CONTAMINANTS AND NUTRIENTS IN FISH FROM THE WOLASTOQ | SAINT JOHN RIVER (NEW BRUNSWICK): SPATIAL AND SPECIES VARIABILITY

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Abstract

Fish provide a rich source of nutrients, like the omega-3 fatty acids (FAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), but can also accumulate harmful contaminants such as mercury (Hg), organochlorine contaminants and trace elements. In rivers, dams can alter the distribution and biogeochemical cycling of contaminants and nutrients, which can sometimes increase their availability to and uptake in fish. However, it is unclear whether FAs covary with contaminants within and among species along dammed systems. This study examined the spatial and species differences in contaminants and nutrients in fish from the Wolastoq | Saint John River (New Brunswick), which has a large hydroelectric dam and supports the subsistence fishing of six First Nation communities. In 2020 and 2021, Smallmouth Bass, Yellow Perch, American Eel and Striped Bass were collected from locations upstream and downstream of the dam and analyzed for Hg, FAs, organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), trace elements and stable isotope ratios of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$), to determine the trophic position and basal carbon source of the fish, respectively. Contaminants varied by species where the lipid-rich American Eel were highest in lipophilic contaminants (OCPs and PCBs) and the top predator Striped Bass was highest in biomagnifying elements like Hg and selenium. Furthermore, EPA was highest in Yellow Perch while greater DHA concentrations were observed in higher-trophic-level fish, both within and among species. Fish from the dam's reservoir were highest in the elements sulfur and phosphorus, and Hg for Yellow Perch. The dam also appeared to alter food web dynamics as fish from the reservoir and just below the dam had higher trophic positions, and reservoir fish were depleted in $\delta^{13}C$. Preliminary risk-benefit analyses indicated that the fish do not provide optimal EPA + DHA intake if they are consumed at levels that are considered safe for contaminants like Hg. Overall, this study suggests

that dams can alter food web dynamics and the uptake of contaminants and nutrients by fish, and that location and species are important factors to include in risk-benefit analyses for fish consumption.

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List of all Abbreviations and Symbols

Abbreviation/Symbol	Definition
ANOSIM	Analysis of similarities
ANOVA	Analysis of variance
As	Arsenic
ASU	Analytical Services Unit (Queen's University)
Ba	Barium
BGE	Background electrolyte
BHT	Butylated hydroxytoluene
Ca	Calcium
CCME	Canadian Council of Ministers of the Environment
CE	Capillary electrophoresis
Co	Cobalt
Cr	Chromium
Cu	Copper
CV	Covariance coefficient
DDT	Dichlorodiphenyltrichloroethane
DHA	Docosahexaenoic acid
DL	Detection limit
DORM4	Fish protein certified reference material for trace metals
dw	Dry weight
EPA	Eicosapentaenoic acid
FA	Fatty acid
Fe	Iron
FF	Far Field site
GC-MS	Gas chromatography mass spectrometry
GC-MS/MS	Gas chromatography tandem mass spectroscopy
GC-MSD	Gas chromatography with inert mass selective detector
GLR	Great Lakes Region
Hg	Mercury
HP	Head Pond site
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma-optical emission spectrometry
IRMS	Isotope ratio mass spectrometry
K	Potassium
MAES	Mactaquac Aquatic Ecosystem Study
MECC	Ministry of the Environment and Climate Change
MeHg	Methylmercury

Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
mpm	Meals per month
MQGS	Mactaquac Generating Station
MSI-NACE-MS	Multisegment injection-nonaqueous capillary electrophoresis-mass spectrometry
MTBE	Methyl tert-butyl ether
Na	Sodium
NF	Near Field site
Ni	Nickel
NPRI	National Pollutant Release Inventory
NSERC	Natural Sciences and Engineering Research Council
OCP	Organochlorine pesticide
OGS	Ontario Graduate Scholarship
P	Phosphorus
Pb	Lead
PCA	Principal component analysis
PCB	Polychlorinated biphenyl
PEL	Probable effect level
S	Sulfur
SD	Standard deviation
Se	Selenium
SJR	Saint John River
SPM	Suspended particulate matter
Sr	Strontium
SRB	Sulfur-reducing bacteria
TEL	Threshold effect level
Ti	Titanium
Tl	Thallium
U.S. EPA	United States Environmental Protection Agency
UPS	Upstream site
V	Vanadium
ww	Wet weight
Zn	Zinc
δ	delta
$\delta^{13}C$	Stable isotopes of carbon
$\delta^{15}N$	Stable isotopes of nitrogen
$\delta^{34}S$	Stable isotopes of sulfur
‰	Per mill/parts per thousand

1.0 Introduction

1.1 Spatial and species patterns of contaminants and nutrients in riverine fish

Canadian rivers have been experiencing contamination and eutrophication from human activities for decades. Forestry, agriculture, leaks from transformers and heat-exchangers, coal-fired power plants and mining are examples of activities that release organochlorine pesticides (OCPs; e.g., dichlorodiphenyltrichloroethane (DDT)), nutrients (e.g., nitrogen (N) and phosphorus (P)), polychlorinated biphenyls (PCBs), mercury (Hg), and trace elements (e.g., selenium (Se); Beyer & Biziuk 2009; Driscoll et al. 2013; Mansouri et al. 2017; Ponton et al. 2022) into riverine ecosystems. Many of these contaminants are persistent in nature and can still be found in soils, sediment and aquatic organisms decades after their release (e.g., Kurek et al. 2019). Some of them also bioaccumulate in organisms (uptake > excretion) and biomagnify through food webs (increase in concentration from prey to predator), and their presence in organisms like fish pose a risk to their health and the health of their consumers, including humans. The high prevalence of these contaminants and nutrients in our society, past and present, make it common for freshwaters to experience multiple sources of contamination, yet many studies only examine a limited number of stressors. More information is needed on how large suites of contaminants and nutrients covary in different fish species along greater spatial scales.

Spatial differences in chemicals and biochemicals in riverine fish are largely influenced by point source inputs and diffuse runoff from land uses. The effects of point source releases have been demonstrated at a watershed scale; e.g., higher levels of Hg, DDT, PCBs and other organochlorine contaminants were observed in young-of-the-year spottail shiners (*Notropis hudsonius*) located near petroleum refineries and chemical plants on the St. Clair River compared

to upstream locations (Gewurtz et al. 2010), and at a national scale; e.g., highest [Se] and [arsenic (As)] in Canadian freshwater fish were observed in mining regions (Ponton et al. 2022). Further, land uses like agriculture cause elevated levels of N and P in the water (Carpenter et al. 1998), and metals like chromium (Cr), lead (Pb), As and cadmium (Cd) are higher in sediment and fish from urbanized areas (Ali et al. 2018; Nour et al. 2019; Ali et al. 2021), both of which are not directly linked to point sources. Factors like high forest land cover can also increase terrestrial Hg deposition (Demers et al. 2007; Denkenberger et al. 2012), which can be released to aquatic ecosystems during forest harvesting due the influx of organic matter from the watershed (Garcia & Carignan 2000; Porvari et al. 2003; Millera Ferriz et al. 2021).

Rivers can also transport contaminants and nutrients in water or bound to sediment to downstream locations and food webs, which can create a gradient of concentrations in fish downstream from the source (Patiño et al. 2012). Also, while sediments act as sinks for contaminants and nutrients, natural (e.g., flooding) or anthropogenic (e.g., dredging) disturbances to sediment can re-expose fish and other biota to these compounds (Eggleton & Thomas 2004; Quesada et al. 2014). However, large river catchments often contain various combinations of point-source inputs, land uses and anthropogenic disturbances, along with variable flow dynamics, which makes the spatial distribution of contaminants and nutrients more complex and challenging to predict.

While point sources and land uses influence the exposure of aquatic biota to chemical and biochemical compounds, their individual uptake by fishes is also dependent on trophic factors such as diet and habitat use, both of which can vary by species and location. Since many of these contaminants bioaccumulate and biomagnify, trophic position is often a good predictor of contaminants like methylmercury (MeHg; the organic, bioaccumulative and more toxic form of

Hg; Lavoie et al. 2013), PCBs (Beyer & Biziuk 2009), DDT (Ikemoto et al. 2008) and some elements like Se and As (Córdoba-Tovar et al. 2022). Increased concentrations of MeHg are often found in fish with greater reliance on autochthonous vs allochthonous carbon because the former has higher concentrations of this contaminant (Riva-Murray et al. 2013; Ponton et al. 2021). Demersal species have a close interaction with sediment-bound contaminants and the benthic food web, both of which can increase their levels of contaminants (as exemplified by the higher concentrations in demersal Cod (*Gadus morhua*) compared to the pelagic Saithe (*Pollachius virens*; Bustnes et al. 2012)). Higher PCBs in fish have been linked to greater reliance on detrital carbon sources within a site, despite higher historical PCB contamination at another site (Lopes et al. 2011), revealing the strong influence these trophic processes have on contaminant concentrations in fish.

The accumulation of contaminants is also dependent on physical characteristics in fish like length, age and growth rates. Length and age are often good predictors of the concentrations of bioaccumulative contaminants as larger fish consume more food to meet their caloric needs, thereby exposing them to greater quantities of contaminants from their diet, and older fish have accumulated contaminants for a longer period of time (van der Oost et al. 2003; Dang & Wang 2012). Faster-growing fish also have lower contaminant concentrations in their tissue as the contaminants are diluted by larger tissue gains compared to slower-growing fish (Luk & Brockway 1997; Wang 2012). This process can similarly occur at lower trophic levels where higher primary productivity dilutes contaminants across a greater biomass of primary producers, which can reduce dietary exposures in higher trophic levels (Pickhardt et al. 2002; Walters et al. 2015). Therefore, factors that can affect fish growth and productivity in systems can also influence the spatial patterns of contaminants in fish.

Nutrients can also vary by fish species depending on physiological requirements. Several elements (often termed as minerals in fish nutrition) are essential to fish, meaning that they play a vital function in these organisms and exposure below certain quantities adversely affects their physiological processes (Mertz 1998). Fish require elements in high (macrominerals; e.g., calcium (Ca), P, sulfur (S), magnesium (Mg), sodium (Na), potassium (K) and chlorine) or low (microminerals; e.g., iron (Fe), Se, copper (Cu), iodine, zinc (Zn) and manganese (Mn)) levels and these elements play important structural, physiological, catalytic, and regulatory roles (Lall 2021). Species have different requirements for certain elements, e.g., Mg requirements are higher in cyprinids compared to salmonids and silurids, which may be related to less efficient uptake from the stomach of cyprinid fish (Antony et al. 2016). However, most research on physiological requirements of fish for these minerals are focused on species commonly used in aquaculture practices and there is less known about their presence in wild, freshwater fish.

1.2 Fatty acids in fish

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are long-chain polyunsaturated fatty acids (FAs) that have been the subject of a lot of research, primarily for their benefits in human health, yet they also play important roles in fish health and functioning. These FAs are produced in aquatic ecosystems, largely by microalgae, and are then taken up by higher-level consumers (Gladyshev et al. 2013). DHA plays a critical role in fish neural development and function, and vision (Sargent et al. 1999; Tocher 2003; Pilecky et al. 2021) while EPA has important metabolic functions in maintaining cardiovascular health, immune and inflammatory responses, and gene expression (Tocher 2015). Deficiencies of EPA and DHA can lead to decreased growth rates, fecundity, reaction time, immune responses, swimming speed and escape behaviour in fish (Izquierdo 1996; Furuita et al. 1998; Benítez-Santan et al. 2007;

Benítez-Santana et al. 2014; Bou et al. 2017; Gesto et al. 2021; Pilecky et al. 2021). While these FAs are critical in all fish, their concentrations can vary considerably between and within species and our understanding of their presence in wild fish is limited.

FA compositions in fish are associated with phylogeny and related life history traits (e.g., evolved feeding mechanisms and environmental niches). Phylogenetic relatedness is one of the strongest predictors of similarity in EPA and DHA concentrations between species (Gladyshev et al. 2018). This has been exemplified in some studies showing greater differences in EPA and DHA content between species, than within a species found in different environmental conditions (e.g., temperature, lake trophic status; Gomes et al. 2016; Sushchik et al. 2018). Variations in FA composition between fish species are hypothesized to be influenced by differences in physiological requirements and acquisition related to life history traits. For example, piscivorous fish tend to have high levels and will selectively retain DHA even when dietary supply is high (Heissenberger et al. 2010; Williams et al. 2014; Gomes et al. 2016; Sushchik et al. 2017; Jardine et al. 2020), likely because DHA is linked to quick muscle movements, swimming, and vision which are necessary to support their predatory lifestyle (Pilecky et al. 2021). In fact, swimming velocity is shown to be one of the highest predictors of DHA in fish (Gladyshev et al. 2018). Furthermore, marine and anadromous fish tend to have higher EPA and DHA concentrations than freshwater fish (Li et al. 2011; Gladyshev et al. 2012a; Gladyshev et al. 2018).

Since EPA and DHA cannot be synthesized *de novo* in vertebrates, and therefore must be obtained from their diet (Parrish 2013), differences in feeding behaviours (e.g., planktivory, omnivory, piscivory) also influence these FAs in fish. Planktivorous fish have high levels of EPA and DHA because their diet of phytoplankton and zooplankton tends to be rich in these FAs (Gladyshev et al. 2015; Strandberg et al. 2015; Gladyshev & Sushchik 2019). Furthermore,

sometimes diet can have a greater influence on EPA and DHA concentrations than phylogenetic relatedness. For example, feeding habit was a better predictor of EPA + DHA concentrations in sport fish from Wisconsin lakes than taxonomy was (Williams et al. 2014). Generally, species are more likely to have distinct EPA and DHA concentrations if they are less phylogenetically related and if their diet and habitat use are also distinct. Nevertheless, the bioaccumulation of these FAs in fish is not always predictable as some FAs are selectively retained (e.g., DHA; Jardine et al. 2020), and therefore, more research is needed to understand the factors affecting their concentrations in fish.

Environmental factors, like trophic status, brownification and pollution, can also influence EPA and DHA concentrations in fish through bottom-up shifts in primary producers. In explanation, the microalgae classes Cryptophyceae, Dinophyceae and Bacillariophyceae are relatively high in these FAs (with some outlier species, e.g., *Cyclotella sp.*) while Chlorophyceae and cyanobacteria cannot produce these long-chain FAs (Gulati & Demott 1997; Petkov & Garcia 2007; Iliev et al. 2011; Gladyshev & Sushchik 2019); therefore, when factors like eutrophication or browning shift algal community composition towards the latter group, less dietary EPA and DHA are available to higher trophic levels (Taipale et al. 2016). Indeed, water trophic status alone (as measured by total P or chlorophyll-a) is not always negatively associated with [EPA] and [DHA] in fish (Gomes et al. 2016; Marques et al. 2020), which reinforces the importance of examining the composition of basal food sources. Pollution can similarly impact FA availability and concentrations in consumers as EPA- and DHA-producing microalgae are sensitive to heavy metal and organic pollution, which can cause a shift in the community composition (Gladyshev et al. 2012b). Thus, environmental factors that influence FAs in fish are

primarily those that influence the community composition of EPA- and DHA-producing microalgae.

1.3 Influence of dams on contaminants and nutrients in fish

Many of the world's rivers are controlled by dams, which can have negative impacts on riverine ecosystems by fragmenting habitats, disrupting hydrological cycles, and producing large amounts of methane and some contaminants like MeHg (Friedl & Wüest 2002; Hall et al. 2005; Scott et al. 2019). As described below, dams can also influence the availability, uptake, and accumulation of contaminants and nutrients in fish by altering trophic processes and biogeochemical cycles and by trapping sediment-bound contaminants and nutrients. These effects become noticeable soon after the flooding of the reservoir and can last throughout the dam's lifespan. Given that the number of hydroelectric dams is projected to increase to help achieve carbon-reduction goals (Grill et al. 2015; Zarfl et al. 2015), it is important to understand how they affect the spatial distribution of contaminants and nutrients in fish within river systems.

Initially, dams can introduce terrestrially-stored contaminants or nutrients into the reservoir through the flooding of the surrounding soil. This phenomenon has been primarily studied for Hg as flooded soils are a hotspot for Hg methylation, the process by which inorganic Hg gets transformed into MeHg, which is facilitated by sulfur-reducing bacteria (SRB; St. Louis et al. 2004). This in turn increases the amount of bioavailable MeHg that can enter the food web and biomagnify to top predators, like fish (Bodaly et al. 2007; Kasper et al. 2012; Bilodeau et al. 2017; Ponton et al. 2021). Long-term studies have shown that Hg concentrations in reservoir fish peak between 2-14 years post dam construction and that it can take up to 20 years for nonpiscivorous fish and up to 31 years for piscivorous fish to return to their pre-flooding Hg concentrations (Bodaly et al. 2007; Bilodeau et al. 2017). This impact is nationally recognized as

reservoirs are one of the primary factors influencing high levels of Hg in fish across Canadian freshwaters (Ponton et al. 2022).

Dams block and reduce water flow, which increases the rate of sedimentation in the reservoir, making them a sink, and potentially a subsequent source, of sediment-bound contaminants and nutrients (Schleiss et al. 2016). For example, dams have significantly reduced the downstream transport of nutrients, like P, and it is predicted that by 2030, 17% of the total P in rivers will be sequestered by reservoir sediments (Maavara et al. 2015). The sediments upstream from dams can also act as a sink for contaminants like trace metals, however, the subsequent release of water from the hypolimnion by the dam can disturb the reservoir sediments and facilitate the transport of sediment-bound contaminants downstream, thereby acting as a source (Vukovic et al. 2014; Dong et al. 2019). The fluxes of nutrients and contaminants from the reservoir to downstream locations also depends on the time-scale under evaluation. For example, seasonally the Fanshawe reservoir (Thames River, ON) fluctuated between a P source and sink to sites downstream, but on an annual basis, the reservoir acted as a net sink retaining between 25-47% of total P (Kao et al. 2022). However, the fluxes of nutrients and contaminants from dams are often not studied in tandem and it is unclear how they covary in upstream and downstream fish.

Dams can also alter biogeochemical cycles of elements, like P and S, through the creation of a lentic, stratified environment in the reservoir. In stratified reservoirs, P can be converted to dissolved reactive P (the bioavailable form of P) and S is reduced to sulfide at the anoxic sediment-hypolimnion interface, and also oxidised to sulfate in the oxygenated epilimnion (Chowdhury & Bakri 2006; Liu et al. 2017; Shi et al. 2020; Yang et al. 2020; Koa et al. 2022). The enhanced cycling of these elements can affect related biochemical processes, e.g., by

increasing Hg methylation due to the higher activity of SRB (Liu et al. 2018; Xiang et al. 2018), and by increasing concentrations of bioavailable P and S in the reservoir and downstream. However, the potential for this P and S to accumulate through food webs in dammed environments has not been examined.

Furthermore, the reduced water flow in the reservoirs can shift food webs from riverine- to lacustrine-energy processes, with increased reliance on autotrophic plankton (Wang 2020; Yang et al. 2022). This shift in basal carbon sources in reservoirs can consequently affect the bioaccumulation of certain contaminants like MeHg (Riva-Murray et al. 2013; Ponton et al. 2021). As discussed above, differences in food web characteristics can also influence accumulation of other organic contaminants, trace elements and fatty acids, however, this has not been well studied in reservoir ecosystems.

1.4 Stable isotopes

As numerous aquatic contaminants, including MeHg, PCBs, OCPs and some trace elements, are persistent in nature and not readily metabolized or excreted by organisms, they tend to accumulate in upper trophic levels of the food web (Ikemoto et al. 2008; Lavoie et al. 2013) and are affected by several factors including diet. Stable isotopes ratios of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) provide a valuable tool to determine the trophic position and basal food source supporting fish, respectively, and to better understand contaminant fate in aquatic systems. $\delta^{15}N$ increases predictably by 2-4‰ between consumers and their diet and is used to determine a consumer's trophic position relative to organisms at the base of the food web (Minagawa & Wada 1984; Post 2002). Ratios of ^{13}C:^{12}C (expressed as $\delta^{13}C$) vary between allochthonous and autochthonous carbon sources and $\delta^{13}C$ changes very little from prey to predator, thus providing information on the basal food sources supporting consumers (Post 2002; Perkins et al. 2014). As

such, measures of stable isotopes in fish and their prey help to understand among and within species variability in the levels of PCBs, DDT, Hg and other elements as diet is the main route of exposure of fish to contaminants (McIntyre & Beauchamp 2007; Ordiano-Flores et al. 2021).

1.5 History of contaminants in the Wolastoq | Saint John River

The Wolastoq | Saint John River (SJR) is the second longest river in eastern North America with a river basin area of over 55,000 km^2 (Kidd et al. 2011). It flows through Maine, Quebec, and then New Brunswick, where it discharges into the Bay of Fundy (SJRBB 1975; Cunjak & Newbury 2005). Industrialization in the river basin rapidly increased in the early to mid 1900s which was characterized by an increase in pulp and paper mills, mining activities, food processing plants, and the construction of large dams (Kidd et al. 2011). The main channel of the river is currently home to three hydroelectric dams: the Grand Falls Generating Station, the Beechwood Generating Station, and the Mactaquac Generating Station (MQGS). MQGS, constructed between 1965-1968, is the largest of the three with a generating capacity of 660 MW and it supplies ~12% of New Brunswick's energy (NB Power 2021). Its construction also produced a large reservoir covering 84 km^2 (Kidd et al. 2011). The industrial activities were suggested to act as sources of contaminants to the Wolastoq | SJR, and the addition of dams likely affected their fate.

Given the links between reservoir creation and Hg in fish, earlier studies have assessed this and other contaminants in fish from the Wolastoq | SJR. The first measures of Hg in freshwater fish from New Brunswick (including from the Wolastoq | SJR) were high, with nine out of 17 species exceeding Canadian guideline for Hg in retail fish (0.5 μg/g ww; Zitko et al. 1971). From the 1970s-1990s, species demonstrating high levels of Hg from the Wolastoq | SJR included Striped Bass (*Morone saxatilis*, range: 0.39 to 3.82 μg/g ww), American Eel (*Anguilla*

rostrata, range: 0.55 to 2.08 µg/g ww), Smallmouth Bass (*Micropterus dolomieu*, range: 0.74 to 0.81 µg/g ww), and Yellow Perch (*Perca flavescens*, range: 0.4 to 1.05 µg/g ww; Zitko et al, 1971; Meth 1972; Dadswell 1975; Prouse & Uthe 1994). Interestingly, some species like American Eel had higher Hg concentrations in the MQGS reservoir compared to other sites (Zitko et al. 1971). Furthermore, OCPs were commonly applied in New Brunswick for agricultural purposes or to control forest pests (Keachie & Côté 1973). Concentrations of DDT were recorded in Striped Bass, which had mean total DDT levels in muscle tissue ranging from 0.19 to 0.44 µg/g ww (Dadswell 1975; Prouse & Uthe 1994). Additionally, PCB Arochlor 1254 (common in industrial uses) was recorded at concentrations ranging from 0.07 to 0.4 µg/g ww in Striped Bass tissue (Dadswell 1975). Although some of these reports had relatively low sample sizes, they indicated alarming concentrations of contaminants in fish, presenting a risk to the fish themselves and their consumers.

Since the implementation of governmental bans or stricter release laws for these contaminants (Hg, DDT, PCBs), more recent reports have shown that Hg concentrations appear to be decreasing in most of the aforementioned species; however levels are still relatively high in Smallmouth Bass and Striped Bass relative to the Canadian guideline on Hg in retail fish (Reinhart & Kidd 2018). Furthermore, some species like Smallmouth Bass had higher Hg concentrations in the MQGS reservoir compared to downstream sites, however this could be attributed to the larger fish caught upstream (Reinhart & Kidd 2018). The small sample sizes yet presence of high Hg concentrations in some fish warrants further investigation. Additionally, some trace elements analyzed in sediment along the river exceeded the Canadian Council of Ministers of the Environment's (CCME) sediment quality guideline for Probably Effect Levels (PELs) and Threshold Effect Level (TELs; Kidd et al. 2019), yet trace element concentrations

have not been examined for fish in this river. Furthermore, there are no recent updates on PCB or OCP concentrations in fish tissue, and no data on the mineral and FA contents of fish from this river. From previous studies, there is some evidence that the MQGS is influencing the contaminant concentrations in fish and therefore it is important to quantify the current state of contaminants and nutrients in fish across a greater spatial scale in this river. Importantly, these fish are consumed by local Indigenous and recreational fishers and therefore, measuring the current concentrations of contaminants and nutrients in these fish will help inform consumers of the risks versus benefits of fish consumption.

1.6 Objectives

In this study, I measured a suite of contaminants (trace elements, Hg, PCBs and OCPs) and nutrients (FAs and select trace elements) in four fish species from the Wolastoq | SJR at sites upstream and downstream from the MQGS to examine: 1) species and spatial differences in contaminants and nutrients in fish in relation to the MQGS; and 2) the relationships between diet (as indicated by stable isotope ratios of $\delta^{15}N$ and $\delta^{13}C$) and the concentrations of contaminants and nutrients in fish.

2.0 Methods

2.1 Study site

Samples were collected from four sites on the Wolastoq | SJR relative to the MQGS: 1) Upstream (UPS), which begins ~100 km upstream and ends ~79 km upstream of MQGS; 2) Head Pond (HP), which lies within the reservoir, extending ~10 km upstream from MQGS; 3) Near Field (NF), which begins at the outflow of MQGS and extends ~18.5 km downstream; and 4) Far Field (FF), which begins ~35 km downstream of the MQGS and ends ~75 km from the river's outflow into the Bay of Fundy (Fig. 1). The UPS site is in the Meductic ecodistrict of the

Valley Lowlands ecoregion, a region characterized by Ordovician calcareous sedimentary rocks and Devonian granitic rocks, with land cover of approximately 65% forest (a mix of hardwood and softwood), 25.2% agriculture, 3.86% water, 2.8% wetlands, 1.4% roads and 1.75% other development (Zelazny 2007). The three other sites (HP, NF and FF) are located in the Aukpaque ecodistrict of the Grand Lowlands ecoregion, comprised of primarily Pennsylvanian sedimentary rocks, with Silurian to Mississippian calcareous and non-calcareous sedimentary strata dominant upriver of Fredericton (in the HP and part of the NF site). This area's land cover is approximately 62% forest (mixed hardwood and softwood), 10.26% agriculture, 10.26% wetlands, 7.98% water, 1.52% roads and 7.98% other development (Zelazny 2007). Throughout the sites, there is an average of ~400-500 mm of precipitation between May-September and an annual average of 1500-1800 degree-days above 5 °C (Zelazny 2007).

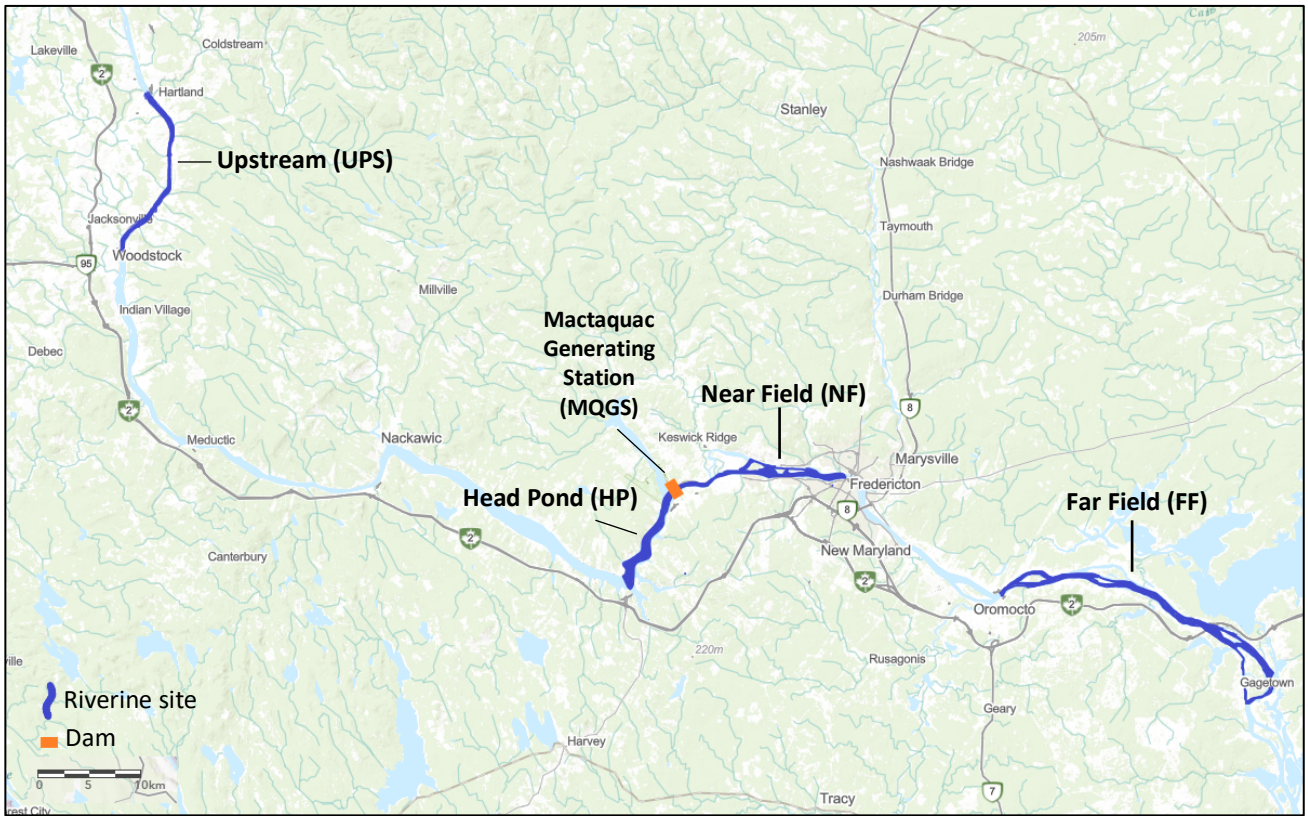


Figure 1. Sampling sites on the Wolastoq | SJR (New Brunswick, Canada).

