significant associations. 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. In the present study, PAHs were not analyzed and no association between kidney histopathologies and total PCBs was observed (data not shown).

Increased MAs in fish tissues has been used as an index of environmental exposure to contaminants (Fournie et al., 2005; Micale and Perdichizzi, 1990; Wolke, 1992). In the present study, liver MA content (count and area) contributed to the separation between Skamania (reference site) and Longview males. Also, spleen MA counts served to discriminate between Columbia City and Longview, and between Columbia City and Skamania males. More specifically, liver MA area was highest in fish from Longview, followed by Skamania and Columbia City, and spleen MA count was highest in males from Columbia City, and males from Skamania had intermediary values. When sum values of contaminant families were used in bivariate correlation analyses, total PCBs and total PBDEs were only weakly ($r < 0.4$) associated with liver macrophage area (data not shown; see also later in Discussion) and the association did not clearly correspond to the identified contaminant gradient—liver MA values for Skamania (reference site) were intermediate between Columbia City and Longview. Thus, either tissue MA levels are not associated with the downstream contaminant gradient in the lower Columbia River or there are tissue MA-inducing contaminants not measured in the present study that follow a non-linear pattern of distribution within the study area.

The health status (biological traits) of female Largescale Sucker did not differ significantly among sites in either year despite some similarities with males in regards to general observations for individual variables (e.g., kidney tubular hyperplasia; Table 2). The failure to identify site-associated differences among female populations could be due to their low sample size (and lower statistical power) relative to male fish. Conversely, this observation with females is consistent with results of previous studies with teleosts that have reported impairments in males, but not in females, after exposure to certain contaminants either in the laboratory (Lema et al., 2008; Muirhead et al., 2006) or in the field (Patiño et al., 2003). In adult fishes, one reason that females may be less sensitive to contaminant exposures than males may be their ability to eliminate lipophilic contaminants via eggs released during spawning (Gundersen et al., 2000).

Incidence (binary) data for external traits and gill condition in males and females were analyzed using univariate analytical approaches. Many individuals of both sexes showed external and gill lesions regardless of collection site. Notably, however, opercular shortening was observed only in fish from Longview and in males the difference among sites was significant. Generally, opercular shortening can be a consequence of dietary imbalances or exposure to contaminants during early development (Beraldo, 2003); for this scenario to apply in this case, the adult fish where this condition was observed would have to have lived in Longview since a very young age. Information on the migratory habits of Largescale Sucker in the lower Columbia River will be necessary for an adequate interpretation of the differences observed in incidences of opercular shortening.

External (skin) parasites were not observed in fish from Skamania, had an intermediate incidence in fish from Columbia City, and showed the highest values in fish from Longview. Results of chi-square analysis did not indicate a significant difference when results were analyzed for males and females separately; however, when data for males and females were combined, differences among sites became statistically significant (Skamania, 0%; Columbia City, 8.3%; Longview, 24%; chi-square analysis, $p = 0.0192$; data not shown). As mentioned earlier, exposure to contaminants can result in higher incidences of parasitic infections (Noga, 2000) and, therefore, the present observations could suggest the existence of an association between the upstream–downstream gradient of contaminant exposures and rates of external parasitic infections in the lower Columbia River. Liver contaminants were not measured in females and, unfortunately, sample sizes were too small for robust examination of the association between male incidence (binary) data and liver organic contaminants in this study.

As mentioned earlier, results of correlation analyses between the primary biological (renal tubular hyperplasia, tubular degeneration, spleen MA count, liver MA area and count, and gill parasites) and liver organic-contaminant drivers (HCB, BDE-47, and BDE-154) resulting from the DFA models failed to identify any associations between the two variable sets. Correlation analysis between the sum values of contaminant families (total DDTs, total OCs excluding DDTs, total PCBs, total PBDEs) and the male biological variables generated by the DFA models also failed to yield significant associations (data not shown). Without additional information, it is difficult to interpret these observations. The apparent lack of connection between overall fish health status and liver organic-contaminant content could indicate that contaminants other than those analyzed in this study (e.g., water soluble contaminants or those that do not accumulate significantly in liver) are responsible for the differences in fish biological condition identified among the sampling sites of this study. Alternatively, there may be non-chemical stressors in the aquatic environment of the lower Columbia River that can also influence fish condition. For example, water turbidity, which can impact fish physiology (Redding et al., 1987), was not measured in the present study.

In summary and conclusion, results of this study revealed the presence of site-associated differences in level of exposure to organic contaminants and the non-reproductive health status of male Largescale Sucker of the lower Columbia River. Biological traits that generally showed an upstream–downstream gradient included renal tubular hyperplasia, gill parasites, opercular shortening and external parasites; liver organic contaminants included HCB, BDE-47, and BDE-154. Although no clear associations between these two variable sets could

be identified, the possible impacts to the health status of fish of other contaminants (e.g., PAHs, heavy metals) or water quality variables not measured in this study cannot be ruled out. The lack of differences among sites in morphometric indices of gonadal condition despite the general downstream increase in organic contaminant concentrations is also noteworthy because this observation is in contrast with field reports for other teleosts. Results of functional sperm analyses reported separately in this issue, however, yielded information suggesting that sperm quality in fish from the present study may have been affected by some of the target contaminants of the study (Jenkins et al., 2014-in this issue). Largescale Sucker can be a useful environmental monitoring tool for the Columbia River basin given their close contact with sediment and role in the food web. To use this tool effectively, however, additional information regarding its physiology, reproduction, migration range and other aspects of its life history will be necessary.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2013.07.112.

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