2.2 Field sampling

2.2.1 Fish collection and processing

Smallmouth Bass, Yellow Perch, Striped Bass and American Eel were collected in the summer and fall of 2020 and 2021. Approximately 20-50 Yellow Perch and Smallmouth Bass were collected at each of the four sites, with efforts to obtain equal sex ratios. These species were selected due to their high abundance at each site and due to their differences in diet where Smallmouth Bass are a 1st rank predator, whereas Yellow Perch are a 1st or 2nd rank predator (Froese & Pauly 2022). Additionally, 14 Striped Bass and 24 American Eel were caught within the NF site, which were selected based on interest from local Indigenous communities. Fish were caught using electro-fishing (Smallmouth Bass and Yellow Perch), angling (Striped Bass, Smallmouth Bass and Yellow Perch), and traps (American Eel), euthanized using procedures approved by the UNB Animal Care Committee (Animal Use Protocol 18029) and kept on ice until processed. The total length (mm; ± 1 mm), fork length (mm; ± 1 mm) and wet weight (g; ± 0.2-2 g, depending on the scale) of the fish were recorded, and they were then dissected and sexed; see Table 1 for the sample sizes, sexes, and mean lengths, weights and ages of fish. A sample of skinless muscle tissue (target of 20 g) was collected, placed in a plastic bag, flash frozen on dry ice, and kept on dry ice until they were stored at -80 °C. The otoliths were also collected for aging purposes. Dissection tools and surfaces were cleaned with 70% ethanol between each fish.

Table 1. Sample sizes (N), sexes and mean (SD) length, weight and age of individual fish samples used for Hg, stable isotope and fatty acid (FA*) analyses for Smallmouth Bass and Yellow Perch collected from all sites and American Eel and Striped Bass collected from the NF site in the Wolastoq | SJR (New Brunswick). The number of fish with no length (NL), no weight (NW) or no age (NA) recorded is presented in brackets next to the SD.

Species	Site	N	Sex			Total length (mm)	Total weight (g)	Age (years)
			M	F	NS			
American Eel	NF	24			24	557 (169)	238 (82; 4 NW)	14 (3; 4 NA)
Striped Bass	NF	14	7	4	3	854 (77; 1 NL)	6600 (1698; 3 NW)	13 (6; 3 NA)
Smallmouth Bass	UPS	48	28	20		401 (49)	998 (367)	8 (3; 2 NA)
	HP	19	9	10		375 (68)	751 (364)	7 (4)
	NF	65	30	29	6	370 (81)	785 (443)	8 (3)
	FF	47	26	21		350 (64)	602 (370)	6 (3)
Yellow Perch	UPS	53	26	26	1	208 (32)	108 (56)	6 (2; 1 NA)
	HP	28	13	15		192 (47)	87 (66)	5 (2)
	NF	60	27	32	1	203 (32)	105 (45)	4 (2)
	FF	53	27	26		186 (24)	79 (35)	8 (2)

*FA analysis was run on a subset of the Smallmouth Bass and Yellow Perch presented in this table; the sample sizes, sexes and mean (SD) length, weight and age of these fish are presented in Table S1.

2.2.2 Benthic invertebrate collection and processing

Amphipods and snails were collected using kick nets from two nearshore locations within each site (except HP) in 2020. Sites were selected based on access to the shoreline and proximity to where fish were caught. The amphipods and snails were cleaned of surficial mud or algae and

placed in filtered river water for 24 hours to clear their guts. After, they were placed in a plastic bag, flash frozen on dry ice and kept on dry ice until they were stored at -80 °C.

Additionally, I used stable isotope data from benthic invertebrates and zooplankton collected in the NF and HP by the Mactaquac Aquatic Ecosystem Study (MAES). For full methods on their collection and analyses see Dolson-Edge et al. (2019). Briefly, invertebrate organisms were collected using a kick net or an Eckman Grab at six locations within the NF and HP and placed on ice until they could be frozen in the laboratory.

2.3 Laboratory

The fish samples were analyzed for ages, total mercury, trace elements, organochlorine pesticides, stable isotopes and fatty acids at several different labs. The locations of these analyses are summarized in Table 2.

Table 2. Sample types and agencies that ran analyses for the various contaminants and nutrients analyzed in fish from this study.

Analysis	Sample type	Agency
Aging	Otolith	Canadian Rivers Institute (New Brunswick) & North/South Consultants Inc. (Manitoba)
Mercury	Freeze-dried tissue of individual fish	Dr. Karen Kidd's lab, McMaster University (Ontario)
Stable isotopes	Freeze-dried tissue of individual fish	Stable Isotope in Nature Laboratory (New Brunswick)
Organochlorine pesticides, polychlorinated biphenyls and trace elements	Freeze-dried tissue of composite fish	Queen's Analytical Services Unit (Ontario)
Fatty acids	Freeze-dried tissue of individual fish	Dr. Britz-McKibbin lab, McMaster University (Ontario)
Fatty acid method comparison	Freeze-dried tissue of individual fish	Dr. Britz-McKibbin lab, McMaster University (Ontario) & Fisheries and Oceans Canada (Winnipeg)

2.3.1 Fish aging

Otoliths were sent to the Canadian Rivers Institute (New Brunswick) and North/South Consultants Inc. (Manitoba) for aging. Otoliths were encased in epoxy until hardened and cut into a transverse section using diamond wafer blades (IsoMet 1000 Precision Saw). The section was sanded, polished and viewed under the microscope to count the annuli. Each annulus corresponds to one year and therefore, the number of annuli corresponded to the age of the fish.

2.3.2 Benthic invertebrate identification and processing

Amphipods and snails were sorted to family using morphological features and the keys "Ecology and Classification of North American Freshwater Invertebrates" (Thorp & Covich 2001) and "North American Freshwater Snails" (Burch 1982), respectively. After, the snails were de-shelled and invertebrates were rinsed with distilled water to remove any debris. Individuals were pooled within family and site to obtain adequate sample masses for stable isotope analysis (2-16 individuals per sample), and samples were freeze-dried and homogenized.

Invertebrates collected by MAES in 2017 were identified to the closest taxonomic group possible, oven-dried at 60 °C for 48 hours and homogenized (Dolson-Edge et al. 2019).

2.3.3 Mercury analysis

A subsample of the fish muscle tissue was analyzed for total mercury (hereafter referred to as Hg; see Table 1 for sample sizes). Samples were placed in acid-washed glass vials and their wet weights (± 0.0001 g) were recorded. The samples were freeze-dried for at least 48 hours, weighed, and homogenized using acid-washed glass stir rods. The percent moisture of the fish was calculated using the wet weights (ww) and dry weights (dw) of the tissue samples. Hg (µg/kg dw) was measured using a Milestone TriCell Direct Mercury Analyzer-80® using U.S. EPA method 7473 (U.S. EPA 2007a). In this method, Hg is quantified using thermal

decomposition, gold amalgamation and atomic absorption spectrophotometry. QA/QC measures included method blanks, two liquid standards with known Hg concentrations, a stability standard, a certified reference material (DORM-4, fish protein, Willie et al. 2012), and sample duplicates run every 10th sample. Method blanks < 0.002 mg/kg Hg and standards/reference material within a ± 20% range were deemed acceptable; if outside range, samples were rerun. The average % recovery for the reference material was 98.1 ± 12.9% (n=73) and the average % difference in duplicate samples was 3.15 ± 4.24% (n=47 pairs).

2.3.4 Stable isotope analysis

Freeze-dried, homogenized tissues of individual fish (see Table 1 for sample sizes) and pooled amphipods and snails were used for stable isotope analyses of $\delta^{13}C$ and $\delta^{15}N$. Samples were weighed (1.00-1.20 mg) and folded into tin capsules. The samples were sent to the Stable Isotope in Nature Laboratory (New Brunswick) where stable isotopes were measured using a Costech 4010 elemental analyzer coupled with a DeltaPlus XP isotope ratio mass spectrometry (IRMS) with Conflo III continuous flow. Isotopic results were expressed in the delta notation (δ), which indicates the ratio between the heavy and light isotopes of an atom, relative to an international standard (Vienna Pee Dee Belemnite and atmospheric air for $\delta^{13}C$ and $\delta^{15}N$, respectively) in parts per thousand (‰). QA/QC measures included certified reference materials (USGS61, N2, CH7), in-house standards (Nicotinamide, Bovine Liver Standard and Muskellunge Muscle Standard), and duplicates run every 10th sample. Mean precision of the certified reference materials was 0.08‰ (SD; n=37) for C and 0.09‰ (SD; n=37) for N and mean precision of in-house reference materials were 0.05‰ (SD; n=63) for C and 0.11‰ (SD; n=63) for N. The average % differences in sample duplicates were 0.19 ± 0.18% for $\delta^{13}C$ and 0.49 ± 0.41% for $\delta^{15}N$ for fish (n=41 pairs), 2.86 ± 3.92% for $\delta^{13}C$ and 3.45 ± 3.16% for $\delta^{15}N$ for

amphipods (n=2 pairs), and 3.94 ± 3.13% for $\delta^{13}C$ and 3.24 ± 1.50% for $\delta^{15}N$ for snails (n=5 pairs).

Invertebrates collected by MAES in 2017 were analyzed using the same methods described above. Analytical precision, measured as the SD of replicate standards, was less than ± 0.2‰ for $\delta^{15}N$ and $\delta^{13}C$. Duplicate determination of actual samples agreed to within ± 0.2‰ for $\delta^{15}N$ and $\delta^{13}C$ (Dolson-Edge et al. 2019).

2.3.5 OCP, PCB and trace element fish sample prep

Freeze-dried homogenized fish tissues were pooled by species and site (two to six fish per pool, six to eight pooled samples per species and site) to obtain adequate mass for analysis. Within species, fish were grouped by length to obtain similar mean lengths of pooled samples across sites (sample sizes and mean lengths, weights, ages and percent lipid of the pooled samples are presented in Table 3). Pooled samples (hereafter referred to as composite samples) were kept frozen until subsampled and analyzed for OCPs, PCBs and trace elements as described below.

Table 3. Sample size (N) and mean (SD) length, weight, age and % lipid of composite samples used for trace element, PCB and OCP analyses of Smallmouth Bass and Yellow Perch collected from all sites and American Eel and Striped Bass collected from the NF site in the Wolastoq | SJR (New Brunswick). Percent lipid (of dried tissues) was measured on the composite samples while lengths, weights and ages were measured on individual fish and then averaged within each composite before an overall average across composites was calculated.

Species	Site	N (composited)	Total length (mm)	Total weight (g)	Age (years)	Percent lipid
American Eel	NF	8 (20)	602 (197)	259 (85)	15 (2)	23.48 (8.74)
Striped Bass	NF	5 (10)	838 (78)	6410 (1642)	12 (4)	4.40 (0.78)
Smallmouth Bass	All sites	24 (75)	382 (65)	823 (381)	8 (3)	2.79 (1.01)
	UPS	6 (20)	398 (43)	971 (295)	8 (2)	3.57 (1.29)
	HP	6 (17)	380 (73)	767 (396)	8 (4)	2.38 (0.64)
	NF	6 (19)	371 (78)	781 (447)	8 (3)	2.62 (0.90)
	FF	6 (18)	377 (74)	775 (436)	8 (4)	2.59 (0.91)
Yellow Perch	All sites	23 (80)	203 (32)	101 (47)	6 (2)	1.45 (0.31)
	UPS	6 (20)	214 (35)	114 (53)	7 (2)	1.33 (0.12)
	HP	6 (20)	204 (37)	99 (54)	5 (1)	1.68 (0.22)
	NF	6 (20)	199 (34)	100 (48)	5 (1)	1.16 (0.29)
	FF	5 (20)	193 (24)	90 (38)	8 (1)	1.66 (0.23)

2.3.6 OCP and PCB analyses

OCP and PCB analyses were conducted by the Queen's University Analytical Services Unit (ASU; Ontario) following U.S. EPA methods 8081B (U.S. EPA 2007b), 3545A-1 (U.S. EPA 2007c), 3500C (U.S. EPA 2007d), and 8082A-1 (U.S. EPA 2007e). Prior to extraction, two

surrogates, DCBP and RS (PCBs), sodium sulphate (5-10 g) and Ottawa sand (5-10 g) were added to each composite (~3-4 g dw). Samples were extracted for six hours using a Soxhlet and 250 mL of Optima grade dichloromethane. The extract was concentrated by roto-evaporation to ~1 mL, reconstituted to 10 mL in dichloromethane, and then a subsample was cleaned up and fractionated with a 1 m gel permeation chromatography column (70 g S-X3 BioBead stationary phase, 100% dichloromethane mobile phase). The fractions containing lipids and OCPs/PCB congeners were collected. Lipids were determined gravimetrically and reported as percent dw (see Table 3 for % lipid of fish). The OCP/PCB congener fraction was concentrated to ~1 mL hexane, separated on Fluorisil into two fractions with hexane and then dichloromethane, and combined for a final volume of 10 mL.

Prior to OCP analysis, subsamples of extracts (100 µL) were spiked with an internal standard. Extracts were run by GC-MSD (gas chromatography with inert mass selective detector) using an Agilent 6890N GC 5975 MS, an HP-5MS capillary column (30 m, 0.25 mm i.d. x 0.25 µm film thickness) and an Enhanced ChemStation software (MSD ChemStation D.02.00.275). The conditions were as follows: Sample volume 1 µL, pulsed splitless injection, temperature programmed ramp, and constant helium carrier gas flow. Data were collected for selected ions within the mass range of 66-510 m/z. Data selection criteria were based on compound retention time, and on the relative intensity of primary and secondary ions for standard reference OCPs and extracted samples. The OCPs quantified (ng/g dw) included DDTs, methoxychlor, etc., and only values above DL are reported herein (see Table S2 for congeners and DLs). Calibration standards containing known concentrations of all reported OCPs were used for quantitation. The acceptable range of % recoveries for samples was 40-150%. QA/QC measures include control samples, blanks and duplicates extracted for 10% of the samples. Average % recovery for

controls was 97.8 ± 12.8% for DDT and its breakdown products (n=8) and 95.5 ± 11.8% for methoxychlor (n=8), and the average % difference in duplicates was 9.84 ± 17.2% for total DDT and 7.98 ± 6.90 for methoxychlor (n=7 pairs).

For PCBs, a portion of the 10 mL extract was concentrated ten times and a 100 µL aliquot was spiked with an internal standard. The samples were analyzed by GC-MS/MS (gas chromatography tandem mass spectroscopy) using a Varian 4000 GC-MS (gas chromatography mass spectrometry), an SGE Forte capillary column (60 m, 0.25 mm i.d. x 0.25 µm film thickness) and Varian MS Workstation V.6 software. The conditions were as follows: Sample volume 1 µL, splitless injection, temperature programmed ramp, and constant helium carrier gas flow. Data were collected for selected ions within the mass range of 150-550 m/z. Data selection criteria were based on compound retention time and on the relative intensity of primary and secondary ions for standard reference congeners and extracted samples. Calibration standards containing known concentrations of all 209 PCB congeners were used for quantitation, and values above DLs were reported in ng/g dw (see Table S3 for a list of PCB congeners and DLs). QA/QC measures included control samples, blanks and duplicates extracted for 10% of the samples. Average % recovery for controls was 91.4 ± 24.0% (n=9) and average % difference in duplicates was 33.1 ± 27.9% for total PCBs (n=9 pairs).

2.3.7 Trace element analyses

Pooled fish samples were analysed for 30 trace elements by the Queen's University ASU. Tissues samples (~0.5 g dw) were prepared for extraction following procedures described in U.S. EPA method 200.7 (U.S. EPA 2001) and acid digested following in-house procedure ASU07 (ASU N.D.) using trace grade solvents. Samples were extracted using 2 mL of concentrated HNO_3 and 6 mL of concentrated HCl heated at 50 °C for 5 hours followed by 95 °C for 2 hours,

and reconstituted to a final volume of 25 mL using double deionized water. The samples were analyzed for some elements using ICP-MS (Inductively Coupled Plasma Mass Spectrometry) using a 7700x ICP-MS spectrometer (Agilent Technologies) and the U.S. EPA method 6020A (U.S. EPA 1998). Boron, P and S were quantified using ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry) using an iCAP 7400 ICP-OES spectrometer (THERMO scientific) and an in-house method. Element concentrations were reported in µg/g dw (see Table S4 for elements and DLs). QA/QC measures included two control samples, a certified reference material (TORT-3), blanks and duplicates extracted for 10% of the samples. Average % recovery of each element ranged from 87.3 to 103% for the controls (n=6) and from 91.1 to 101% for the certified reference material (n=4), and an average % difference in duplicates ranged from 0 to 79.4% (n=6 pairs).

2.3.8 Fatty acid analysis

Approximately 200 samples that reflected a range in Hg levels (n = ~20 per species and site) were measured for the FAs EPA and DHA. Approximately 10 g of frozen fish tissue from individuals was pulverized over liquid nitrogen to obtain a homogenized sample. A portion of this homogenate was freeze-dried and stored at -80 °C until analysis.

One hundred µL of 2.5 M sulfuric acid and 100 µL of 0.01% v/v butylated hydroxytoluene (BHT; lipophilic antioxidant) in toluene were added to the vials containing ~5 mg of freeze-dried muscle from an individual fish. The mixture was incubated at 80 °C for one hour and 500 µL of 2.5 µM myristic acid-d27 (recovery standard) in methyl tert-butyl ether (MTBE) was then added to the mixture. The mixture was vigorously shaken (3000 rpm) for 15 minutes at room temperature followed by the addition of 200 µL of deionized water to induce phase separation. The samples were then centrifuged at 3000 g at 4 °C for 15 minutes to

sediment protein at the bottom of the vial followed by a biphasic water layer and an ether (MTBE) layer. A fixed volume (400 µL) was collected from the upper MTBE layer and transferred into a new vial, which was dried under a gentle stream of nitrogen gas at room temperature. The samples were diluted three times by repeating the addition of the recovery standard, vigorous shaking, centrifuging, and collection/drying of the upper MTBE layer two more times. Fish extracts were then stored at -80 °C and, before analysis, were reconstituted in 75 µL of acetonitrile/isopropanol/water (70:20:10) with 10 mM ammonium acetate and 50 µM stearic acid-d35 (internal standard). Samples were further diluted 5- or 10-fold before they were run on the instrument.

FA analysis was completed using Multisegment Injection-Nonaqueous Capillary Electrophoresis-Mass Spectrometry (MSI-NACE-MS) using an Agilent 6230B time-of-flight mass spectrometer with an electrospray ionization source equipped with an Agilent G7100A capillary electrophoresis (CE) unit (Agilent Technologies Inc., Mississauga, ON). Details on the instrumental methods are described in Azab et al. (2019). Changes to the methods were as follows: The bare fused-silica capillaries were 120 cm long, the CE separations occurred at an isocratic pressure application of 5 mbar (0.5 kPa), the background electrolyte (BGE) was 35 mM ammonium acetate in 70% v/v acetonitrile, 15% v/v MeOH, 5% v/v isopropanol with an apparent pH of 8, the fish extracts were injected hydrodynamically at 50 mbar (5 kPa) alternating between 5 s for each sample plug and electrokinetically at 30 kV for 75s for the BGE spacer plug for a total of thirteen discrete samples analyzed within one run of 30 minutes. Concentrations of EPA and DHA were reported in µM/mg dw and converted to µg/g ww using the molecular masses of EPA and DHA and the % moisture of the fish. QA/QC measures included a pooled fish sample (prepared by combining an equal amount of all fish samples) analyzed with every

run, and duplicate samples. A covariance coefficient (CV) of < 20% for the pooled samples is deemed analytically acceptable; the CV for EPA and DHA and were 8.5% and 6.5%, respectively (n=18 runs). Average % difference in duplicates were 19.56 ± 15.59% for EPA and 21.01 ± 18.97% for DHA (n=14 pairs).

2.3.9 Fatty acid method comparison

Other studies examining FAs in fish typically use GC-MS, so to ensure my data were comparable, I conducted an inter-method comparison. A subset of my FA samples (24 fish) was sent to Fisheries and Oceans Canada (Winnipeg, MA) where FAs were extracted using the modified Folch procedure (Folch 1957) and quantified (μg/g dw) using GC-MS. Full method details are described in Giraldo et al. (2016). The average % difference in samples run across both methods was 30.96 ± 31.11% for EPA and 24.62 ± 20.9% for DHA (1:1 comparisons are shown in Fig. S1).

2.4 Statistics

Spatial and species comparisons of all analytes used ww concentrations, which were calculated from dw concentrations using the % moisture of the individual fish. For composite samples, the ww concentration was calculated using the mean % moisture of the individual fish within the composite sample. Similarly, the length, weight and age of the composite samples were the mean of the fish within the composite samples (Table 3).

2.4.1 Spatial and species differences

Spatial and species differences were tested differently based on the analyte. For results from composite samples (elements, PCBs and OCPs), spatial and species differences were tested on spatially- or species-ranked ww concentrations due to the low samples sizes. Spatial differences in ranked concentrations within Smallmouth Bass and Yellow Perch across all four

sites, and species differences in ranked concentrations in the four fishes collected in the NF site were tested using analyses of variance (ANOVAs), and species differences in ranked concentrations between Smallmouth Bass and Yellow Perch were tested on concentrations pooled across the sites using t-tests ($p < 0.05$). For analytes measured in individual fish samples (Hg, EPA, DHA and trophic position (as determined by $\delta^{15}N$, described below)) where sample sizes were larger, spatial and species differences were run on raw data using non-parametric Kruskal-Wallis tests (non-parametric ANOVA) and Mann-Whitney-Wilcoxon tests (non-parametric t-test), due to the non-normal distribution of the data ($p < 0.05$ for Shapiro-Wilk's test) and the unequal variance between comparison groups ($p < 0.05$ for Levene's test), even when the data were $\log_{10}$ adjusted.

2.4.2 Covariates

The potential covariates length, weight and age were considered when examining spatial differenced in analytes within Smallmouth Bass and Yellow Perch. For the elements, PCBs and OCPs analyzed on composite samples, site comparisons were not size adjusted as fish were selected to obtain similar size ranges across sites, and there were no significant differences in ranked lengths or weights between sites within each species (ANOVA – $p > 0.05$; Table 3). Smallmouth Bass also did not significantly differ in ranked age across sites, however, Yellow Perch did (ANOVA – $p = 0.001$) and Yellow Perch from the HP and NF were on average two and three years younger, respectively, than fish from the UPS and FF sites (Table 3). Nevertheless, because composite samples were made and sample sizes were low, age was not accounted for in site differences of elements and chlorinated organics.

For Hg, EPA, DHA and trophic position data generated for individual fish, I used non-parametric Spearman correlations to look for significant relationships between the analytes and

the potential covariates and tested for differences in the potential covariates across sites using Kruskal-Wallis tests. Hg and trophic position were positively correlated with length, weight and age in both Smallmouth Bass and Yellow Perch (Spearman – $p < 0.05$; Fig. S2-S7) and length, weight and age all significantly differed between sites within both species (Kruskal-Wallis – $p < 0.05$; Table 1). DHA was negatively correlated with age in Yellow Perch and with length, weight and age in Smallmouth Bass (Spearman – $p < 0.05$; Fig. S8-S10), but only age significantly differed between sites in Yellow Perch for the subset of fish analyzed for FAs (Kruskal-Wallis – $p < 0.0001$; Table S1). EPA did not significantly correlate with length, weight or age in either species (Spearman – $p < 0.05$; Fig. S11-S13). To account for all covariates when assessing spatial differences in Hg and trophic position in both species, and age when assessing spatial differences in DHA for Yellow Perch, Hg, trophic position and DHA were length-, weight- or age-adjusted accordingly. To do so, I generated linear models between the $\log_{10}$ transformed response variables (to meet assumptions of normality and homoscedasticity in the model), covariates and site (e.g., $\log_{10}$Hg ~ length + site) and determined the least square mean (LSM) for each site from this model at the mean length, weight or age for that species; the LSM for each site was considered the covariate-adjusted response variable. I then looked for site differences in the LSMs using a Tukey posthoc test with alpha set at 0.05. When covariates influenced spatial differences within the Smallmouth Bass and Yellow Perch compared to the raw data, statistics are presented for the adjusted data, however when they did not, the statistics are presented for the raw data (these results are explained below). All length-, weight- and age-adjusted Hg, DHA and trophic positions are presented in Table S5.

2.4.3 Hg and trace elements

Elements were presented as "nutritious" for human consumption if they had a Canadian Recommended Dietary Intake (Health Canada 2006) and when fish from this study had concentrations that were lower than Ontario's Guide to Eating Fish "do not eat" consumption advisory (MECC 2015) (Table 4). Conversely, elements were presented as "toxic" (non-nutritious) for human consumption if they did not have a Canadian Recommended Dietary Intake or when fish from this study had concentrations that were above Ontario's "do not eat" consumption advisory (Table 5).

Individual elements were statistically analyzed if > 60% of the values for a species were above the DL; for any samples < DL in this retained group, a value of half of the DL was substituted prior to further analyses. Spatial and species differences in individual trace elements were analyzed using the methods described above and the P-values were Bonferroni adjusted to account for multiple comparisons (30 trace metals). For Hg, spatial differences in Yellow Perch and Smallmouth Bass were analyzed using age-adjusted concentrations as age influenced the spatial pattern of Hg compared to the raw data, whereas length and weight did not (Table S5).

To look for differences in overall element composition between sites and species, I ran principal component analyses (PCAs) on the centered element concentrations (except for Hg). Some element concentrations were $\log_{10}$ transformed to help meet assumptions of normality and homoscedasticity. However, in some cases $\log_{10}$ transformations helped the element concentrations approach, but not meet assumptions of normality and homoscedasticity and therefore, I used a non-parametric analysis of similarities (ANOSIM) to test for differences in element compositions between sites and species ($p < 0.05$).

2.4.4 OCPs

DDT compounds and methoxychlor were the only OCPs with > 60% of samples above DL within a species (Table S2); for methoxychlor, a value of half of the DL was substituted for the remaining samples but this was not needed for total DDT. For total DDT, concentrations were calculated by adding all the DDT compounds above DL together for each sample. Spatial and species differences in total DDT and methoxychlor were tested using the same methods described above. Total DDT concentrations in fish from this study were compared to Ontario's Fish Guide "do not eat" consumption advisory in Table S6.

2.4.5 PCBs

Total PCBs were calculated by summing together the values of all of the PCB congeners above the DL for each sample. For species where > 60% of samples were above DL for total PCBs, a value of half of the average DL for the congeners detected in other fish from this species was substituted for the remaining samples below DL. Spatial and species differences in total PCBs were tested using the same methods described above. Total PCB concentrations in fish from this study were compared to Ontario's Fish Guide "do not eat" consumption advisory in Table S6.

2.4.6 Fatty acids

Spatial and species differences of EPA and DHA were analyzed using methods described above. In terms of covariates, spatial differences in DHA were analyzed using raw data in Smallmouth Bass and Yellow Perch, as age-adjusting the concentrations in Yellow Perch did not influence the spatial patterns compared to the raw data (Table S5).

2.4.7 Stable isotopes

Fish $\delta^{13}C$ values were not lipid corrected as there were no significant relationships observed between $\delta^{13}C$ and C:N within species (Spearman – $p > 0.05$). For $\delta^{15}N$ and $\delta^{13}C$ biplots, I included a subset of the benthic invertebrates and zooplankton collected by the MAES in 2017 that had overlapping $\delta^{13}C$ values with the fish to identify potential food sources; this consisted of the following groups: Baetidae, Bivalva, Chironomidae, Copepoda, Ephemeroptera, Gammarus, Gastropoda, Gyrinidae, Hemiptera, Hirudinae, Oligochaeta, Tipulidae and Trichoptera.

Nutrient inputs from agriculture or sewage can increase $\delta^{15}N$ values in food webs (Savage 2005; Diebel et al. 2009) and therefore, the $\delta^{15}N$ in fish from this study were adjusted to baseline $\delta^{15}N$ values for each site, which were calculated by averaging the $\delta^{15}N$ values from amphipods and gastropods at each site (collected by myself in 2020 or by the MAES team in 2017), as they were common taxa across all sites. Baseline-adjusted $\delta^{15}N$ values were then calculated for the fish by subtracting the site's baseline $\delta^{15}N$ from the $\delta^{15}N$ of the individual fish, and this value was used as a proxy for trophic position. Spatial and species differences in trophic positions were analyzed as described above, and differences in trophic position between Smallmouth Bass and Yellow Perch within each site were also tested using Mann-Whitney-Wilcoxon tests ($p < 0.05$). Additionally, spatial differences in trophic positions in Yellow Perch and Smallmouth Bass were analyzed on age-adjusted values as age influenced the spatial pattern of trophic positions in Yellow Perch compared to the raw data, whereas length and weight did not (Table S5).

3.0 Results

3.1 Elements: Species differences

Both nutritious (Table 4) and toxic (Table 5) elements differed significantly among species from the Wolastoq | SJR. When data were pooled across sites, Yellow Perch were higher than Smallmouth Bass in Ca ($p < 0.0001$), cobalt (Co; $p < 0.0001$), Mn ($p < 0.0001$), strontium (Sr; $p < 0.0001$), thallium (Tl; $p = 0.011$), titanium (Ti; $p = 0.0097$) and Zn ($p < 0.0001$), and lower in Cu ($p < 0.0001$) and Hg ($p < 0.0001$). Qualitatively, Smallmouth Bass were higher in As as ~78% of Yellow Perch were below DL, while Yellow Perch were higher in barium (Ba) and vanadium (V) as ~96% and 50%, respectively, of Smallmouth Bass were below DL.

Table 4. Mean (SE) concentrations of "nutritious" elements (µg/g ww) in fish muscle are presented for American Eel and Striped Bass collected from the NF site, and for Smallmouth Bass and Yellow Perch collected from all sites on the Wolastoq | SJR. Concentrations are given for elements where > 60% of the samples within a species were above DL (half of the DL was substituted for the remaining samples below DL).

Species	N (# composited)	Mean length (mm)	Calcium	Chromium	Copper	Iron	Magnesium	Manganese
"Do not eat"				>14	>600			>640
American Eel	8 (20)	602	233 (27.8)	0.76 (0.06)	0.25 (0.016)	17.8 (6.15)	200 (4.84)	0.96 (0.23)
Striped Bass	5 (10)	838	74.1 (10.1)	0.79 (0.11)	0.18 (0.0057)	4.81 (0.50)	270 (6.89)	0.12 (0.010)
Smallmouth Bass	24 (75)	381	228 (22.1)	0.68 (0.10)	0.22 (0.0063)	5.05 (0.57)	275 (3.99)	0.14 (0.010)
UPS	6 (20)	398	162 (38.9)	0.98 (0.20)	0.22 (0.061)	6.90 (0.91)	279 (7.10)	0.09 (0.0065)
HP	6 (17)	340	229 (29.5)	0.15 (0.04)	0.21 (0.013)	2.47 (0.62)	267 (9.69)	0.17 (0.025)
NF	6 (20)	371	330 (45.3)	1.09 (0.15)	0.24 (0.016)	7.61 (0.78)	285 (8.09)	0.16 (0.019)
FF	6 (18)	377	189 (36.1)	0.49 (0.10)	0.21 (0.011)	3.21 (0.46)	268 (6.22)	0.13 (0.0066)
Yellow Perch	23 (80)	203	626 (52.9)	0.83 (0.10)	0.16 (0.0048)	6.14 (0.59)	262 (2.93)	0.90 (0.13)
UPS	6 (20)	214	548 (52.4)	0.98 (0.30)	0.15 (0.0077)	7.54 (1.48)	253 (5.08)	0.91 (0.23)
HP	6 (20)	204	590 (72.9)	0.37 (0.06)	0.14 (0.0020)	3.57 (0.84)	263 (3.05)	0.61 (0.075)
NF	6 (20)	199	608 (109)	1.03 (0.14)	0.19 (0.0054)	6.88 (0.81)	273 (6.03)	0.50 (0.046)
FF	6 (20)	193	784 (180)	0.95 (0.14)	0.15 (0.0042)	6.67 (0.76)	260 (6.97)	1.57 (0.35)

Table 4 (continued)

Species	N (# composited)	Mean length (mm)	Molyb-denum	Phosphorus	Potassium	Selenium	Sodium	Sulfur	Zinc
"Do not eat"						>24			>1400
American Eel	8 (20)	602	-	1920 (44.9)	2780 (86.6)	0.16 (0.0077)	407 (29.5)	2640 (55.3)	17.9 (2.39)
Striped Bass	5 (10)	838	-	2522 (89.9)	4077 (86.5)	0.36 (0.016)	235 (7.78)	2609 (27.1)	2.70 (0.083)
Smallmouth Bass	24 (75)	381	-	2330 (41.3)	3714 (38.0)	0.16 (0.0090)	264 (4.24)	2182 (62.7)	3.32 (0.081)
UPS	6 (20)	398	-	2139 (23.0)	3604 (73.9)	0.11 (0.011)	268 (8.67)	1903 (28.1)	3.59 (0.14)
HP	6 (17)	340	-	2506 (100)	3727 (95.6)	0.16 (0.0089)	254 (8.41)	2451 (113)	3.23 (0.15)
NF	6 (20)	371	-	2324 (70.97)	3796 (85.4)	0.18 (0.018)	278 (8.38)	1950 (69.0)	3.49 (0.17)
FF	6 (18)	377	-	2353 (47.0)	3726 (28.6)	0.20 (0.012)	257 (6.17)	2424 (37.2)	2.97 (0.045)
Yellow Perch	23 (80)	203	0.039 (0.0061)	2289 (34.0)	3582 (37.2)	0.15 (0.0055)	263 (7.04)	2171 (52.5)	4.07 (0.052)
UPS	6 (20)	214	0.050 (0.015)	2197 (41.6)	3531 (55.6)	0.13 (0.0046)	256 (6.64)	2047 (40.4)	3.99 (0.10)
HP	6 (20)	204	0.0051 (0.000059)	2492 (22.2)	3757 (42.1)	0.14 (0.0026)	239 (12.7)	2544 (42.7)	3.99 (0.13)
NF	6 (20)	199	0.053 (0.0069)	2272 (62.4)	3618 (58.5)	0.14 (0.0057)	252 (7.81)	2034 (50.7)	4.15 (0.10)
FF	6 (20)	193	0.050 (0.0081)	2176 (44.0)	3392 (56.7)	0.19 (0.066)	312 (5.14)	2036 (63.8)	4.16 (0.063)

Table 5. Mean (SE) concentration (µg/g ww) of "toxic" elements in fish muscle are presented for American Eel and Striped Bass collected from the NF site, and Smallmouth Bass and Yellow Perch collected from all sites on the Wolastoq | SJR. Concentrations are given for elements where > 60% of the samples within a species were above DL (half of the DL was substituted for the remaining samples below the DL).

Species/ consumption advisory	**N (# composited)**	**Mean length (mm)**	**Arsenic**	**Barium**	**Cobalt**	**Nickel**	**Strontium**
"Do not eat"			>8			>120	
American Eel	8 (20)	602	0.30 (0.027)	-	0.012 (0.0019)	0.067 (0.015)	0.32 (0.038)
Striped Bass	5 (10)	838	0.31 (0.020)	-	0.0025 (0.00038)	0.036 (0.0056)	0.088 (0.040)
Smallmouth Bass	24 (75)	381	0.31 (0.066)	-	0.0028 (0.00029)	0.028 (0.0028)	0.32 (0.040)
UPS	6 (20)	398	0.0056 (0.000031)	-	0.0034 (0.00037)	0.037 (0.0071)	0.21 (0.069)
HP	6 (17)	340	0.12 (0.044)	-	0.0014 (0.00029)	0.020 (0.0058)	0.29 (0.058)
NF	6 (20)	371	0.49 (0.13)	-	0.0044 (0.00041)	0.027 (0.0021)	0.52 (0.079)
FF	6 (18)	377	0.62 (0.099)	-	0.0020 (0.00029)	0.028 (0.0048)	0.25 (0.064)
Yellow Perch	23 (80)	203	-	0.074 (0.0091)	0.0046 (0.00041)	0.024 (0.0017)	0.98 (0.13)
UPS	6 (20)	214	-	0.073 (0.014)	0.0058 (0.00062)	0.024 (0.0032)	0.75 (0.11)
HP	6 (20)	204	-	0.041 (0.0064)	0.0023 (0.00030)	0.027 (0.0029)	0.55 (0.070)
NF	6 (20)	199	-	0.091 (0.018)	0.0045 (0.00048)	0.026 (0.0041)	1.39 (0.31)
FF	6 (20)	193	-	0.094 (0.026)	0.0059 (0.00085)	0.020 (0.0040)	1.28 (0.34)

Table 5 (continued)

Species/ consumption advisory	N (# composited)	Mean length (mm)	Thallium	Titanium	Vanadium	N	Mean length (mm)	Mercury
"Do not eat"								>1.8
American Eel	8 (20)	602	-	0.21 (0.040)	0.014 (0.0049)	24	557	0.38 (0.021)
Striped Bass	5 (10)	838	-	0.13 (0.0085)	-	14	854	1.20 (0.083)
Smallmouth Bass	24 (75)	381	0.0014 (0.00010)	0.13 (0.0033)	-	179	374	0.62 (0.025)
UPS	6 (20)	398	0.0012 (0.00010)	0.13 (0.0067)	-	48	401	0.41 (0.044)
HP	6 (17)	340	0.00091 (0.000076)	0.12 (0.0049)	-	19	374	0.71 (0.075)
NF	6 (20)	371	0.0020 (0.000092)	0.14 (0.0065)	-	65	369	0.67 (0.036)
FF	6 (18)	377	0.0013 (0.000089)	0.14 (0.0068)	-	47	350	0.71 (0.052)
Yellow Perch	23 (80)	203	0.0019 (0.00019)	0.15 (0.0023)	0.010 (0.0010)	194	198	0.32 (0.0088)
UPS	6 (20)	214	0.0014 (0.00014)	0.15 (0.0070)	0.013 (0.0016)	53	208	0.34 (0.019)
HP	6 (20)	204	0.0011 (0.000067)	0.15 (0.0038)	0.0045 (0.00054)	28	192	0.36 (0.029)
NF	6 (20)	199	0.0030 (0.00030)	0.15 (0.0025)	0.010 (0.0010)	60	203	0.26 (0.011)
FF	6 (20)	193	0.0021 (0.00033)	0.14 (0.0044)	0.013 (0.0020)	53	186	0.33 (0.013)

*Aluminium, antimony, beryllium, boron, cadmium, lead, silver, tin and uranium had < 60% of samples above DL for all species.

At the NF site where all four fish species were caught, elemental compositions differed (ANOSIM: $p = 0.0001$) and were most distinct in Striped Bass and American Eel compared to Smallmouth Bass and Yellow Perch, which had overlapping ellipses in the PCA (Fig. 2). Elements that were positively associated with Smallmouth Bass, Yellow Perch and Striped Bass in principal component one (Dim1) included Mg, P and K, while elements positively associated with American Eel on this axis were Cu, Na, Ni, Fe, Mn and Zn. In principal component two (Dim2), elements that were positively associated with Smallmouth Bass and Yellow Perch were Ca, Cr and Sr, while Se was positively associated with Striped Bass along this axis (American Eel highly overlapped both directions along this axis). Specifically, elements that were significantly higher in Striped Bass than the other species were Hg ($p < 0.0001$; Fig. 3a), K ($p < 0.0001$; Fig. S14k) and Se ($p = 0.001$, Fig. 3d), and those lower were Ca ($p < 0.0001$; Fig. S14c), Co ($p < 0.0001$; Fig. S14e), Sr ($p < 0.0001$; Fig. S14l) and Zn ($p < 0.0001$; Fig. S14o). Furthermore, American Eel were highest in Co ($p < 0.0001$), Na ($p < 0.0001$, Fig. 3d) and Zn ($p < 0.0001$; Fig. S14o), and lowest in Mg ($p = 0.0003$; Fig. S14g), P ($p < 0.0001$, Fig. 3b) and K ($p < 0.0001$; Fig. S14k). In addition, Yellow Perch were only highest in Sr ($p < 0.0001$; Fig. S14l) and Smallmouth Bass were not significantly highest or lowest in any elements compared to the other species (Fig. S14a-o). Qualitatively Yellow Perch were lowest in As, being the only species with values below DL, and highest in Ba and, along with Smallmouth Bass, Tl (Fig. 3c) based on the high number of samples below DL in other species.

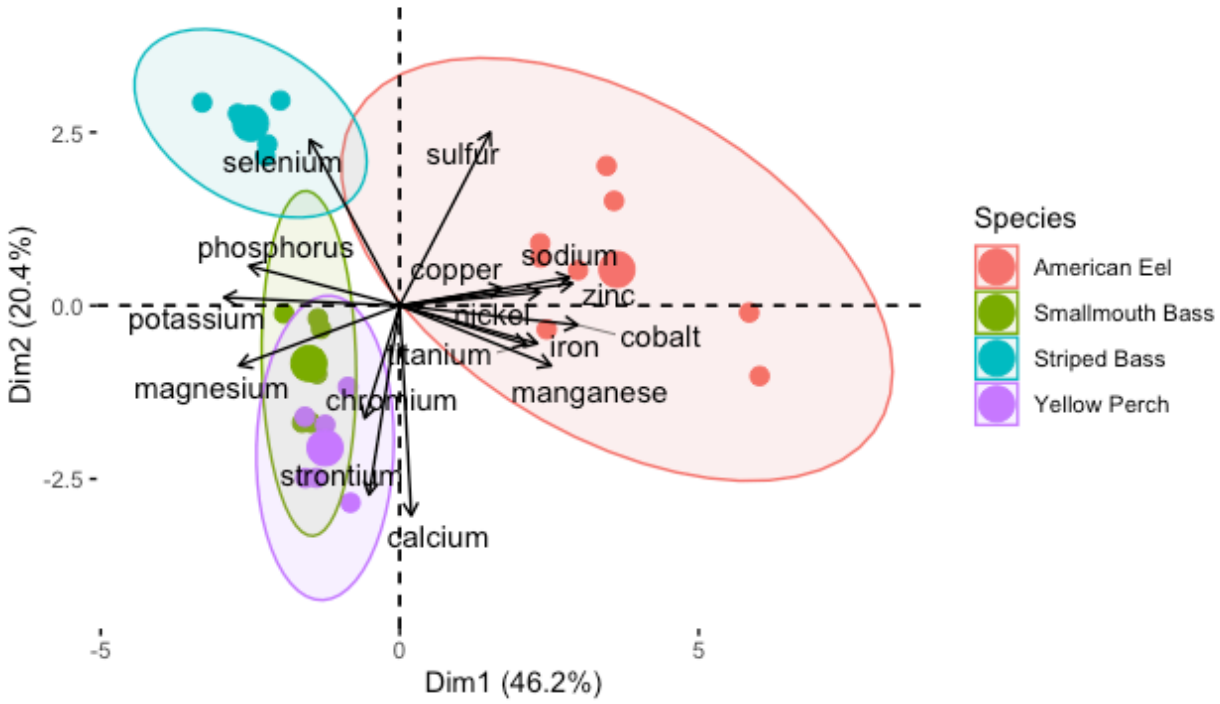


Figure 2. Principal component analysis biplots of the centered element concentrations in muscle tissue of American Eel, Smallmouth Bass, Striped Bass and Yellow Perch collected from the NF site on the Wolastoq | SJR. Elements included are those where > 60% of samples within each species were above DL (16 elements); half of the DL was substituted for the remaining samples below DL. Arrows show the loading of each element, points show the scores of each composite fish, large points show the central score for each species, and ellipses show the 95% confidence intervals. Principal components one (Dim1) and two (Dim2) explained 46.2% and 20.4% of the variability in element compositions between species, respectively. Highly overlapping ellipses indicate more similar element compositions between species and less overlap indicates more distinct element compositions between species.

There were significant site by species interactions between Yellow Perch and Smallmouth Bass for four out of the 17 elements that could be statistically compared (i.e. > 60% of samples above DL). For simplicity, I interpreted site differences within species and these results are as follows.

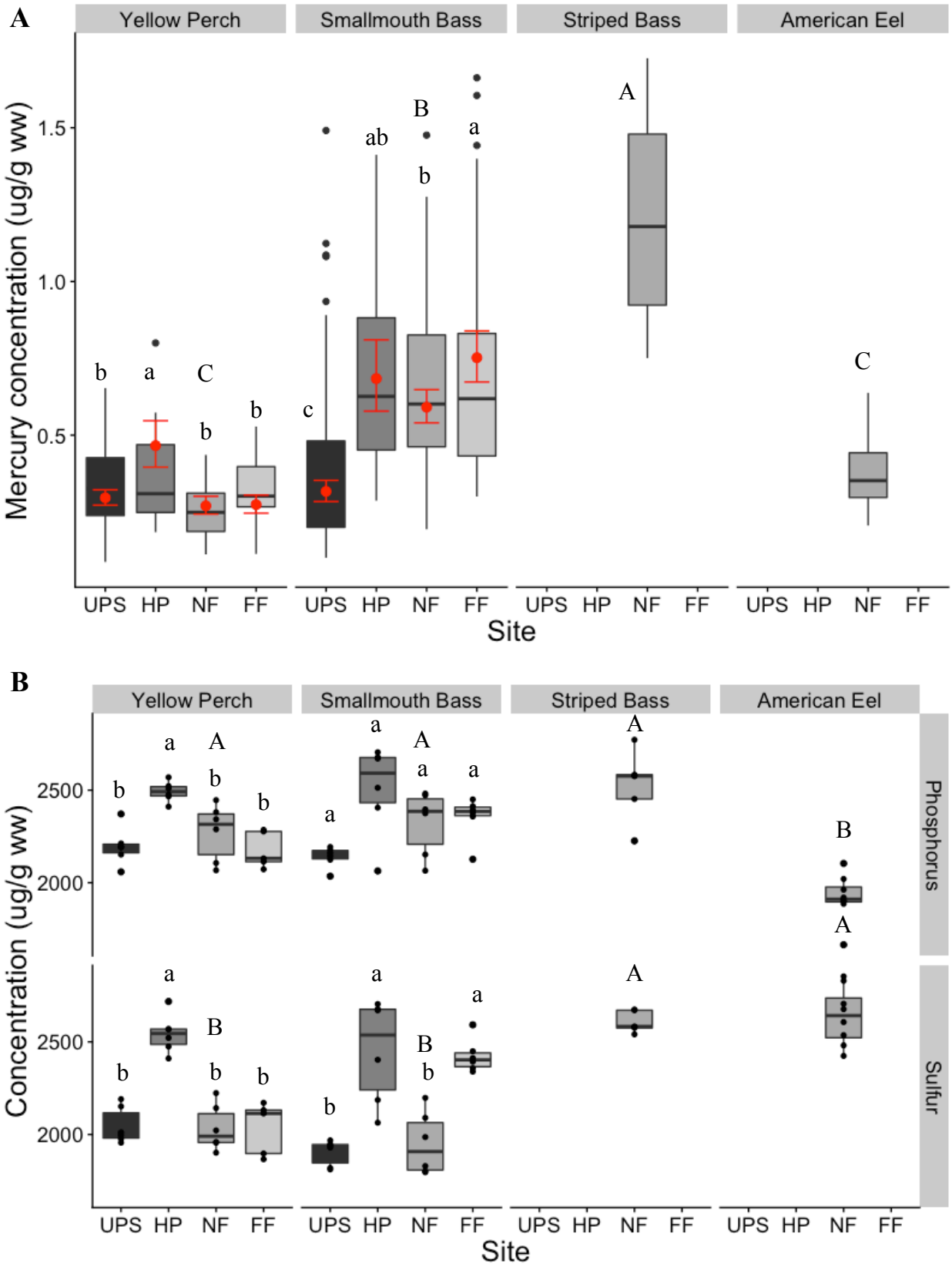
3.2 Elements: Spatial differences within Smallmouth Bass and Yellow Perch

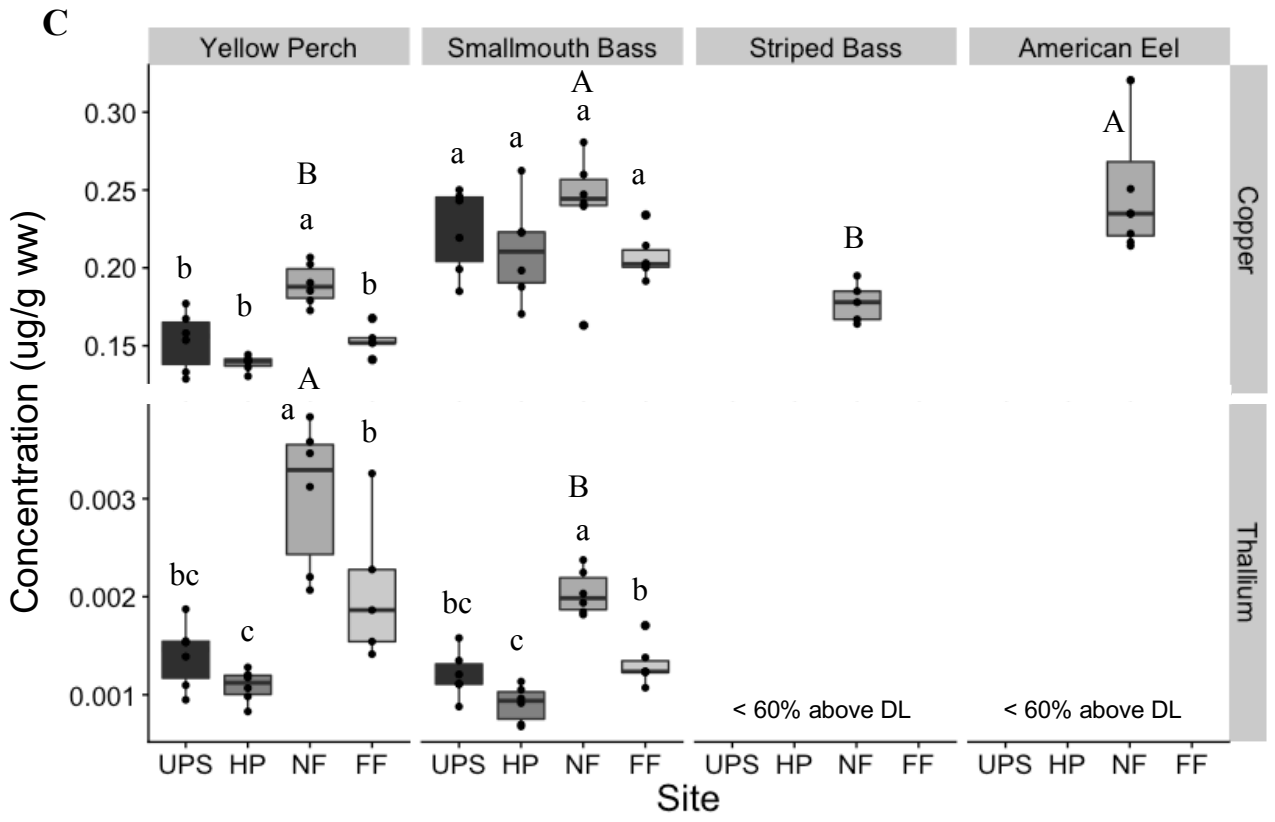
Both nutritious (Table 4) and toxic/non-nutritious (Table 5) elements also differed between sites within Smallmouth Bass and Yellow Perch. The composition of elements were

significantly different between sites within Smallmouth Bass (ANOSIM: $p = 0.0018$) and Yellow Perch (ANOSIM: $p = 0.0001$) (Fig. 4). In both species, S and P separated most strongly from all other elements, based on Dim1, and these elements had positive associations with the HP. However, the sites sorted out differently between the species in the PCAs. In Yellow Perch, the HP site was distinct from the other three sites based on Dim1 while in Smallmouth Bass, fish from the HP and FF were more similar and were distinct from the NF and UPS fish, which sorted together on Dim1. This was influenced by elements like Sr, Na and Ca that were positively associated with fish from the FF site in Yellow Perch but not in Smallmouth Bass, which also uniquely had As positively associated with the FF. However, the elements included in the PCAs differed between Yellow Perch and Smallmouth Bass, based on their DLs, thereby influencing the spatial comparisons of element compositions between these species.

Individual elements in Smallmouth Bass and Yellow Perch also showed interesting differences within species, among sites. At the UPS site, Smallmouth Bass were lowest in age-adjusted Hg ($p < 0.0001$; Fig. 4a), while Yellow Perch were not significantly highest or lowest in any elements (Fig. S14a-o). In the HP, Yellow Perch were highest in age-adjusted Hg ($p < 0.0001$) and P and S were highest in both species, although only significantly for Yellow Perch ($p = 0.009$ and $p = 0.013$ for S and P, respectively; Fig. 3b). Additionally, Yellow Perch were highest in K ($p = 0.026$; Fig. S14k) and lowest in Co ($p = 0.002$; Fig. S14e), molybdenum (Mo; $p = 0.007$; Fig. S14i) and V ($p = 0.003$; Fig. S14n) at this site. At the NF site, Tl was the greatest in both species (Smallmouth Bass: $p < 0.0001$; Yellow Perch: $p = 0.0002$; Fig. 3c), and Yellow Perch also had highest Cu concentrations at this site ($p = 0.002$; Fig. 3c). Finally, Se was highest at the FF site for Yellow Perch ($p = 0.016$) and Smallmouth Bass, although not significantly higher than all other sites for this species (Fig. 3d). Yellow Perch were also highest in Na at this

site ($p = 0.013$; Fig 3d) and Smallmouth Bass were highest in As, although not significantly (Fig. S14a).


A
Yellow Perch
Smallmouth Bass
Striped Bass
American Eel
Mercury concentration (ug/g ww)
1.5
1.0
0.5
b
a
C
b
c
ab
B
b
a
b
A
C
UPS HP NF FF
Site
B
Yellow Perch
Smallmouth Bass
Striped Bass
American Eel
Concentration (ug/g ww)
2500
2000
Phosphorus
Sulfur
Site


C
Yellow Perch
Smallmouth Bass
Striped Bass
American Eel
Copper
Thallium
Concentration (ug/g ww)
0.30
0.25
0.20
0.15
0.003
0.002
0.001
< 60% above DL
< 60% above DL
UPS HP NF FF
Site

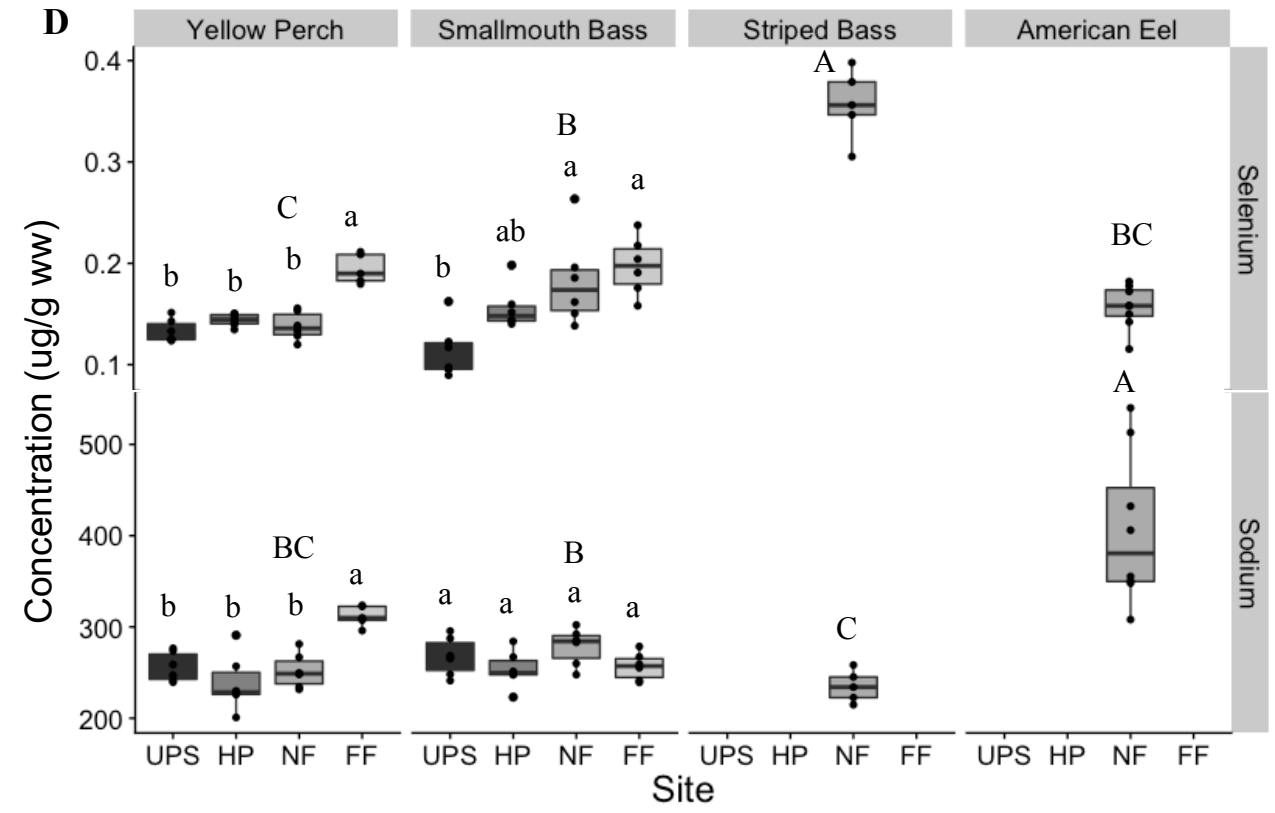

D
Yellow Perch
Smallmouth Bass
Striped Bass
American Eel
Selenium
Sodium
Concentration (ug/g ww)
0.4
0.3
0.2
0.1
500
400
300
200
UPS HP NF FF
Site

Figure 3. Spatial and species differences of select element concentrations (µg/g ww) in fish muscle tissue collected from sites along the Wolastoq | SJR, listed from upstream to downstream left to right. Capital letters represent significant differences in ww concentrations between species at the NF, and lower case letters represent significant differences in age-adjusted concentrations (A) or ww concentrations (B-D) between sites within the species Yellow Perch and Smallmouth Bass, as determined using Tukey's posthoc test ($p < 0.05$). A) Boxplots show ww Hg concentrations across sites and species, red points represent Hg concentrations adjusted to the mean age across sites in Yellow Perch (mean age = 6) and Smallmouth Bass (mean age = 8), and red error bars represent 95% CI around the age-adjusted mean; B-D) boxplots show concentrations of select elements across sites and species and points represent individual composite samples.

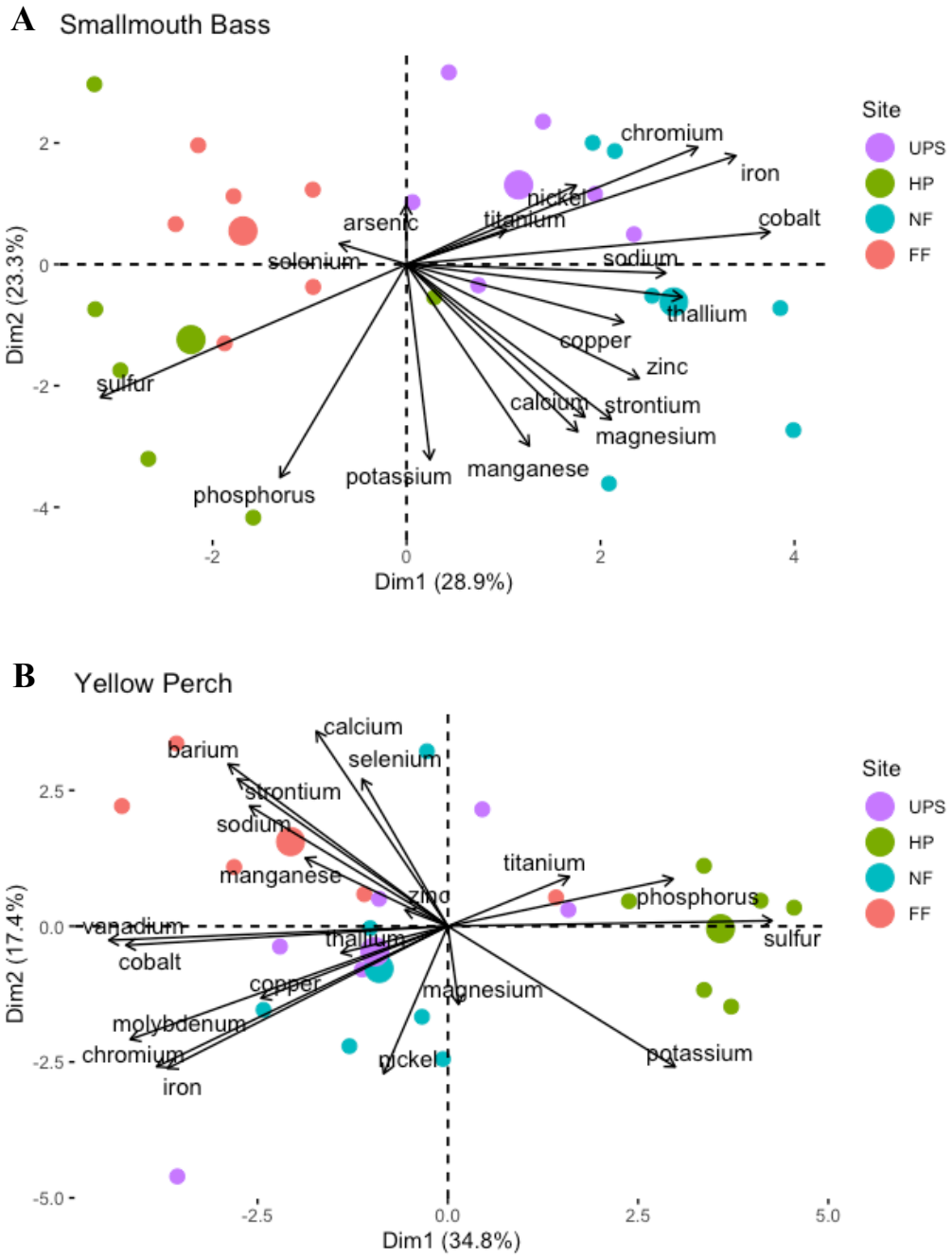


Figure 4. PCA biplots of the elements in A) Smallmouth Bass and B) Yellow Perch muscle tissue collected from the Wolastoq | SJR, showing the loading of each element (arrows), the scores of each composite fish (points), and the central score for each site (large points). Elements included were those where > 60% of composite samples were above DL within Smallmouth Bass (18 elements included) and Yellow Perch (20 elements included); half of the DL was substituted for the remaining samples below. Dim1 explained 28.9% and 34.8%, and Dim2 explained 23.3% and 17.4%, of the variation in element compositions between sites within Smallmouth Bass and Yellow Perch, respectively.

3.3 OCPs: Species and spatial differences

DDT and its breakdown products, and methoxychlor were the only OCPs that had > 60% of samples above DL within the species (Table S2). Among fish from the NF site, species differed in total DDT ($p < 0.0001$) whereby American Eel had the highest concentrations, followed by Striped Bass and Smallmouth Bass, while Yellow Perch had the lowest levels (Fig. 5). Species differences in % lipid followed a similar order as total DDT, with American Eel > Striped Bass > Smallmouth Bass > Yellow Perch ($p < 0.0001$; Table 3). For methoxychlor, American Eel and Yellow Perch had < 60 % of samples above DL and there was no significant difference in concentrations between Smallmouth Bass and Striped Bass in the NF (Fig. S15).

Total DDT and methoxychlor in Smallmouth Bass and Yellow Perch also sometimes varied between sites. Total DDT in Yellow Perch was lower in the NF compared to the UPS and HP sites ($p = 0.0018$), but no among-site differences were found for Smallmouth Bass. In Smallmouth Bass, methoxychlor was lower in the FF than the UPS site with the NF and HP falling in between ($p = 0.0413$; Fig. S15). Percent lipid in Yellow Perch significantly differed between sites ($p = 0.002$), yet incongruently to the differences in total DDT (i.e., Near Field was the highest in % lipid but lowest in total DDT), while Smallmouth Bass did not differ in percent lipid between sites (Table 3).

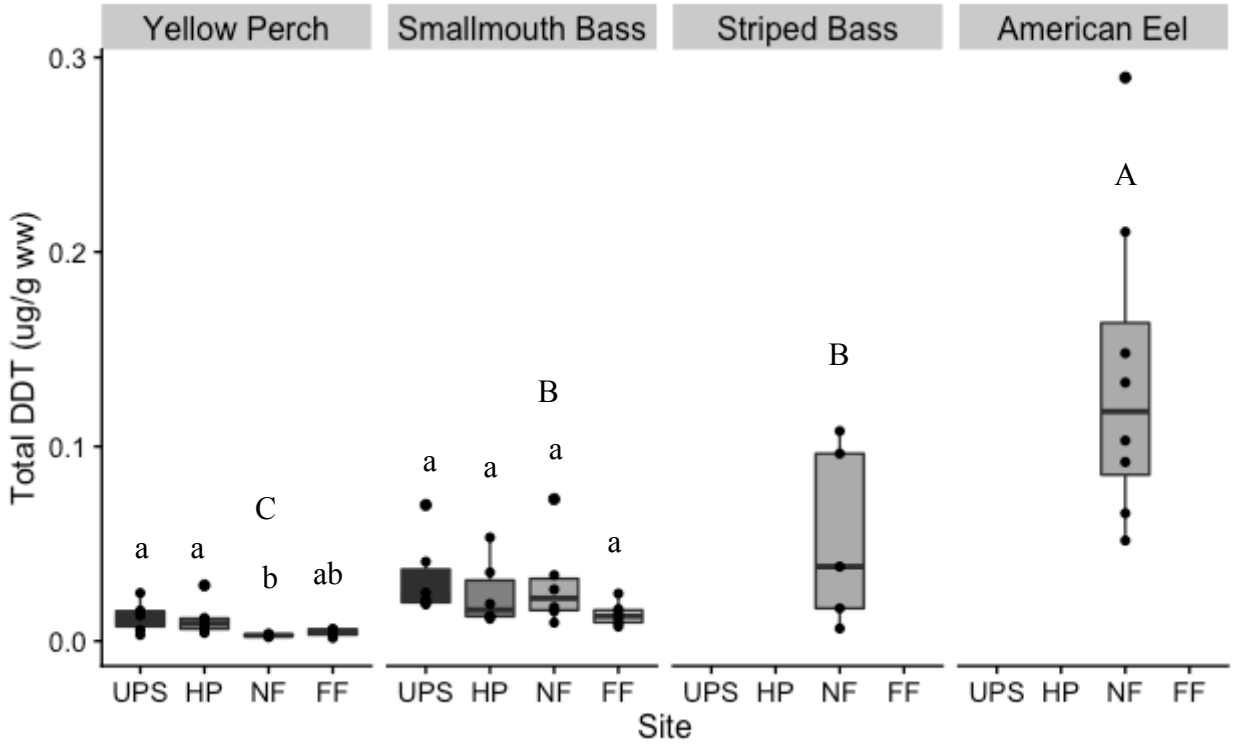


Figure 5. Boxplot showing the spatial and species differences of total DDT (µg/g ww) in fish muscle tissue from sites on the Wolastoq | SJR. Points represent individual composite samples, lower case letters represent significant differences in concentrations between sites within Yellow Perch and Smallmouth Bass, and capital letters represent significant differences in concentrations between species at the NF, determined using a Tukey's posthoc test ($p < 0.05$).

3.4 PCBs: Species and spatial differences

Total PCBs differed between species but did not differ across sites. In the NF, qualitatively Yellow Perch had lowest total PCBs as only one sample was above DL at this site. Among the other species, American Eel had significantly higher total PCBs than Smallmouth Bass ($p = 0.007$), with Striped Bass falling in between (Fig. 6). However, there were no significant differences in total PCBs across sites within Smallmouth Bass (Yellow Perch had < 60% samples above DL).

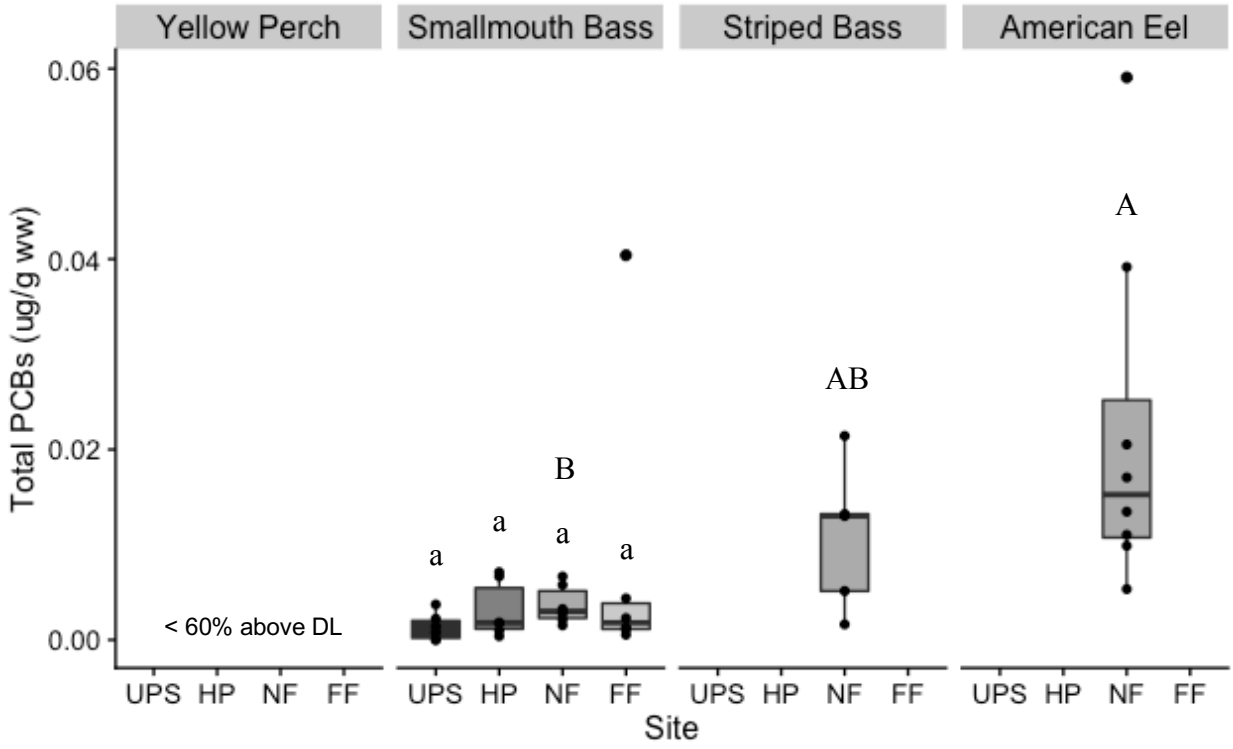


Figure 6. Boxplot showing the spatial and species differences of total PCBs (µg/g ww) in fish muscle tissue collected from the Wolastoq | SJR. Points represent individual composite samples, lower case letters represent significant differences in concentrations across sites within Smallmouth Bass, and capital letters represent significant differences in concentrations between species at the NF site, determined using a Tukey's posthoc test ($p < 0.05$).

3.5 FAs: Spatial and species differences

The FAs EPA and DHA also varied significantly among species. Within the NF, Yellow Perch had the highest EPA concentrations ($p < 0.0001$; Fig. 7a), but the fish species did not significantly differ in DHA (Fig. 7b). When concentrations in Smallmouth Bass and Yellow Perch were pooled across sites, DHA was highest in Striped Bass and Smallmouth Bass, American Eel had intermediate levels and Yellow Perch had the lowest values ($p = 0.0001$), whereas EPA was significantly highest in Yellow Perch, followed by American Eel, Striped Bass and Smallmouth Bass ($p < 0.0001$; Fig. S16).

EPA and DHA concentrations also varied between sites for Smallmouth Bass and Yellow Perch. Smallmouth Bass had highest EPA in the UPS site and the lowest concentrations in the

HP (although not significantly different from the FF; $p < 0.0001$), while Yellow Perch did not significantly differ in EPA concentrations among sites. For DHA, Yellow Perch had highest concentrations in the HP and lowest concentrations in the FF (although not significantly different from the UPS; $p < 0.0001$), while Smallmouth Bass did not significantly differ in DHA among sites.

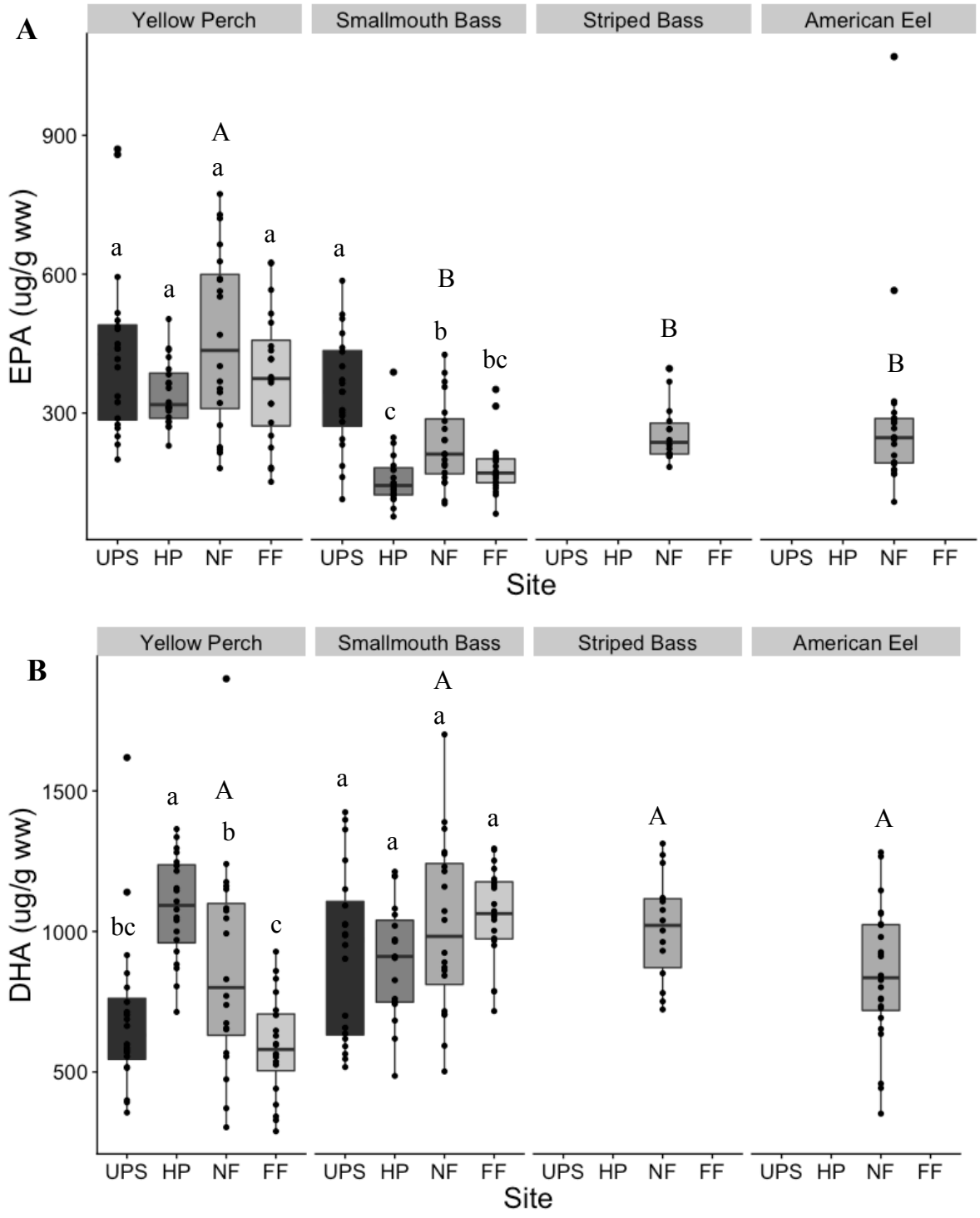


Figure 7. Boxplots showing the spatial and species differences in A) EPA and B) DHA concentrations (µg/g ww) across sites and species from the Wolastoq | SJR. Points represent individual fish, lower case letters represent significant differences between sites within Smallmouth Bass and Yellow Perch, and capital letters represent significant differences between species at the NF, as determined using a Tukey's posthoc test ($p < 0.05$).

3.6 Stable Isotopes: Spatial and species differences

Stable isotope biplots are presented for benthic invertebrates, zooplankton and the four fish species, and results are described qualitatively here (Fig. 8). In the NF, $\delta^{15}N$ in fish were ordered from Striped Bass > Smallmouth Bass > American Eel > Yellow Perch and $\delta^{13}C$ values were more positive in Striped Bass compared to the other fish species. Of the benthic invertebrates in the NF, scrapers like gastropods (including families Hydrobiidae and Planorbidae) and amphipods (including genera Gammarus and Hyalella) had more positive $\delta^{13}C$ values while filter feeders like bivalves had more negative $\delta^{13}C$ values. Furthermore, predatory and detritivorous invertebrates like Tipulidae, Hirudinae and Oligocheata had higher $\delta^{15}N$ values compared to other benthic invertebrates.

Within Smallmouth Bass and Yellow Perch, unadjusted $\delta^{15}N$ varied across sites and were ordered from HP > NF > FF > UPS in both species. $\delta^{13}C$ in Smallmouth Bass was most negative in the HP, intermediate in the NF and FF, and most positive at UPS while in Yellow Perch, $\delta^{13}C$ was more negative in the HP and FF and more positive at NF and UPS. Between the two species, within each site, Smallmouth Bass usually had higher unadjusted $\delta^{15}N$ values compared to Yellow Perch (except in the HP) and both species had similar $\delta^{13}C$ values, although Yellow Perch had more negative $\delta^{13}C$ values in the UPS and FF sites compared to Smallmouth Bass. Amphipods and gastropods had similar $\delta^{15}N$ values across all sites, while both organisms had more negative $\delta^{13}C$ values in the HP compared to other sites.

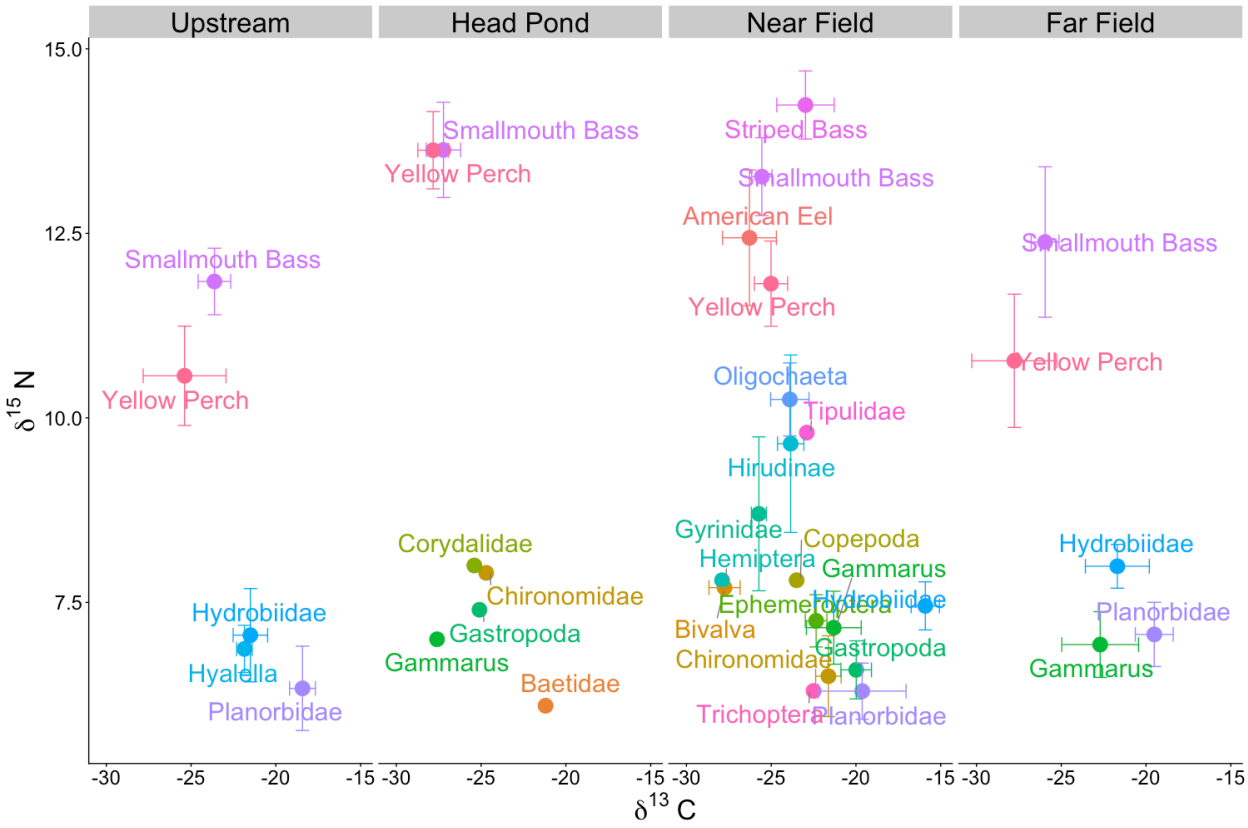


Figure 8. Stable isotope biplot of $\delta^{15}N$ and $\delta^{13}C$ (‰) in fish, benthic invertebrates and zooplankton collected in 2017, 2020 and 2021 within the four sites on the Wolastoq | SJR. Points represent site means for the taxonomic classifications and error bars represent standard deviation.

Trophic positions of these fishes also varied among species, among sites within species and among species within sites. For fish collected in the NF, trophic positions were ordered from Striped Bass > Smallmouth Bass > American Eel > Yellow Perch ($p < 0.0001$; Fig 9). Age-adjusted trophic positions also differed across sites within Yellow Perch ($p < 0.0001$) and Smallmouth Bass ($p < 0.0001$) which were ordered from HP = NF > UPS = FF for both species. Within each site, except the HP, Smallmouth Bass assumed a higher trophic position than Yellow Perch ($p < 0.0001$ for the three sites).

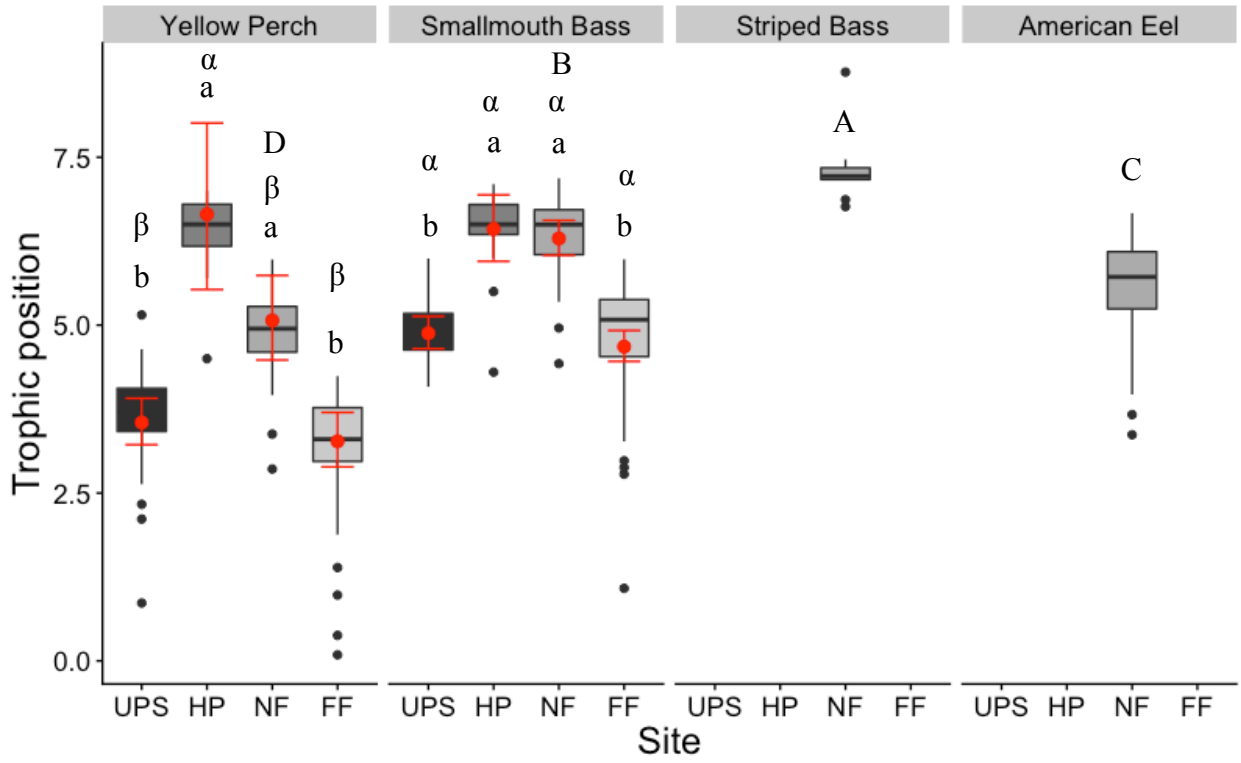


Figure 9. Trophic position of fish across sites and species, as calculated by subtracting the baseline $\delta^{15}N$ value for each site from the fish $\delta^{15}N$ values. Lower case letters represent significant differences in age-adjusted trophic position between sites within Yellow Perch (mean age = 6) and Smallmouth Bass (mean age = 8), capital letters represent significant differences between species at the NF, as determined by Tukey's posthoc test ($p < 0.05$), and Greek letters represent significant differences between Smallmouth Bass and Yellow Perch within sites, as determined by Mann-Whitney-Wilcoxon ($p < 0.05$).

4.0 Discussion

The contaminant and nutrient concentrations in fish were influenced by species and location, and the spatial and species patterns observed differed by the contaminant or nutrient. Element compositions were more similar in Smallmouth Bass and Yellow Perch compared to Striped Bass and American Eel at the NF, but Smallmouth Bass and Yellow Perch also showed significant differences in numerous elements when sites were pooled. Some elements in fish (e.g., P, S and Hg) had higher concentrations in the MQGS reservoir, which suggests that the dam is influencing the distribution of certain contaminants and nutrients in the Wolastoq | SJR.

PCBs and OCPs showed differences at the species level where the fattiest fish (American Eel) were highest in these contaminants. For FAs, Yellow Perch had the highest EPA concentration of the species collected in the NF and also showed the greatest variability in DHA concentrations. Spatial and species patterns of certain contaminants, as well as nutrients like DHA, could be related to the relative trophic position and basal carbon source of the fish, which the dam appeared to also be influencing.

4.1 Reservoir effects

Some elements (e.g., P and S) were higher in fish from the reservoir than those collected upstream or downstream, which included age-adjusted Hg in Yellow Perch. Various studies have shown that nutrients like P and S can accumulate in fine sediments trapped by dams (Friedl and Wüest 2002; Harrison et al 2010; Kao et al. 2022), however, to my knowledge, no studies have looked at the concentrations of these elements in reservoir fish. Nevertheless, the stratification of water in the reservoir can alter the biogeochemical cycling of these elements, creating more bioavailable forms of P and S (Chowdhury and Al Bakri 2006; Scott et al. 2019; Liu et al. 2017; Shi et al. 2020; Yang et al. 2020; Koa et al. 2022). In turn, this can affect primary productivity as exemplified by algal blooms caused by high P loading from the trapped sediments (Chowdhury and Al Bakri 2006). It is not known how dietary P and S affect concentrations of these elements in wild fish, however feeding trials in aquaculture have demonstrated increased concentrations in fish tissue with higher dietary supply (Lall 2021). The accumulation of P and S in fish from the HP is complemented by sediment data collected for the Wolastoq | SJR in 2014 and 2015, whereby P increased with increasing proximity to the dam and both elements had higher average concentrations in sites within the reservoir (P = 1292, S = 734 mg/kg dw) compared to sites downstream from the dam (P = 590, S = 221 mg/kg dw; Kidd et al. 2019). Therefore, the results

herein suggest that dams influence the accumulation of P and S reservoir food webs and future studies that include elemental composition of lower-trophic-level organisms would inform the mechanisms underpinning the higher P and S in the HP fish.

Results from my study suggest that the HP is still affecting levels of Hg in the fish of the Wolastoq | SJR. More specifically, Yellow Perch in the HP had higher Hg when concentrations were adjusted to the mean age. However, the lack of pre-dam Hg concentrations in fish limits my ability to make such conclusions. For Smallmouth Bass, the higher levels of Hg in the reservoir and in downstream sites could be due to abiotic or biotic downstream transfer of Hg from the reservoir or other local point source inputs (see below). High levels of Hg are commonly observed in reservoir fish due to the high Hg-methylation capacity in these environments (Bodaly et al. 2007; Bilodeau et al. 2017). In addition, elevated Hg in fish downstream from hydroelectric dams occurs and is related to increased release of MeHg in dissolved water and suspended particulate matter (SPM) from the reservoir to downstream locations (Schetagne et al. 2000; Hylander et al. 2006; Kasper et al. 2012). Schetagne et al. (2000) also examined the contribution of drifting biotic samples from the reservoir (phytoplankton, zooplankton, benthic invertebrates and fish) to the total Hg transport downstream from the dam and found that although the biotic sources had higher Hg concentrations compared to SPM, they had a significantly lower overall contribution to downstream exports due to their very low biomass per volume of water flowing through the dam compared to the high biomass of SPM. Nevertheless, the relative contribution of abiotic vs biotic Hg transfer will depend on the river and the dam in focus. The MQGS could be allowing higher amounts of fish to pass through the turbines and thereby providing a higher-Hg diet to downstream predators; the potential for Hg transfer

downstream from the MQGS and the pathways associated with this could be addressed in future studies.

The dam also appears to be influencing the aquatic food web whereby Smallmouth Bass and Yellow Perch from the HP had more negative $\delta^{13}C$ (along with the FF in Yellow Perch) and both species had highest trophic positions in the HP and NF. Black et al. (2003) also showed depleted $\delta^{13}C$ in fish from a reservoir ecosystem, which was related to predominant reliance on the pelagic food web in this environment, even by fish that are generally considered obligate benthivores (e.g., Cyprinids, Catostomids, and Cottids). Previous studies on the Wolastoq | SJR also demonstrated that food webs from the HP and just below the dam had the largest niche widths, based on a greater range of $\delta^{13}C$ and $\delta^{15}N$ values, compared to sites upstream and downstream, which suggests the dam could be altering food web processes away from the natural state of the river (Dolson-Edge et al. 2019). The limited data on lower-trophic-level biota in my study make it difficult to predict the direction of food web shifts, however the shift in $\delta^{13}C$ values within fish species suggest that energy pathways are being affected by the dam nonetheless. Altered flow dynamics associated with dams have been shown to increase trophic positions in top predators by altering foraging behaviours (Ruhí et al. 2016). In the Wolastoq | SJR, the MQGS blocks the passage of migrating fish, like the small anadromous Gaspereau (Alewife (*Alosa pseudoharengus*) and Blueback Herring (*Alosa aestivalis*)), which therefore accumulate below the dam and one million are manually transported above the dam each year (Samways et al. 2019). These fish could provide an abundant, higher-level prey item to Smallmouth Bass and Yellow Perch in the HP and NF, possibly explaining their higher trophic positions. Overall, the physical fragmentation of the Wolastoq | SJR by the MQGS appears to be

altering food web dynamics; this can subsequently influence contaminant and nutrient uptake by fish.

4.2 Spatial patterns in contaminants

Some of the spatial patterns of contaminants in fish observed herein could be due to other anthropogenic influences, such as point source inputs or land uses. For example, the spatial differences in Hg concentrations could also be attributed to industrial release near the sites. AV Nackawic, a dissolving grade pulp located ~45 km upstream from the HP site, is one of the largest Hg-emitters in New Brunswick, releasing 36.44 kg of Hg directly into the Wolastoq | SJR between 2006 to 2019 (NPRI 2022). UPS is upstream from this facility, and would therefore have lower exposure to the effluent; this could be another reason why fish at this site have lower Hg than the same species downstream. Furthermore, Hg in sediment measured in 2014 and 2015 was higher downstream of this facility (99 µg/kg dw) compared to sites upstream from this facility (31 µg/kg dw) or sites downstream from the dam (23 µg/kg dw; Kidd et al. 2019).

In contrast to Hg, there are no recorded releases of Tl or Cu from facilities along the Wolastoq | SJR from 1994 to present (NPRI 2022); therefore, the high concentrations of these elements in NF fish could be related to land use. The land surrounding the NF is built-up as its located in the City of Fredericton. Soil erosion, industrial run-off and high traffic can mobilize elements like Tl and Cu into aquatic ecosystems (Peter & Viraraghavan 2005; Banas et al. 2010; Couture et al. 2011), potentially explaining the higher levels in Yellow Perch and Smallmouth Bass from this site. Although Tl was higher in Smallmouth Bass and Yellow Perch from the NF, concentrations were still low (range = 0.0007-0.002 µg/g ww) and are lower than ranges found in Arctic Char from a remote Arctic lake (0.004-0.025 µg/g ww; Gantner et al. 2009). High Cu concentrations in fish were observed in urban lakes and rivers in Bangladesh and China (mean

concentrations ranged from 1.1–7.2 and 0.23-1.13 µg/g ww, respectively), which were higher than concentrations in this study (species range: 0.16-0.25 µg/g ww) and likely reflective of the higher populations in the former basins (Islam et al 2015; Bi et al. 2018).

In the FF, Se, As and Hg concentrations may be high in fish due to the release of these elements by a previous coal mining and powerplant facility (Grand Lake Generating Station) located next to Grand Lake, which connects to the Wolastoq | SJR at this site (New Brunswick Museum 2013; NPRI 2022). Between 1997 and 2010 (when the facility closed) this facility released a total of ~19,107 kg of As into the water, with additional releases to land and air, and ~887 kg of Hg into the air (NPRI 2022). Although no Se release data were available for this facility, coal mining and burning are major contributors of Se release into the environment (Etteieb et al. 2020) and these activities are among the strongest predictors of Se and As accumulation in fish from Canadian freshwaters (Ponton et al. 2022); therefore, it is probable that this facility acted as a source. Although these elements were released one decade prior, they can still be relevant to study in fish presently as they are persistent in sediments; for example, Se concentrations have remained relatively elevated in sediment, benthic invertebrates and fish ten years after exposure via waste-water effluent from a coal-fired electric generating facility on Belews Lake (North Carolina; Lemly 1997).

4.3 Species patterns in contaminants and minerals

Species differences in elements and organic contaminants could be caused by variable trophic positions, habitat use, lipid content or, for certain elements, physiological requirements. Total DDT and PCBs in species were reflective of their % lipid (i.e., species with higher % lipid had higher concentrations) as these organic contaminants are stored in lipid tissues (Hebert & Keenleyside 1995). Concentrations often need lipid correction (normalizing concentrations to

lipid content in fish) to see trends of bioaccumulation (Hebert & Keenleyside 1995; Gray 2002) which was apparent in my data as species at higher trophic positions did not necessarily have higher organic contaminants. Nevertheless, I reported ww concentrations as they are more valuable for assessing the safe consumption of fish. For bioaccumulative elements like Hg and Se (Hamilton 2004; Wang 2012), species differences were related to their relative trophic positions (i.e. Striped Bass were highest in these elements, Smallmouth Bass were intermediate, and American Eel and Yellow Perch were lowest).

Other differences in elements between species could be related to habitat use and other life history traits. Habitat can influence an organism's interaction with metals in the environment; for example, demersal fish can have higher exposure to metal-enriched sediments, which can increase their accumulation of metals (Roach et al. 2008; Bustnes et al. 2012). This phenomenon could explain the higher levels of Co, Zn and, although not significantly, Fe and Ti in American Eel in the Wolastoq | SJR as it is a demersal species. Furthermore, a review of the factors influencing nutrient compositions (including Fe and Zn) in fish determined that phylogenetic relatedness was associated with similarities in nutrient profiles and eel species were in the 80th percentile of high Zn concentrations (Vaitla et al. 2018). The distinct element compositions of American Eel and Striped Bass compared to Yellow Perch and Smallmouth Bass, which were more similar in PCAs, could be related to the migratory behaviour of the former two species. Striped Bass are anadromous (born in freshwater, migrate to ocean and return to freshwater to spawn) and American Eel are catadromous (born in ocean, migrate to freshwater and return to ocean to spawn) and therefore, they can accumulate metals from a broader range of habitats, including marine environments. To better understand the uptake of metals from the Wolastoq | SJR vs marine sources, stable isotope ratios of sulfur ($\delta^{34}S$) in muscle

tissue or Sr to Ca ratios (Sr:Ca) could be used to determine habitat use (Fry & Chumchal 2011; Yang et al. 2011; Walther & Limburg 2012).

4.4 Spatial and species differences in fatty acids

Differences in FA concentrations among fish species and within species among sites are also explained by physiological requirements and ecological factors. When Smallmouth Bass and Yellow Perch data were pooled across sites, DHA was highest in Striped Bass and Smallmouth Bass, American Eel had intermediate levels, and Yellow Perch were the lowest, whereas EPA was significantly highest in Yellow Perch, followed by American Eel, Striped Bass and Smallmouth Bass. Interestingly, DHA appears to be positively associated with the species' trophic positions, and the different trophic positions among sites Yellow Perch, whereas EPA is somewhat negatively associated with trophic position in these fishes. DHA is more selectively retained in higher trophic levels than EPA (Heissenberger et al. 2010; Strandberg et al. 2015; Sushchik et al. 2017; Jardine et al. 2020), which may explain the positive association between trophic position and DHA, but not EPA. The higher levels of EPA in Yellow Perch could be related to a diet high in benthic invertebrates, which tend to be enriched in EPA but lower in DHA compared to other food sources like phytoplankton and zooplankton (Heissenberger et al. 2010; Sushchik et al. 2017). The lower trophic position and $\delta^{15}N$ in Yellow Perch compared to the other fish species supports that they are eating lower-trophic-level prey items, but the limited $\delta^{13}C$ data for benthic and pelagic food sources across sites in this study make it difficult to confirm whether Yellow Perch rely more on the benthic food web; future studies could investigate this. Specifically for Smallmouth Bass, higher levels of EPA in the UPS site could be related to shifts in basal carbon food sources as $\delta^{13}C$ was enriched in fish from this site. Smallmouth Bass from the UPS had high amounts of crayfish observed in stomach contents

(pers. obs.) and crayfish tend to be richer in EPA than DHA (Harlioğlu et al. 2012; McInerney et al. 2022). To better understand which factors are influencing FA compositions in fish, EPA and DHA concentrations in food sources, along with other factors like water trophic status, temperature and plankton community, could be measured in future studies on this system.

4.5 Risk-benefit analysis of fish from the Wolastoq | SJR

Of the contaminants measured in this study, Hg presented the greatest concern to consumers as it was the only contaminant that restricted the frequency of fish consumption based on the meals per month (mpm) consumption advisories from Ontario's Guide to Eating Fish (MECC 2015). Therefore, I performed a preliminary risk-benefit analysis of fish consumption by determining the length ranges associated with different Hg mpm consumption advisories for the general population, for each species and site, and then determined the EPA + DHA concentration a consumer would obtain if they consumed these sizes of fish at their maximum allowable frequency (see brief methods and results in text of SI section 5.2 and Table S7). These EPA + DHA concentrations were then compared against an optimal intake of 250 mg of EPA + DHA per day to reduce risks of coronary heart disease (Mozaffarian & Rimm 2006; EFSA 2010; Williams et al. 2017; Laird et al. 2018; Strandberg et al. 2018). My results showed that the average daily EPA + DHA intake provided by any fish across all size ranges in this study did not meet this 250 mg/day recommendation. Species and sites also differed in their risks and benefits; for example, Striped Bass had the highest Hg concentrations and therefore the most restrictive mpm consumption advisories, which limited consumer EPA + DHA intake. Furthermore, Smallmouth Bass from the UPS site were advised for more frequent consumption at larger sizes compared to the other sites, which is reflective of the lower Hg concentrations at this site, and

allowed for greater EPA + DHA intake (although levels were still low compared to daily intake recommendations).

The overall low benefit of eating these fish species from the Wolastoq | SJR is due to the low EPA + DHA and high Hg concentrations, which limit their consumption. Numerous risk-benefit analyses have been performed for fish species from the Great Lakes Region (GLR) and average EPA + DHA concentrations in Smallmouth Bass and Yellow Perch across these studies were 2010 µg/g and 1571 µg/g ww, respectively, compared to 1208 µg/g and 1213 µg/g, respectively, across sites in my study (Neff et al. 2014; Williams et al. 2014; Williams et al. 2017; Strandberg et al. 2020). Strandberg et al. (2020) examined a suite of contaminants (total PCBs, Hg, dioxins/furans and OCPs) and EPA + DHA concentrations in Smallmouth Bass and Yellow Perch from the GLR. The mpm advisories (based on the contaminant that restricted consumption the most) allowed for more frequent consumption of fish at similar sizes to those in my study because of the lower contaminant levels and, therefore, a higher potential for safe EPA + DHA intake at recommended levels. Although there are less data on the FA concentrations for wild American Eel and Striped Bass, EPA + DHA concentrations have been reported at 13400 ug/g ww and 6400 ug/g ww, respectively (Exler & Weihrauch 1976), which are higher than those found in my study (means = 1130 and 1269 µg/g ww, respectively). Overall, this preliminary analysis indicated that these fish do not provide optimal EPA + DHA levels if they are consumed safely at their Hg consumption advisory. Furthermore, the sensitive population (children under 15 and women of child-bearing age) has more restrictive concentrations for safe Hg consumption, and therefore their mpm consumption advisories and daily EPA + DHA intake from these fish would be even lower. Further risk-benefit analyses could examine the potential protective effects of Se, which binds to Hg and is suggested to reduce its toxicity (Gochfeld &

Burger 2021), or the counteractive effects of EPA and DHA to Hg toxicity for specific endpoints like coronary heart disease and IQ (Ginsberg & Toal 2009).

4.6 Conclusion

This study suggests that species and location are two important factors that affect the contaminant and nutrient concentrations in fish from the Wolastoq | SJR. The varying concentrations in fish from this river could be related to point source releases, land use, trophic processes and physiological requirements. The MQGS appeared to be altering Hg, S and P uptake in fish, which could be because these elements were present in sediments that were trapped by the dam or because the reservoir provided suitable conditions for converting these elements into bioavailable forms. Furthermore, this study suggests that the dam was altering food web dynamics through shifts in basal carbon sources and trophic positions, which could also impact the uptake of contaminants and nutrients by fish. This was the first study to examine FA concentrations in fish from this river and interestingly, DHA concentrations were greater in higher-trophic-level fish, however, these fish also had higher concentrations of bioaccumulative contaminants, of which Hg was the greatest concern for human consumers. Preliminary risk-benefit analyses indicated that these fish do not provide optimal levels of EPA + DHA if their Hg consumption advisory is followed, which means consumers would need to obtain their EPA + DHA elsewhere if they choose to follow the recommended intakes. These data can be used to monitor changes of contaminants and nutrients in this system and inform local consumers of the risks and benefits of consuming fish.

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5.0 Supplemental Information

5.1 SI Tables

Table S1. Sample sizes, sexes and mean (SD) lengths, weights and ages of the subset of Smallmouth Bass and Yellow Perch used for FA analysis. The number of fish with no age (NA) recorded is presented in brackets next to the SD.

Species	Site	N	Sex			Total length (mm)	Total weight (g)	Age (years)
			M	F	NS			
Smallmouth Bass	Upstream	20	12	8		402 (43)	994 (316)	8 (3; 2 NA)
	Head Pond	19	9	10		375 (68)	751 (364)	7 (4)
	Near Field	20	12	7	1	369 (77)	774 (435)	8 (4)
	Far Field	20	11	9		371 (71)	737 (448)	7 (4)
Yellow Perch	Upstream	20	7	13		213 (33)	111 (50)	7 (3)
	Head Pond	20	10	10		194 (49)	91.1 (70)	4 (2)
	Near Field	20	10	9	1	198 (33)	98.1 (46)	5 (2)
	Far Field	20	10	10		187 (22)	82.3 (35)	8 (2)

Table S2. OCPs, detection limits (ng/g dw) and percent of composite fish samples above DL within each species. Analyses were performed at the Queen's ASU (Kingston, ON).

OCPs	Detection limit	% of samples above DL			
		American Eel	Smallmouth Bass	Striped Bass	Yellow Perch
alpha-BHC	<2.0	0	0	0	0
beta-BHC	<4.0	0	0	0	0
gamma-BHC	<4.0	0	0	0	0
delta-BHC	<4.0	0	0	0	0
heptachlor	<2.0	0	0	0	0
aldrin	<4.0	0	0	0	0
heptachlor epox iso B	<2.0	0	0	0	0
2,4-DDE	<3.0	0	0	0	0
endosulfan I	<10.0	0	0	0	0
dieldrin	<4.0	0	0	0	0
4,4-DDE	<3.0	100	100	100	100
2,4-DDD	<6.0	0	4	0	0
endrin	<5.0	0	0	0	0
Endosulfan II	<5.0	0	0	0	0
4,4-DDD	<6.0	100	71	80	13
2,4-DDT	<4.0	0	4	20	0
endrin aldehyde	<4.0	0	0	0	0
endosulfan sulfate	<6.0	0	0	0	0
4,4-DDT	<4.0	100	50	80	0
endrin ketone	<4.0	0	0	0	0
methoxychlor	<5.0	0	63	80	35

Table S3. PCB congeners, detection limits (ng/g dw) and percent of composite fish samples above DL within each species. Analyses were performed at the Queen's ASU (Kingston, ON).

IUPAC Name	IUPAC #	Detection limit	% of samples above DL			
			American Eel	Smallmouth Bass	Striped Bass	Yellow Perch
2,2',5-Trichlorobiphenyl	18	<0.05	0	25	0	13
2,4,4'-Trichlorobiphenyl	28	<0.05	0	0	0	0
2,3',4,6-Tetrachlorobiphenyl 2,2',5,5'-Tetrachlorobiphenyl	69+52	<0.10	25	0	0	4.3
2,2',4,5'-Tetrachlorobiphenyl	49	<0.05	12.5	0	0	0
2,3,5,6-Tetrachlorobiphenyl 2,4,4',6-Tetrachlorobiphenyl 2,2',4,4'-Tetrachlorobiphenyl 2,2',4,5-Tetrachlorobiphenyl	65+75+47+48	<0.20	12.5	0	0	0
2,4,4',5-Tetrachlorobiphenyl	74	<0.05	0	0	0	0
2,3',4,4'-Tetrachlorobiphenyl	66	<0.05	12.5	4.2	0	0
3,4,4',5-Tetrachlorobiphenyl	81	<0.05	0	4.2	0	0
3,3',4,4'-Tetrachlorobiphenyl	77	<0.05	0	0	0	0
2,2',4,5,5'-Pentachlorobiphenyl	101	<0.05	100	62.5	100	26.1
2,2',4,4',5-Pentachlorobiphenyl	99	<0.05	100	50	80	4.3
2,3,3',4',6-Pentachlorobiphenyl	110	<0.05	100	62.5	100	13
2,3',4,4',5'-Pentachlorobiphenyl	123	<0.05	0	0	0	0
2,3',4,4',5-Pentachlorobiphenyl	118	<0.05	100	54.2	60	4.3
2,3,4,4',5-Pentachlorobiphenyl	114	<0.05	0	0	0	0
2,3,3',4,4'-Pentachlorobiphenyl 3,3',4,5,5'-Pentachlorobiphenyl	105+127	<0.10	100	37.5	80	0
3,3',4,4',5-Pentachlorobiphenyl	126	<0.05	0	0	0	0
2,2',4,4',5,5'-Hexachlorobiphenyl	153	<0.05	100	66.7	100	17.4
2,2',3,4,5,5'-Hexachlorobiphenyl	141	<0.05	100	8.3	0	4.3

2,2',3,4,4',5'-Hexachlorobiphenyl	138	<0.05	100	87.5	100	39.1
2,2',3,3',4,4'-Hexachlorobiphenyl	128+162	<0.10	37.5	4.2	0	0
2,3,3',4',5,5'-Hexachlorobiphenyl						
2,3',4,4',5,5'-Hexachlorobiphenyl	167	<0.05	0	0	0	0
2,3,3',4,4',5-Hexachlorobiphenyl	156	<0.05	37.5	0	0	0
2,3,3',4,4',5'-Hexachlorobiphenyl	157	<0.05	0	0	0	0
3,3',4,4',5,5'-Hexachlorobiphenyl	169	<0.05	0	0	0	0
2,2',3,3',5,5',6-Heptachlorobiphenyl	178	<0.05	0	29.2	0	0
2,2',3,4,4',5,6'-Heptachlorobiphenyl	182+187	<0.10	12.5	33.3	0	0
2,2',3,4',5,5',6-Heptachlorobiphenyl						
2,2',3,4,4',5',6-Heptachlorobiphenyl	183	<0.05	12.5	33.3	0	0
2,2',3,4,4',5,5'-Heptachlorobiphenyl	180	<0.05	37.5	29.2	0	4.3
2,2',3,3',4,4',5-Heptachlorobiphenyl	170	<0.05	25	33.3	0	0
2,3,3',4,4',5,5'-Heptachlorobiphenyl	189	<0.05	0	0	0	0
2,2',3,3',4,5',6,6'-Octachlorobiphenyl	201	<0.05	0	0	0	0
2,2',3,4,4',5,5',6-Octachlorobiphenyl	203	<0.05	0	0	0	0
2,2',3,3',4,4',5,6-Octachlorobiphenyl	195	<0.05	0	0	0	0
2,2',3,3',4,4',5,5'-Octachlorobiphenyl	194	<0.05	0	4.2	0	0

2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	206	<0.05	0	0	0	0

Table S4. Trace elements, detection limits (μg/g dw) and percent of composite fish samples above DL within each species. Analyses were performed at the Queen's ASU (Kingston, ON).

Element	Detection limit	% of samples above DL			
		American Eel	Smallmouth Bass	Striped Bass	Yellow Perch
Aluminum	<10	50	0	0	0
Antimony	<0.1	12.5	0	0	0
Arsenic	<0.5	100	66.7	100	21.7
Barium	<0.1	50	0	0	100
Beryllium	<0.005	0	0	0	0
Boron	<10	0	0	0	0
Cadmium	<0.005	25	4.2	0	47.8
Calcium	<20	100	100	100	100
Chromium	<0.1	100	100	100	100
Cobalt	<0.01	100	70.8	80	95.6
Copper	<0.5	100	100	100	100
Iron	<10	100	87.5	100	95.6
Lead	<0.05	50	0	20	4.3
Magnesium	<2.0	100	100	100	100
Manganese	<0.2	100	100	100	100
Molybdenum	<0.05	0	50	0	73.9
Nickel	<0.05	100	91.7	100	95.6
Phosphorus	<20	100	100	100	100
Potassium	<15	100	100	100	100
Selenium	<0.1	100	100	100	100
Silver	<0.01	0	0	0	0
Sodium	<15	100	100	100	100
Strontium	<0.2	100	100	80	100
Sulfur	<25	100	100	100	100
Thallium	<0.0025	0	100	0	100

Tin	<0.1	0	0	0	0
Titanium	<0.2	100	100	100	100
Uranium	<0.0025	25	0	0	0
Vanadium	<0.02	62.5	54.2	0	95.6
Zinc	<2.0	100	100	100	100

Table S5. Hg (µg/g ww), DHA (µg/g ww) and trophic position adjusted to the mean length (L), weight (W) and age (A) in Smallmouth Bass and Yellow Perch. Adjusted data are presented for response variables that had significant relationships with L, W or A (Spearman – $p < 0.05$), and where the fish differed in these covariates between sites, within species (Kruskal-Wallis tests – $p < 0.05$). The mean L, W and A were 198 mm, 96 g, and 6 years-old for Yellow Perch, respectively, and 373 mm, 792 g, 8 years-old for Smallmouth Bass, respectively.

Species	**Site**	**Hg**			**DHA**	**Trophic position**		
		L	**W**	**A**	**A**	**L**	**W**	**A**
Smallmouth Bass	Upstream	0.291	0.283	0.317	–	4.81	4.79	4.88
	Head Pond	0.638	0.664	0.684	–	6.38	6.42	6.43
	Near Field	0.617	0.610	0.591	–	6.32	6.31	6.29
	Far Field	0.709	0.738	0.752	–	4.68	4.71	4.68
Yellow Perch	Upstream	0.295	0.299	0.296	646	3.47	3.54	3.55
	Head Pond	0.347	0.346	0.466	1090	6.43	6.43	6.65
	Near Field	0.234	0.234	0.270	808	4.81	4.83	5.07
	Far Field	0.339	0.336	0.274	552	2.65	2.87	3.27

Table S6. Ontario's "do not eat" fish consumption advisories for the general population, and mean concentration (µg/g ww) and standard error of total DDT and PCBs in fish muscle of American Eel and Striped Bass collected from the NF site, and Smallmouth Bass and Yellow Perch collected from all sites on the Wolastoq | SJR. Concentrations are given for total DDT and PCBs where > 60% of the samples within a species were above DL (half of the DL was substituted for the remaining samples below).

Species/ consumption advisory	Site	N (# composited)	Mean length (mm)	Total DDT	Total PCBs
"Do not eat"				>5	>0.844
American Eel	NF	8 (20)	602	0.14 (0.028)	0.022 (0.0064)
Striped Bass	NF	5 (10)	838	0.053 (0.021)	0.011 (0.0035)
Smallmouth Bass	All sites	24 (75)	381	0.025 (0.0037)	0.0041 (0.0016)
	UPS	24 (75)	381	0.033 (0.0082)	0.0014 (0.00059)
	HP	6 (20)	398	0.024 (0.0069)	0.0031 (0.0012)
	NF	6 (17)	340	0.029 (0.0094)	0.0037 (0.00084)
	FF	6 (20)	371	0.014 (0.0025)	0.0083 (0.0064)
Yellow Perch	All sites	23 (80)	203	0.008 (0.0015)	-
	UPS	6 (20)	214	0.013 (0.0031)	-
	HP	6 (20)	204	0.012 (0.0036)	-
	NF	6 (20)	199	0.0030 (0.00027)	-
	FF	6 (20)	193	0.0044 (0.00087)	-

5.2 Risk-benefit analysis

5.2.1 Risk-benefit analysis methods

I performed simple regression analyses between $\log_{10}$ Hg concentration (µg/g ww) and total length (mm) for each site and species and determined the fish length ranges at specific Hg thresholds (i.e., those associated with a mpm consumption advisories for the general population, e.g., < 0.15 ug/g ww = 32 mpm, 0.15-0.29 ug/g ww = 16 mpm, etc.) using the R package chemCal (CRAN, 2021; version 0.2.2). Although the relationship between $\log_{10}$ Hg and length was not significant in Striped Bass and American Eel ($p > 0.05$), the relationship was still positive and the same methods were used for this preliminary analysis. I then determined the average EPA + DHA concentration of the fish that fell within the specific length ranges to determine how many mg of EPA + DHA were in a 227 g meal (i.e., the meal size used in Ontario's mpm advisories) and then calculated a consumer's daily EPA + DHA intake (mg/day) if they consumed the fish at their maximum allowable frequency.

5.2.2 Risk-benefit analysis table

Table S7. Length ranges (L; mm) associated with Hg mpm consumption advisories for the general population (from Ontario's Fish Guide), calculated for each species and site, and the associated average (SD) EPA + DHA daily intake (mg/day) a consumer would obtain if they consumed the fish at their maximum allowable frequency. Blanks indicate that the predicted fish length ranges were outside those collected in this study. No fish fell within the 32 mpm or "do not eat" consumption advisories.

Species	**Site**	**Meals per month consumption advisory**														
		16			**12**			**8**			**4**			**2**		
		N	L	EPA + DHA	N	L	EPA + DHA	N	L	EPA + DHA	N	L	EPA + DHA	N	L	EPA + DHA
Smallmouth Bass	UPS	5	275-384	142 (50)	12	385-429	117 (40)	3	430-493	81 (25)	–	–	–	–	–	–
	HP	–	–	–	2	249-295	118 (16)	7	296-362	73 (11)	10	363-476	28 (7)	–	–	–
	NF	–	–	–	1	171-251	97	6	252-365	89 (24)	13	366-561	35 (11)	–	–	–
	FF	–	–	–	1	201-257	135	6	258-337	83 (11)	12	338-474	35 (6)	1	475-554	18
Yellow Perch	UPS	8	116-202	145 (40)	7	203-239	103 (60)	5	240-290	60 (8)	–	–	–	–	–	–
	HP	9	65-173	179 (22)	5	174-218	133 (23)	5	219-282	85 (7)	1	283-391	28	–	–	–
	NF	20	51-272	160 (66)	–	–	–	–	–	–	–	–	–	–	–	–
	FF	6	<171	126 (42)	12	171-247	85 (27)	–	–	–	–	–	–	–	–	–
Striped Bass		–	–	–	–	–	–	–	–	–	7	365-854	40 (8)	6	855-1140	18 (3)
American Eel		–	–	–	20	203-730	103 (20)	4	731-1475	66 (43)	–	–	–	–	–	–

5.3 SI Figures

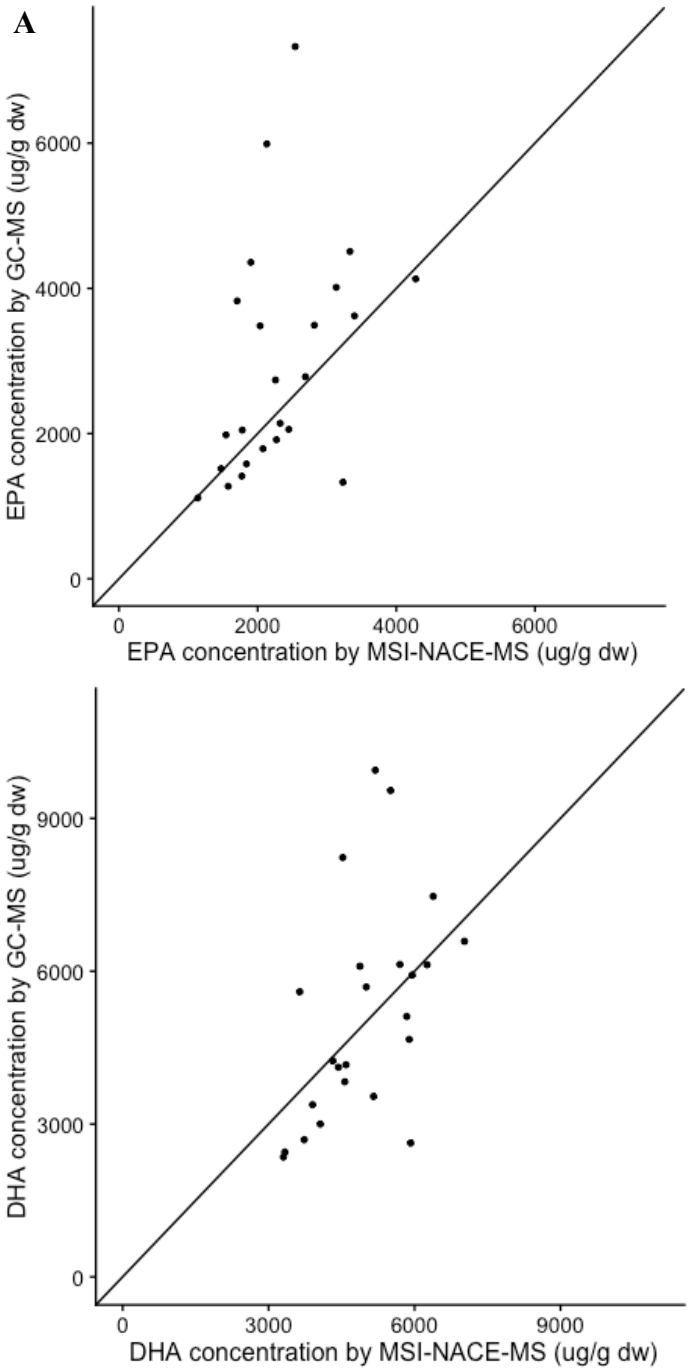


Figure S1. A) EPA and B) DHA concentrations (μg/g dw) analyzed in a subset of 24 fish by both GC-MS and MSI-NACE-MS.

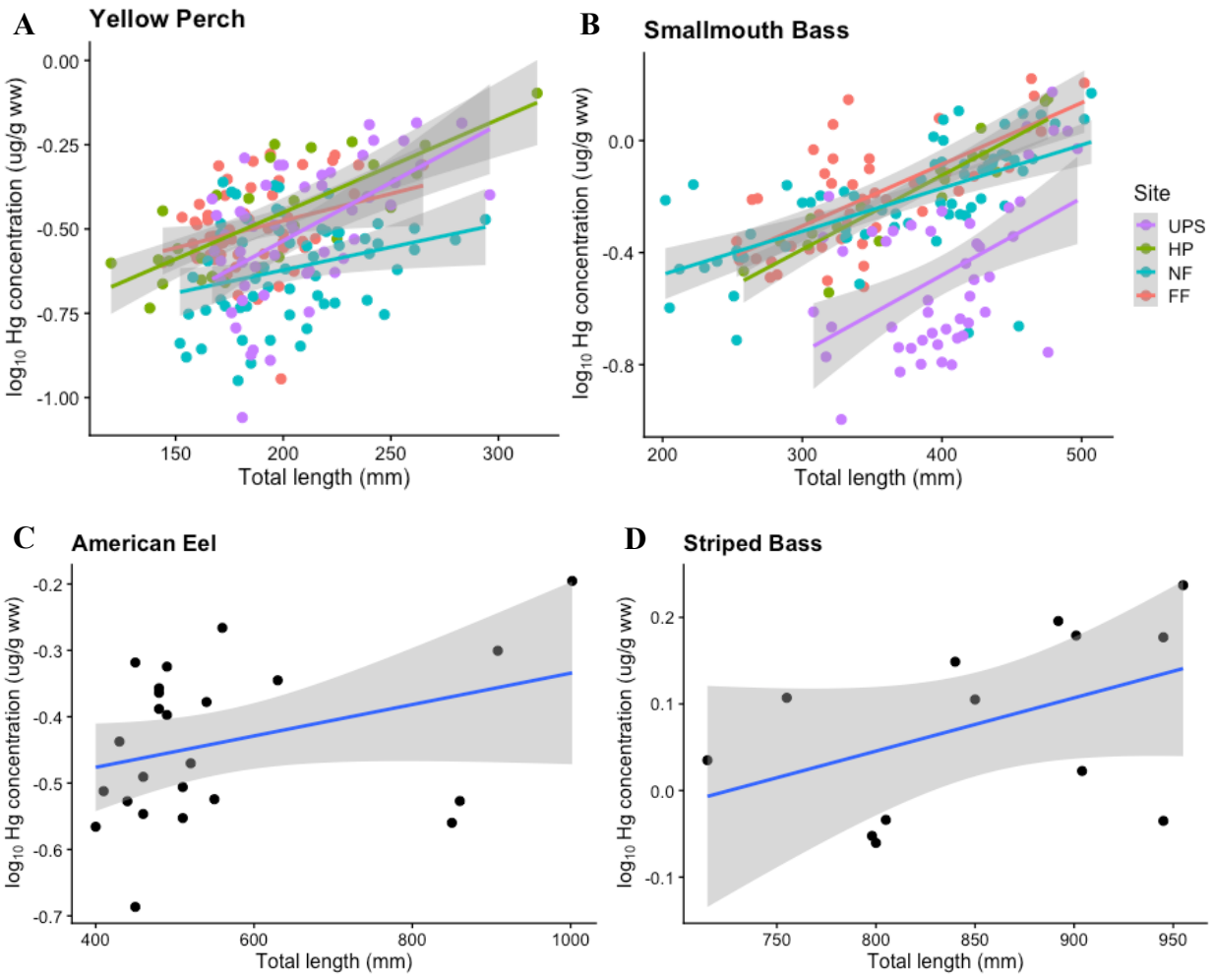


Figure S2. Relationship between $\log_{10}$ Hg (µg/g ww) and total length (mm) in Yellow Perch (A) and Smallmouth Bass (B) collected from all sites and American Eel (C) and Striped Bass (D) collected from the NF in the Wolastoq | SJR.

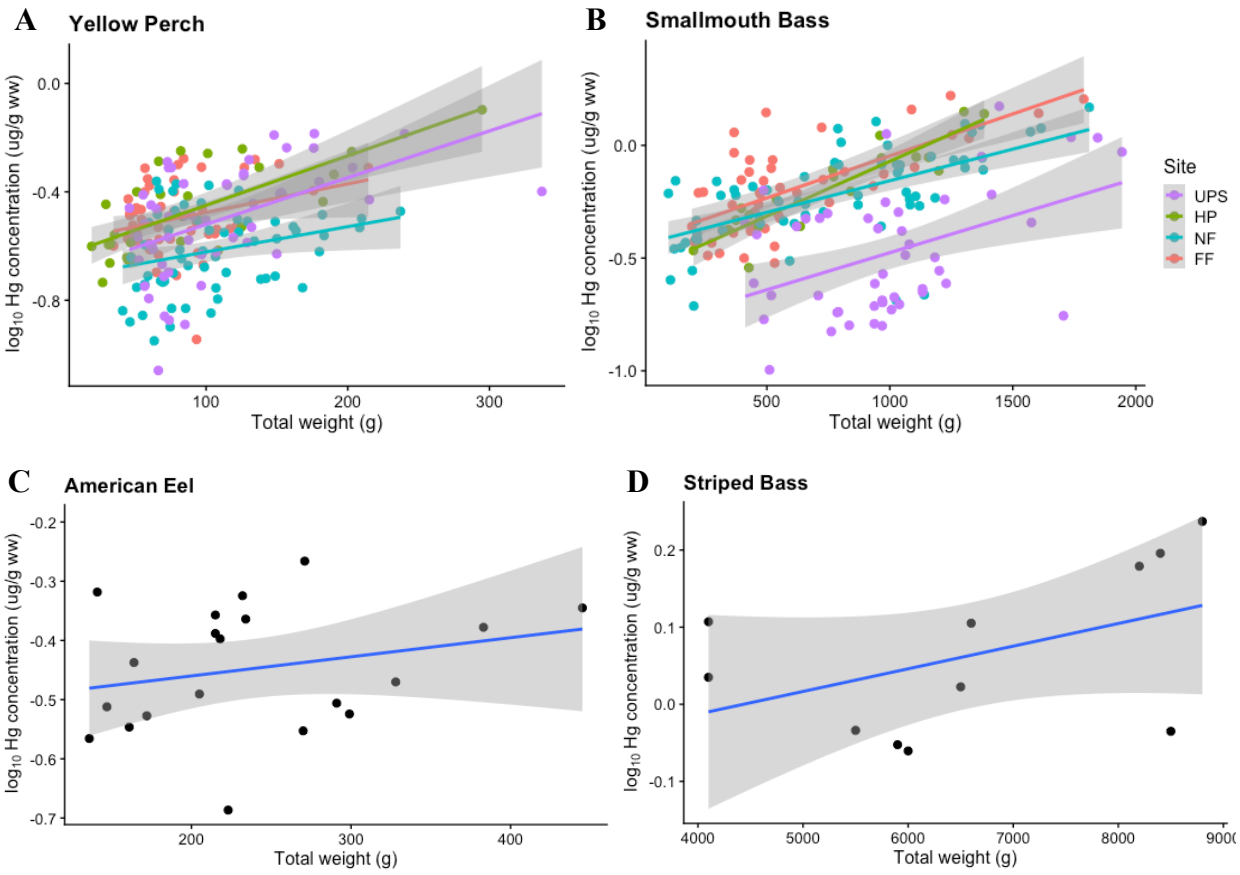


Figure S3. Relationship between $\log_{10}$ Hg (µg/g ww) and total weight (g) in Yellow Perch (A) and Smallmouth Bass (B) collected from all sites and American Eel (C) and Striped Bass (D) collected from the NF in the Wolastoq | SJR. The four longest American Eel and three longest Striped Bass were not weighed and are therefore not included in these relationships.

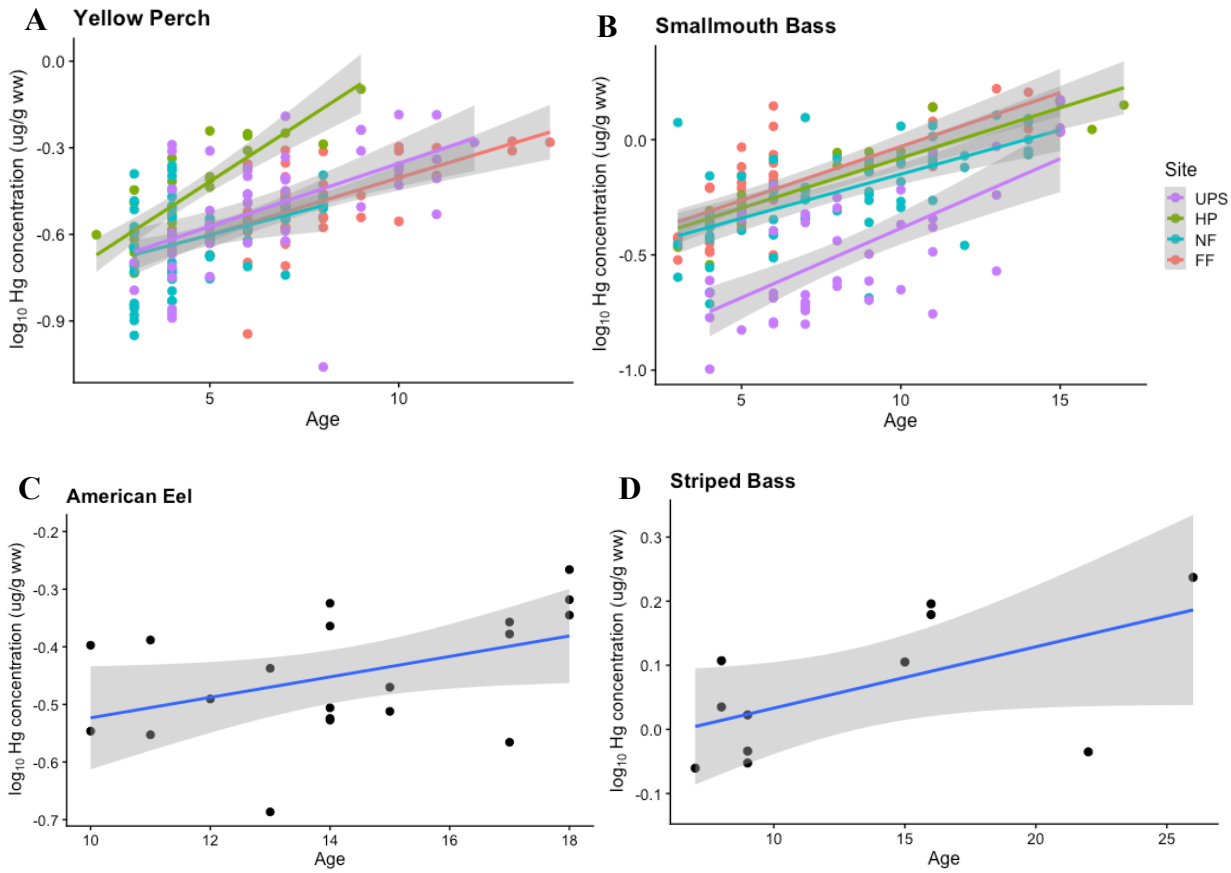


Figure S4. Relationship between $\log_{10}$ Hg (µg/g ww) and age (years-old) in Yellow Perch (A) and Smallmouth Bass (B) collected from all sites and American Eel (C) and Striped Bass (D) collected from the NF in the Wolastoq | SJR. The four longest American Eel and three longest Striped Bass were not aged and are therefore not included in these relationships.

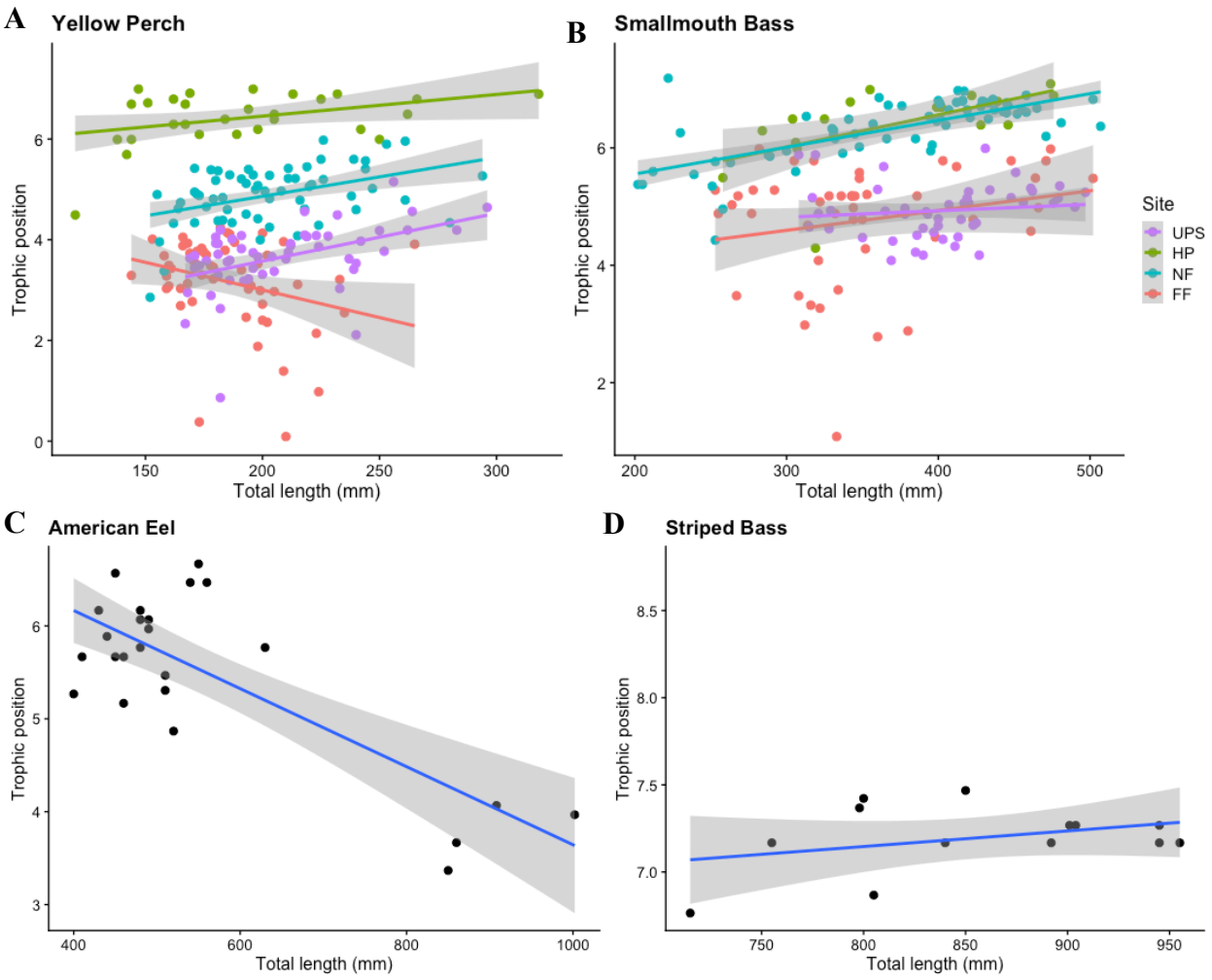


Figure S5. Relationship between trophic position and total length (mm) in Yellow Perch (A) and Smallmouth Bass (B) collected from all sites and American Eel (C) and Striped Bass (D) collected from the NF in the Wolastoq | SJR.

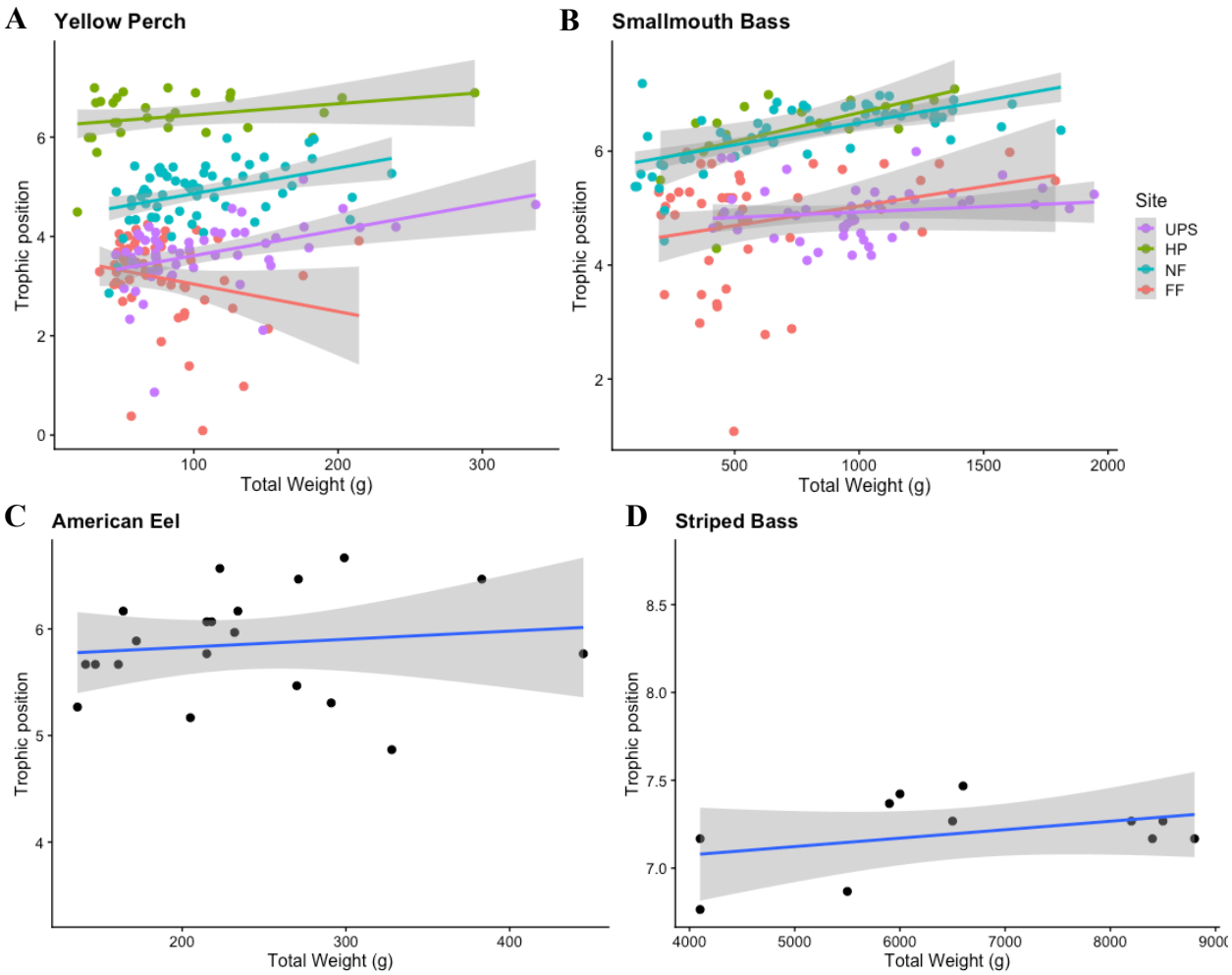


Figure S6. Relationship between trophic position and total weight (g) in Yellow Perch (A) and Smallmouth Bass (B) collected from all sites and American Eel (C) and Striped Bass (D) collected from the NF in the Wolastoq | SJR. The four longest American Eel and three longest Striped Bass were not weighed and are therefore not included in these relationships.

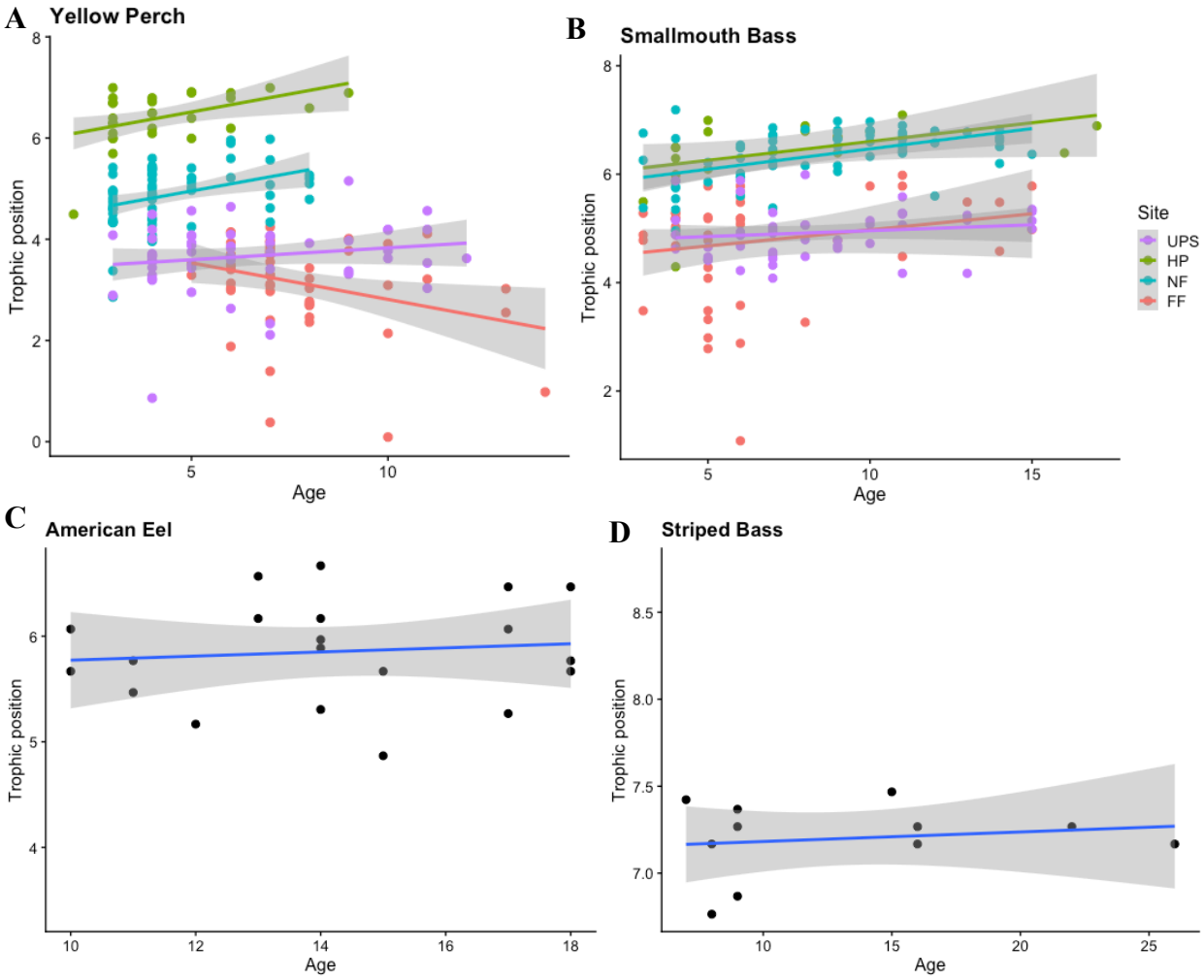


Figure S7. Relationship between trophic position and total age (years-old) in Yellow Perch (A) and Smallmouth Bass (B) collected from all sites and American Eel (C) and Striped Bass (D) collected from the NF in the Wolastoq | SJR. The four longest American Eel and three longest Striped Bass were not aged and are therefore not included in these relationships.

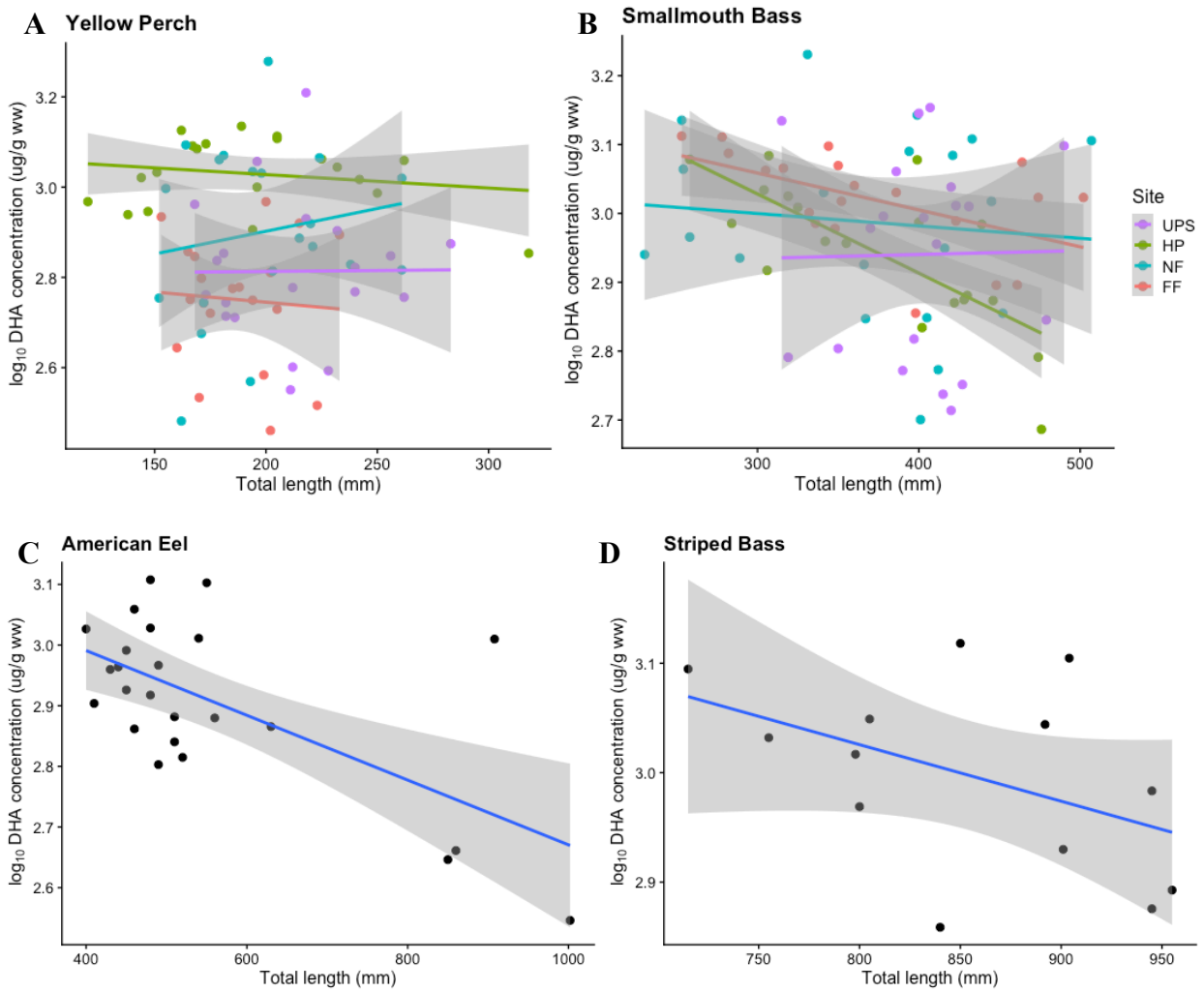


Figure S8. Relationship between $\log_{10}$ DHA (µg/g ww) and total length (mm) in Yellow Perch (A) and Smallmouth Bass (B) collected from all sites and American Eel (C) and Striped Bass (D) collected from the NF in the Wolastoq | SJR.

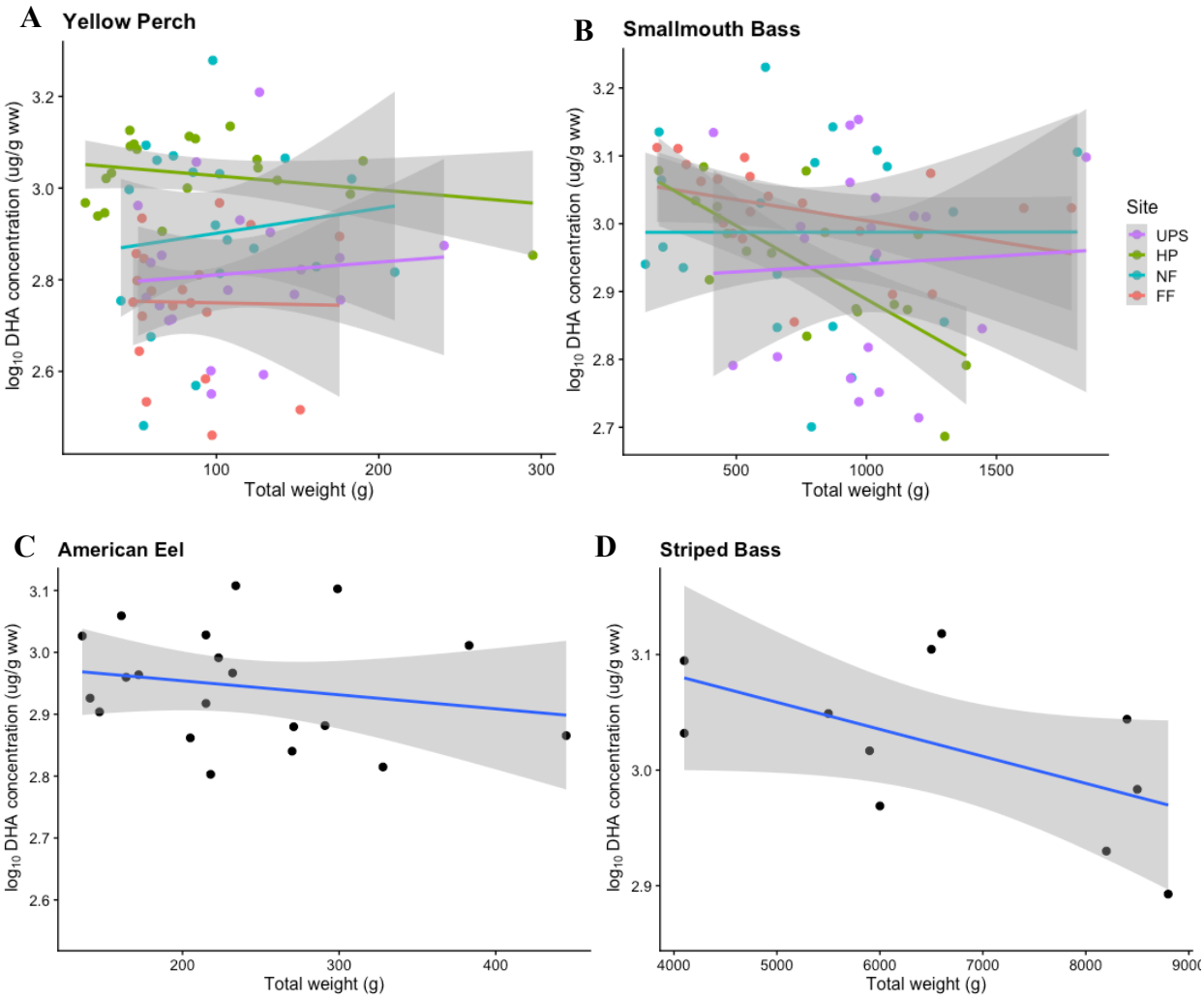


Figure S9. Relationship between $\log_{10}$ DHA (µg/g ww) and total weight (g) in Yellow Perch (A) and Smallmouth Bass (B) collected from all sites and American Eel (C) and Striped Bass (D) collected from the NF in the Wolastoq | SJR. The four longest American Eel and three longest Striped Bass were not weighed and are therefore not included in these relationships.

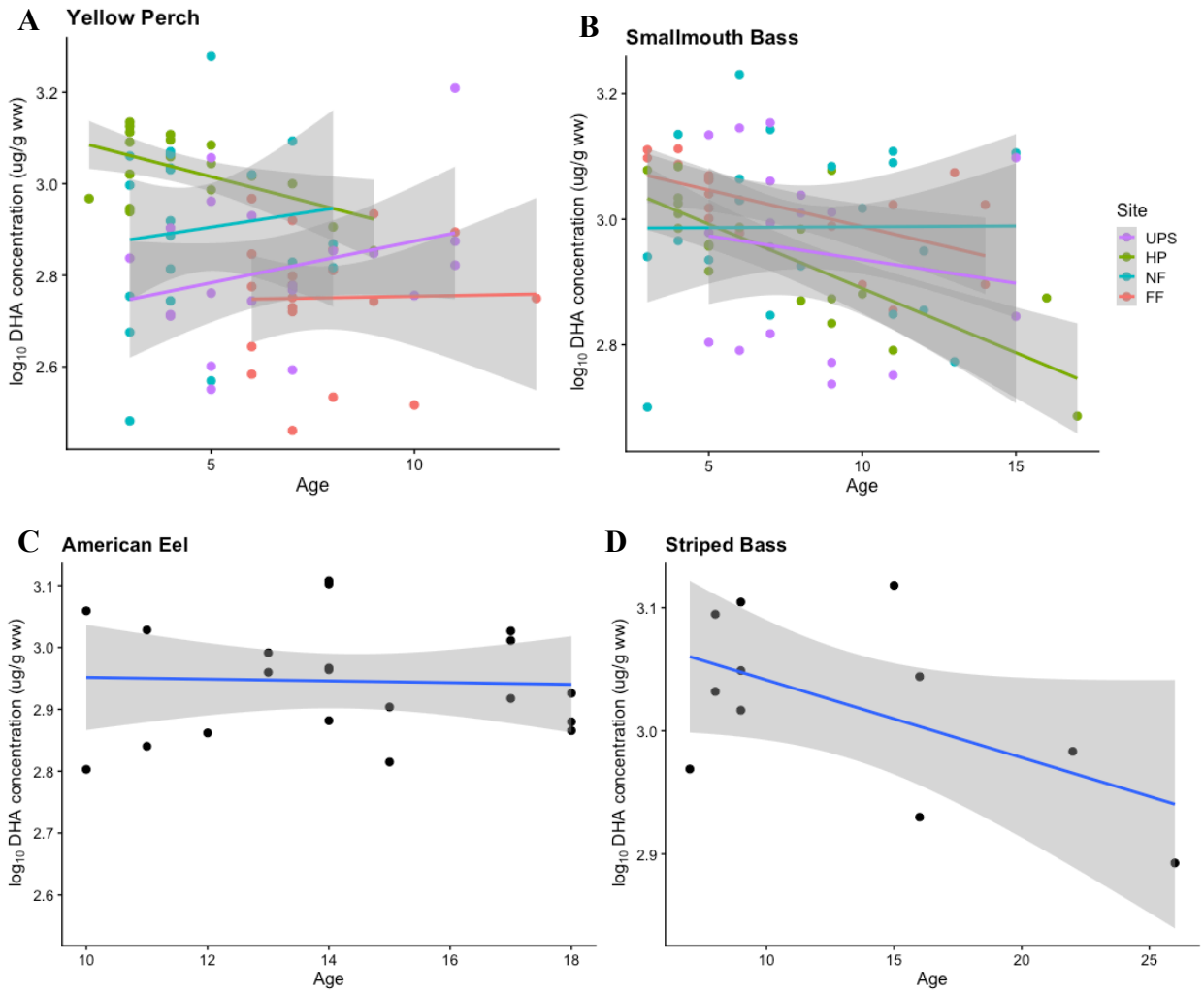


Figure S10. Relationship between $\log_{10}$ DHA (µg/g ww) and age (years-old) in Yellow Perch (A) and Smallmouth Bass (B) collected from all sites and American Eel (C) and Striped Bass (D) collected from the NF in the Wolastoq | SJR. The four longest American Eel and three longest Striped Bass were not aged and are therefore not included in these relationships.

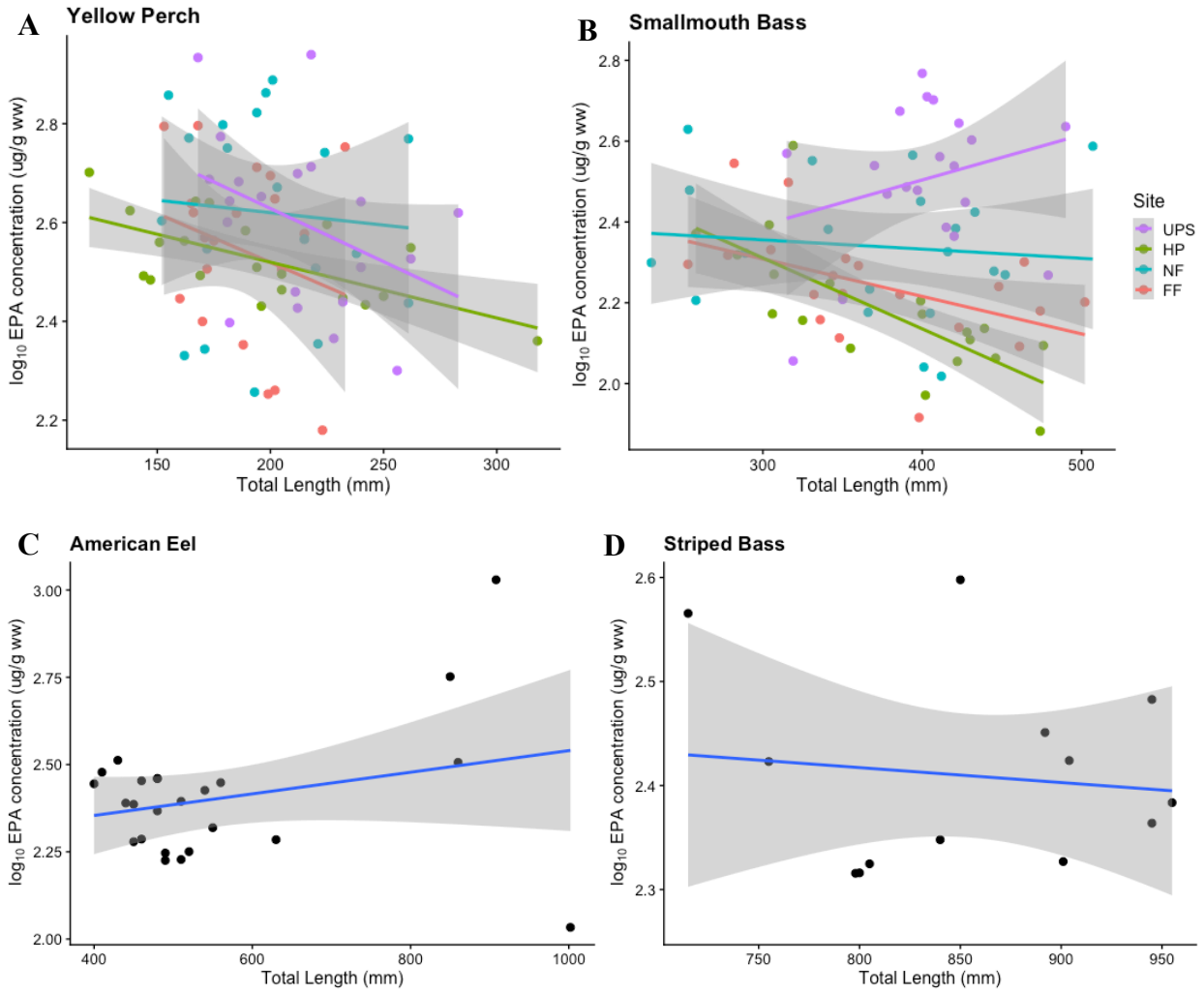


Figure S11. Relationship between $\log_{10}$ EPA (µg/g ww) and total length (mm) in Yellow Perch (A) and Smallmouth Bass (B) collected from all sites and American Eel (C) and Striped Bass (D) collected from the NF in the Wolastoq | SJR.

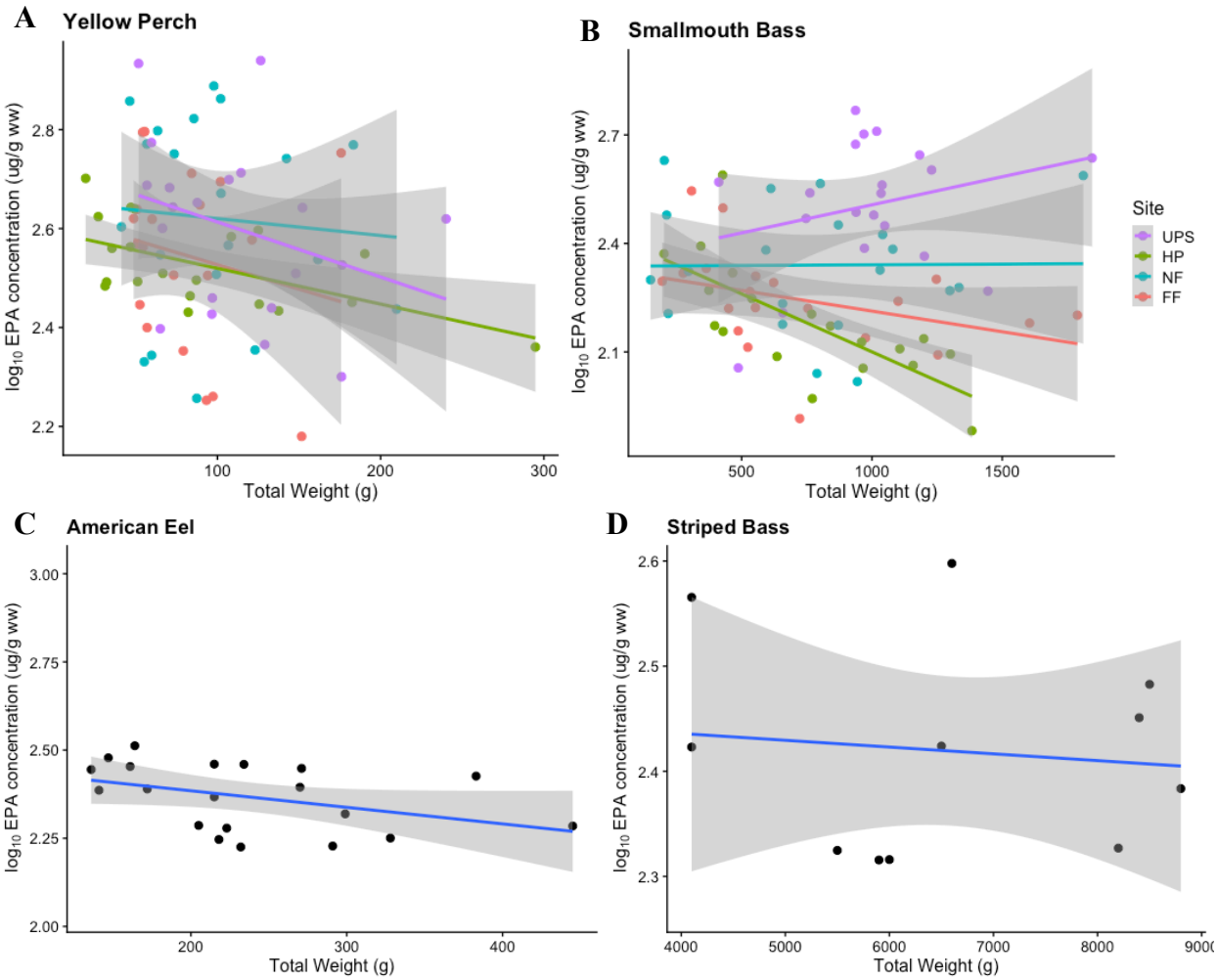


Figure S12. Relationship between $\log_{10}$ EPA (µg/g ww) and total weight (g) in Yellow Perch (A) and Smallmouth Bass (B) collected from all sites and American Eel (C) and Striped Bass (D) collected from the NF in the Wolastoq | SJR. The four longest American Eel and three longest Striped Bass were not weighed and are therefore not included in these relationships.

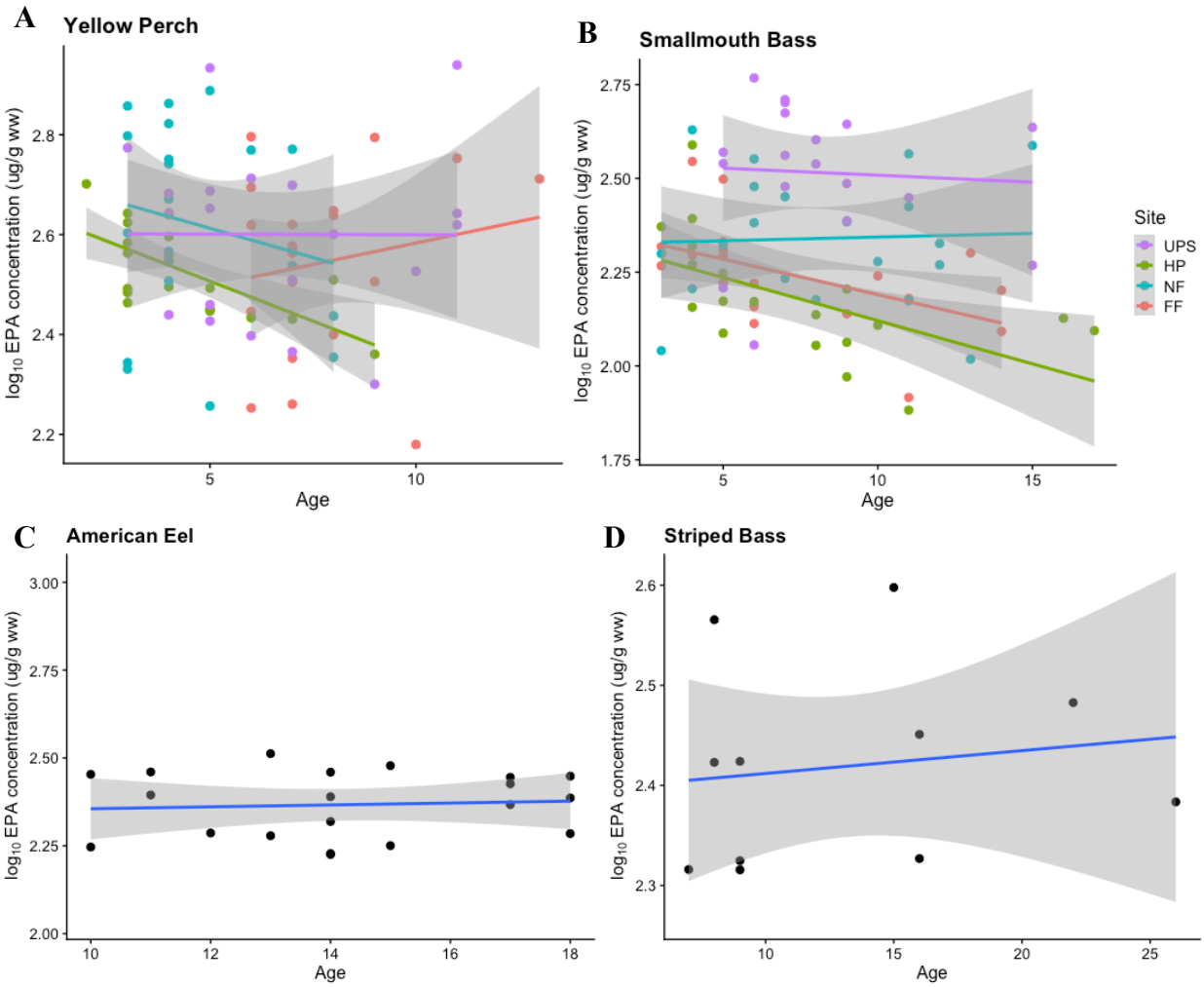
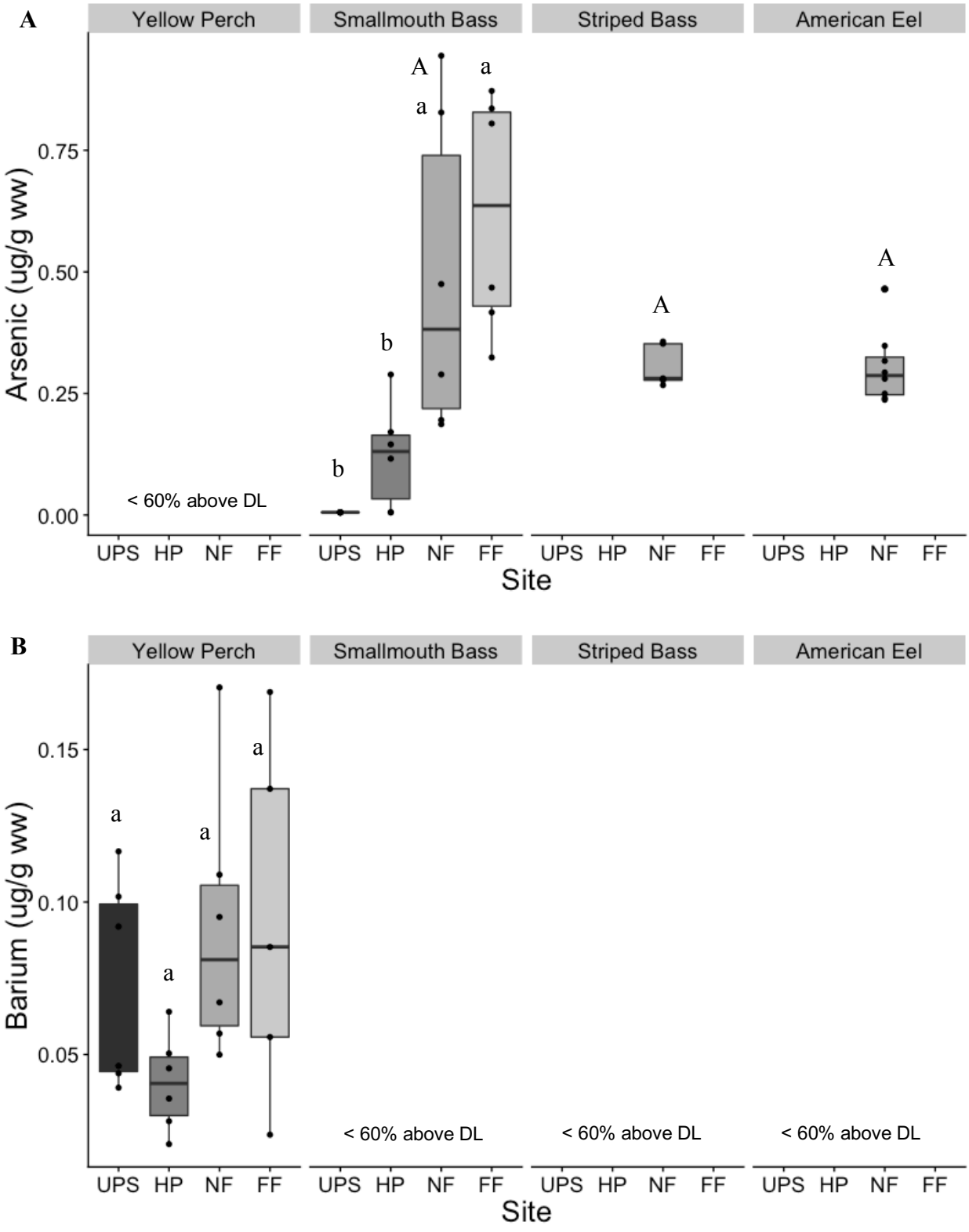
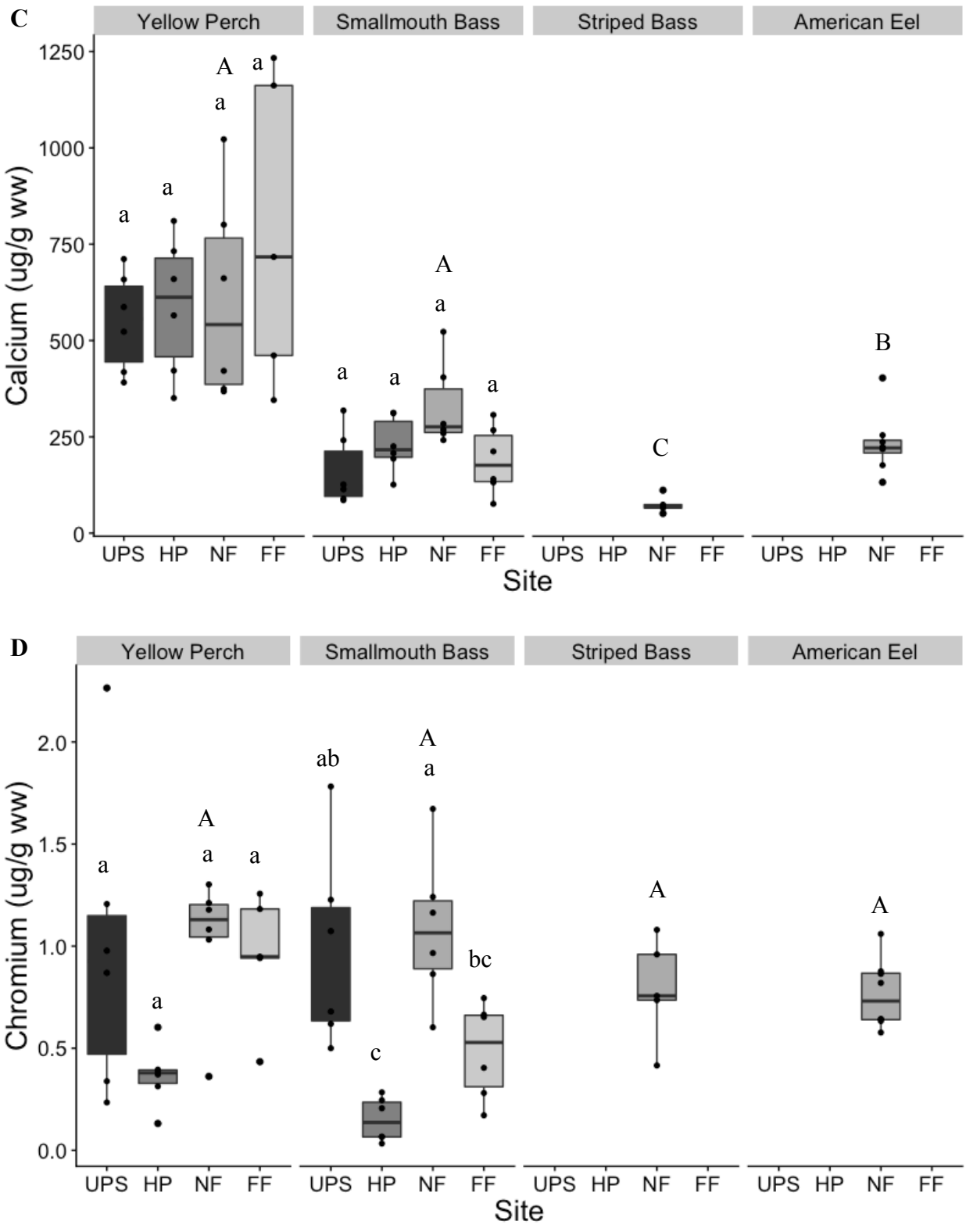
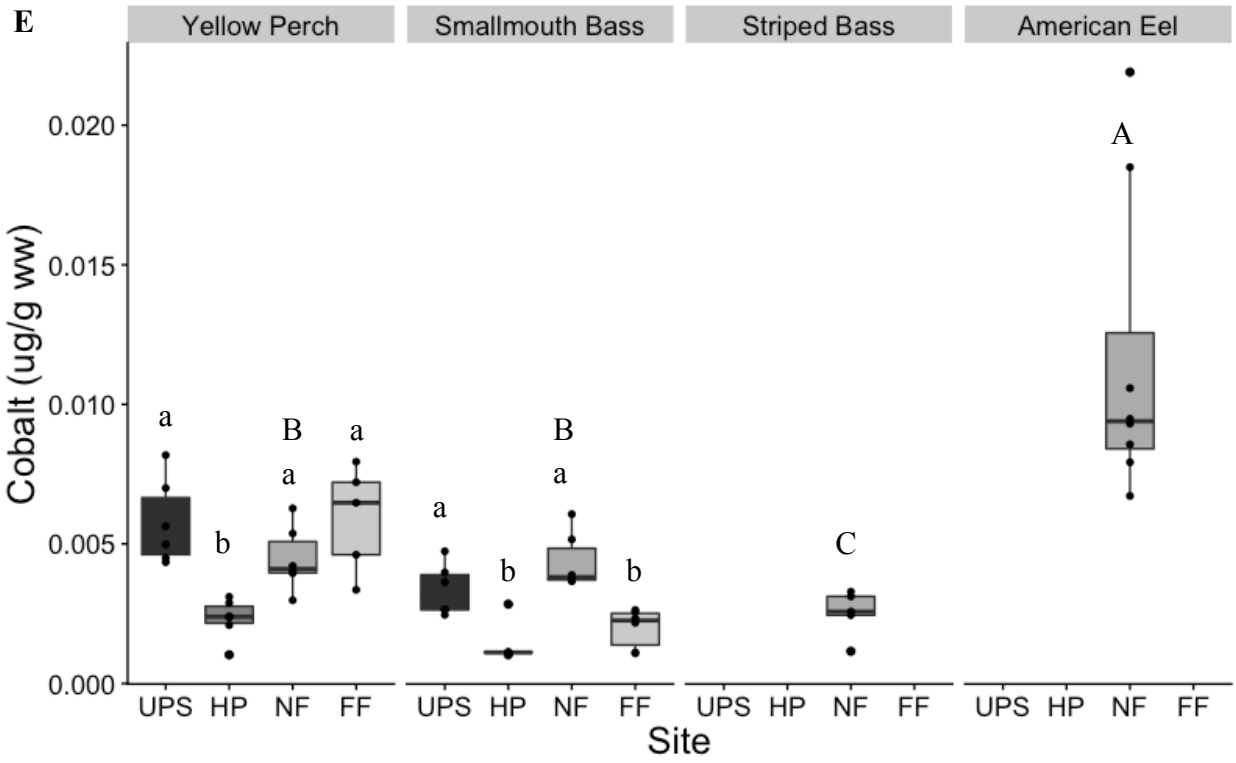
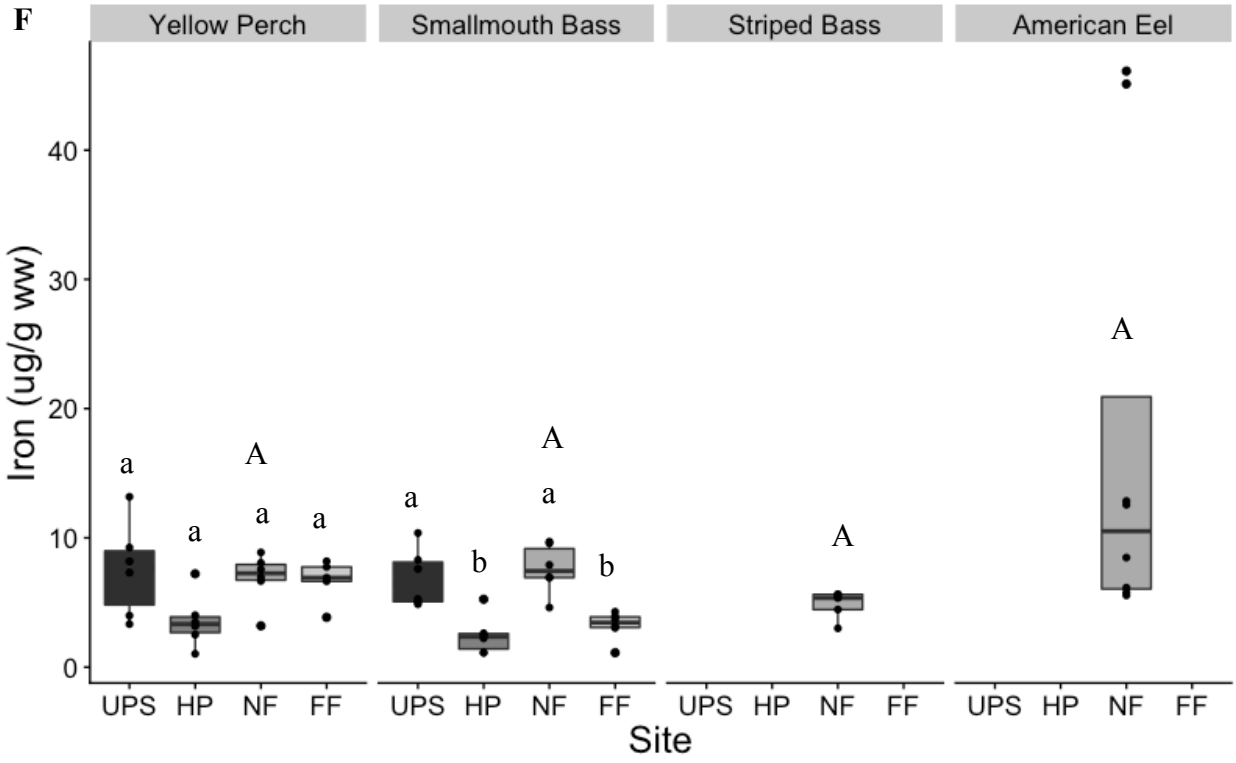


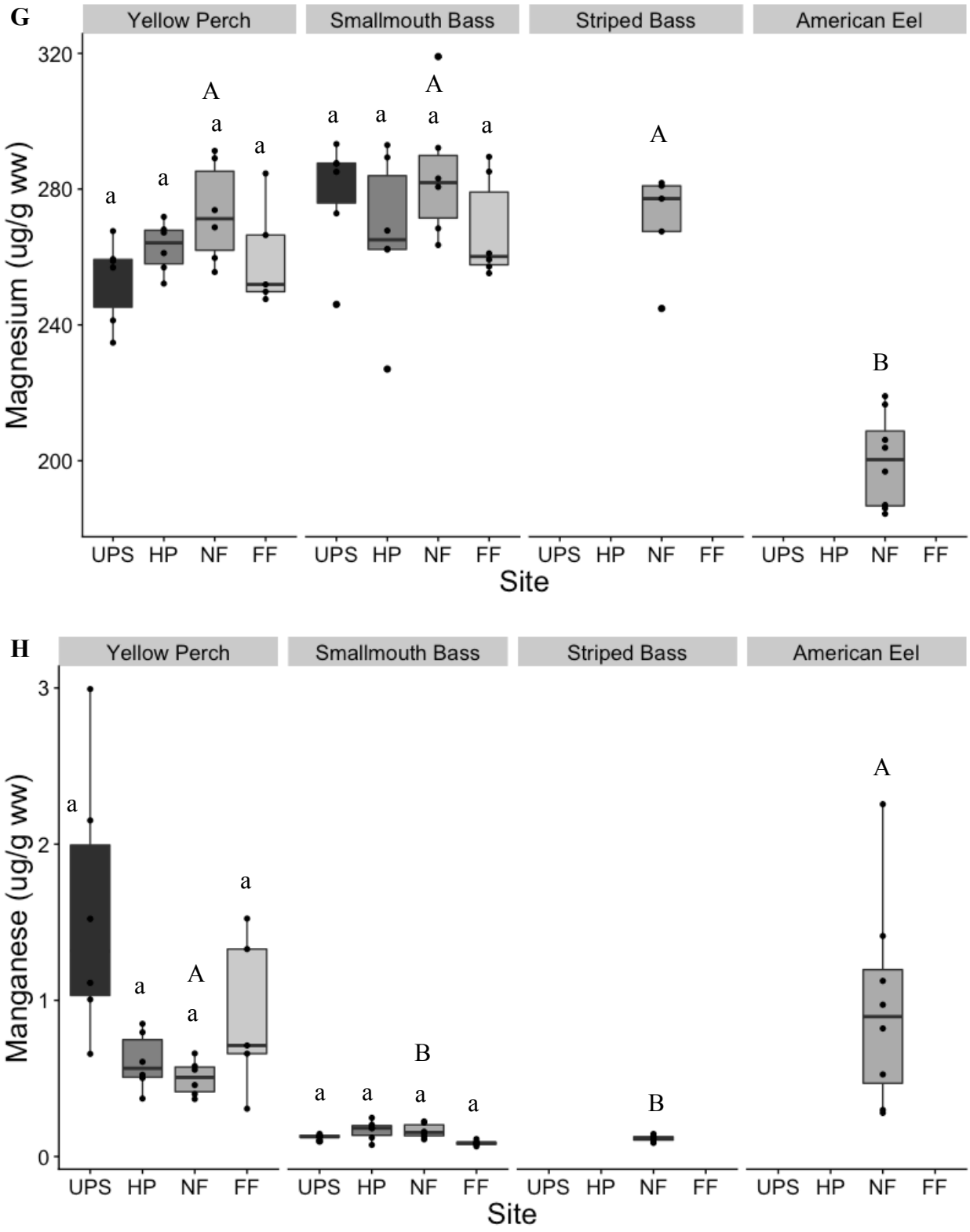
Figure S13. Relationship between $\log_{10}$ EPA (µg/g ww) and age (years-old) in Yellow Perch (A) and Smallmouth Bass (B) collected from all sites and American Eel (C) and Striped Bass (D) collected from the NF in the Wolastoq | SJR. The four longest American Eel and three longest Striped Bass were not aged and are therefore not included in these relationships.

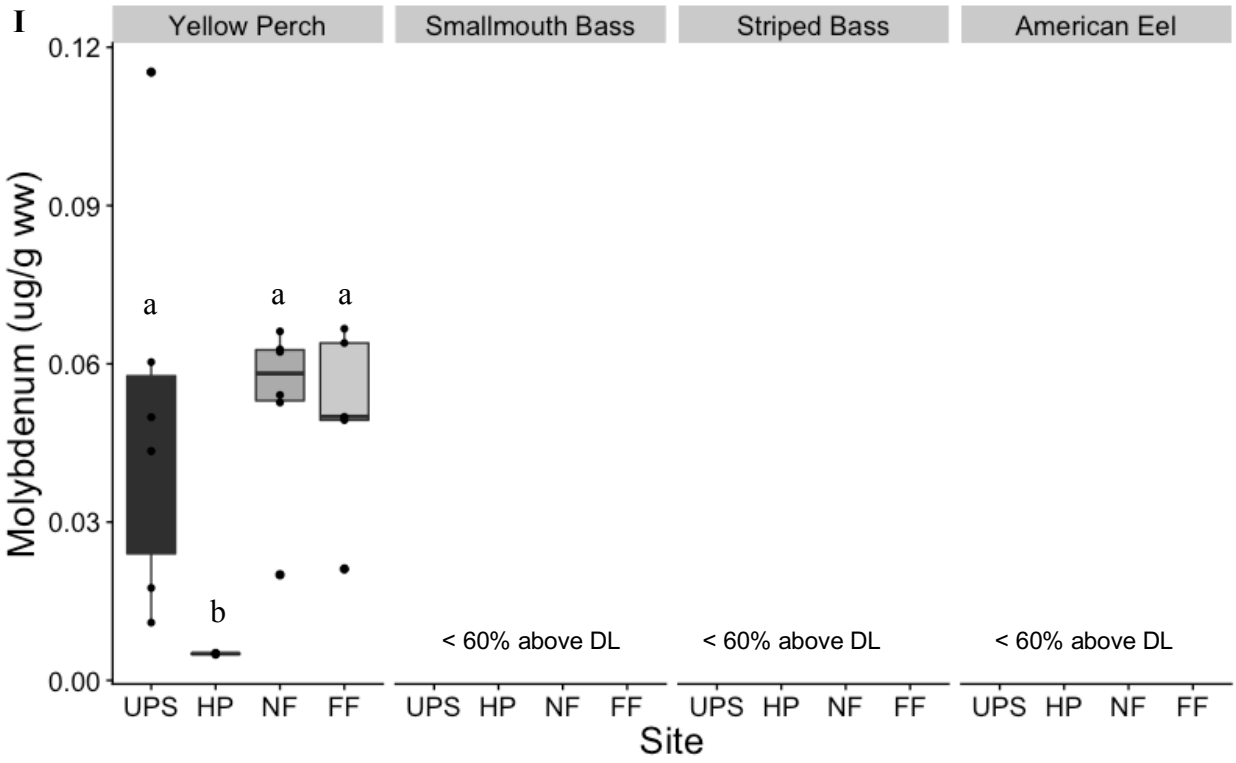

A
Yellow Perch
Smallmouth Bass
Striped Bass
American Eel
Arsenic (ug/g ww)
0.00
0.25
0.50
0.75
< 60% above DL
b
b
A
a
A
a
A
UPS HP NF FF
Site
B
Yellow Perch
Smallmouth Bass
Striped Bass
American Eel
Barium (ug/g ww)
0.05
0.10
0.15
a
a
a
a
< 60% above DL
< 60% above DL
< 60% above DL
UPS HP NF FF
Site

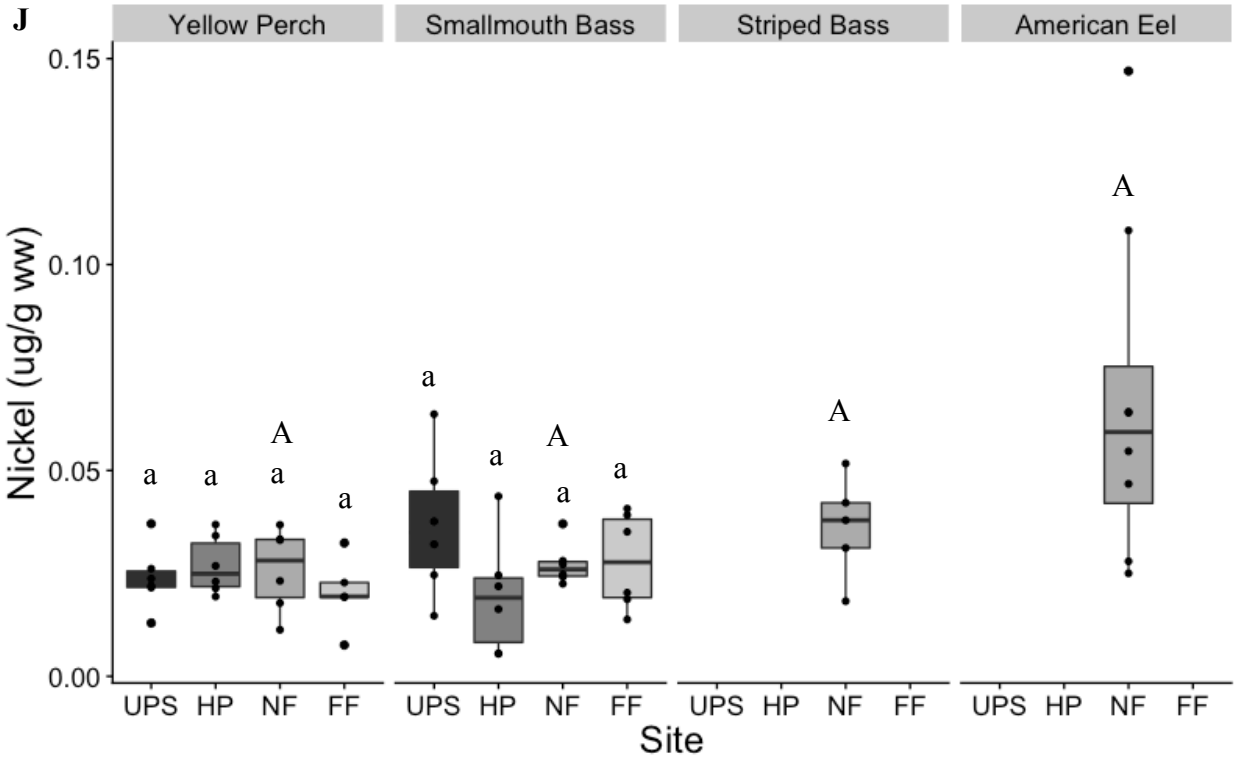

C
Yellow Perch
Smallmouth Bass
Striped Bass
American Eel
Calcium (ug/g ww)
1250
1000
750
500
250
0
a
a
A
a
a
a
A
a
a
a
C
B
UPS HP NF FF
Site
D
Yellow Perch
Smallmouth Bass
Striped Bass
American Eel
Chromium (ug/g ww)
2.0
1.5
1.0
0.5
0.0
a
a
A
a
a
ab
A
a
c
bc
A
A
UPS HP NF FF
Site

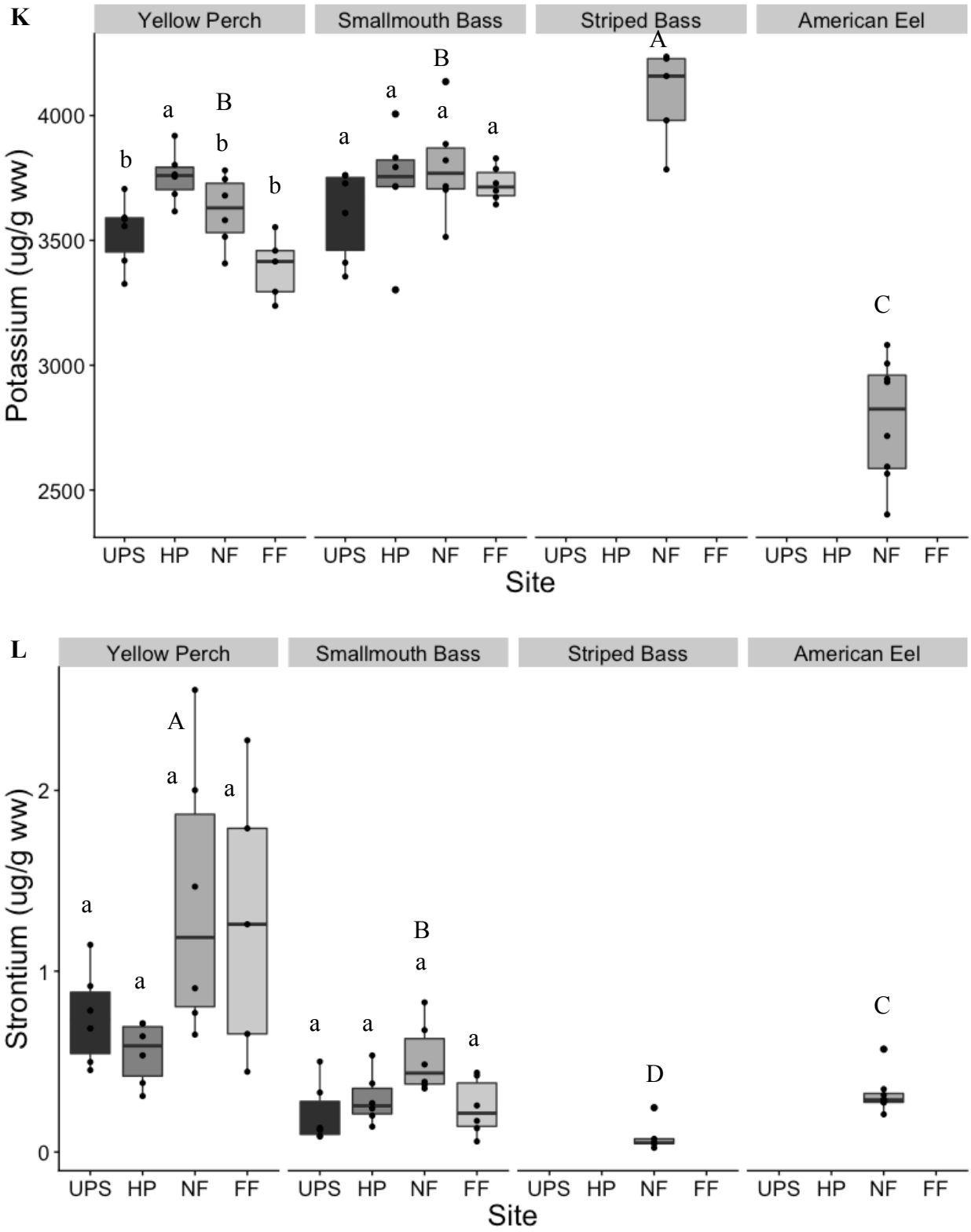

E
Yellow Perch
Smallmouth Bass
Striped Bass
American Eel
Cobalt (ug/g ww)
0.020
0.015
0.010
0.005
0.000
a
b
B
a
a
a
b
B
a
b
C
A
UPS HP NF FF
Site

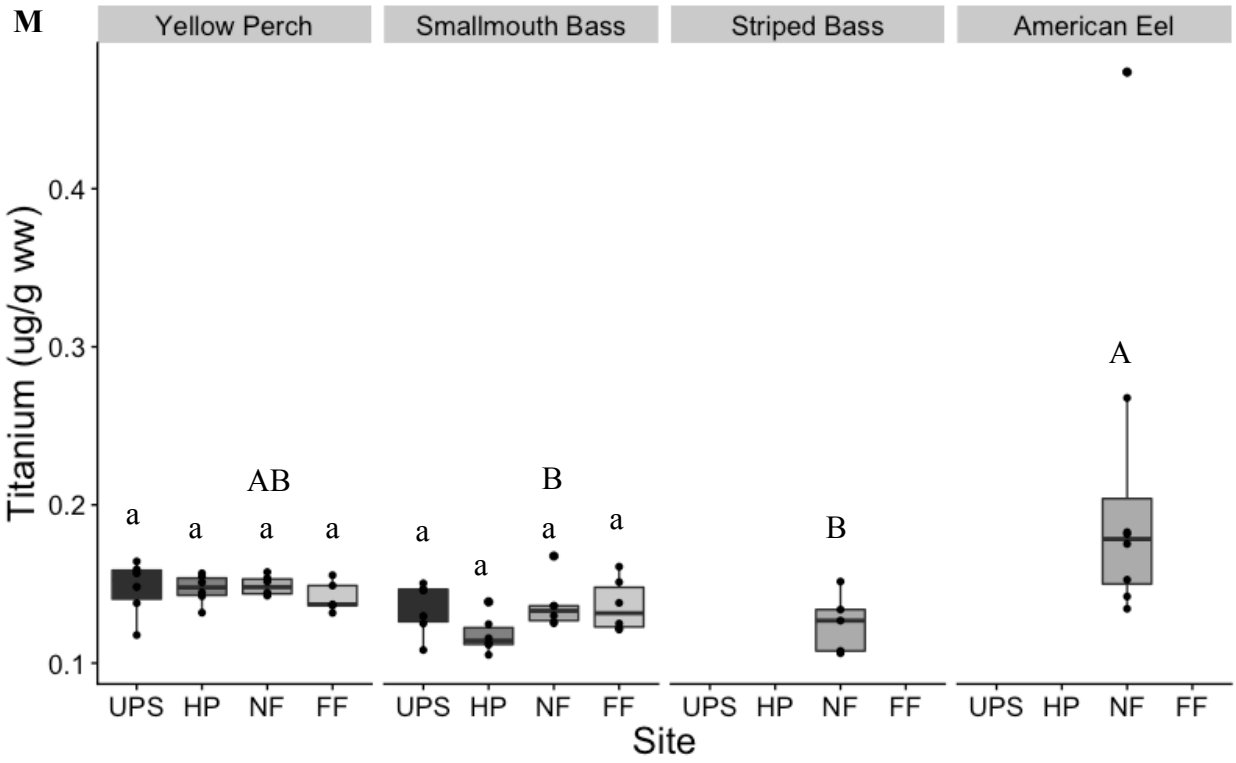

F
Yellow Perch
Smallmouth Bass
Striped Bass
American Eel
Iron (ug/g ww)
40
30
20
10
0
a
a
A
a
a
a
b
A
a
b
A
A
UPS HP NF FF
Site

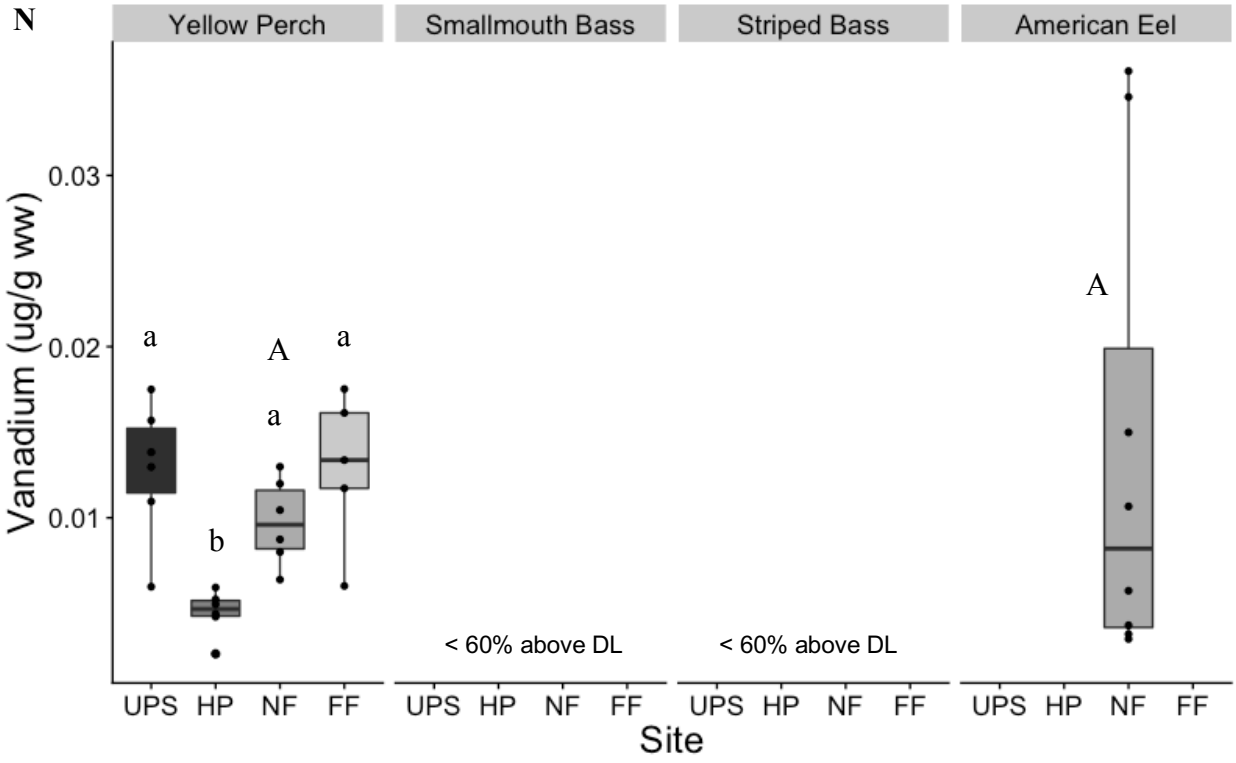

G
Yellow Perch
Smallmouth Bass
Striped Bass
American Eel
Magnesium (ug/g ww)
320
280
240
200
UPS HP NF FF
Site
H
Manganese (ug/g ww)
3
2
1
0


I
Yellow Perch
Smallmouth Bass
Striped Bass
American Eel
Molybdenum (ug/g ww)
0.12
0.09
0.06
0.03
0.00
a
b
a
a
< 60% above DL
< 60% above DL
< 60% above DL
UPS HP NF FF
Site


J
Yellow Perch
Smallmouth Bass
Striped Bass
American Eel
Nickel (ug/g ww)
0.15
0.10
0.05
0.00
a
a
A
a
a
a
A
a
A
A
UPS HP NF FF
Site


K
Yellow Perch
Smallmouth Bass
Striped Bass
American Eel
Potassium (ug/g ww)
4000
3500
3000
2500
b
a
B
b
b
a
a
B
a
a
A
C
UPS HP NF FF
Site
L
Yellow Perch
Smallmouth Bass
Striped Bass
American Eel
Strontium (ug/g ww)
2
1
0
a
a
A
a
a
a
B
a
a
D
C
UPS HP NF FF
Site


M
Yellow Perch
Smallmouth Bass
Striped Bass
American Eel
Titanium (ug/g ww)
0.4
0.3
0.2
0.1
a
a
AB
a
a
a
B
a
a
B
A
UPS HP NF FF
Site


N
Yellow Perch
Smallmouth Bass
Striped Bass
American Eel
Vanadium (ug/g ww)
0.03
0.02
0.01
a
b
A
a
a
< 60% above DL
< 60% above DL
A
UPS HP NF FF
Site

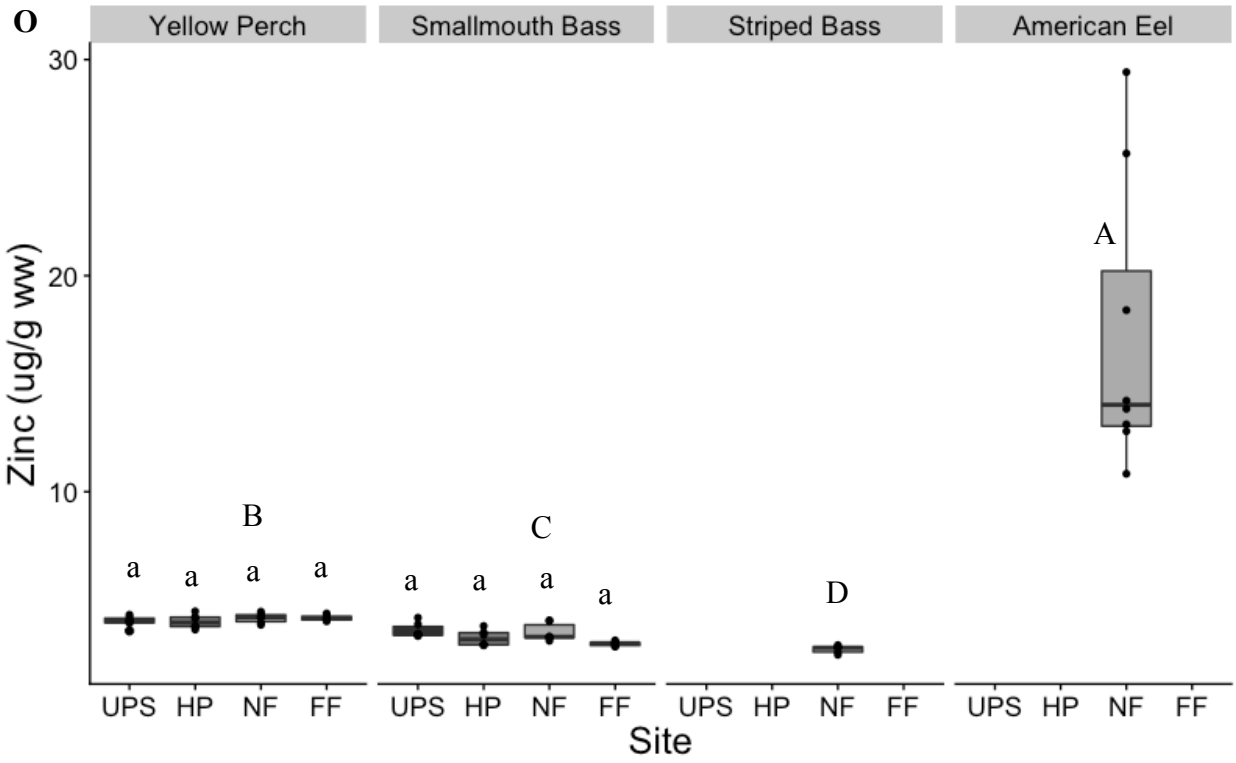


Figure S14. Element concentrations (µg/g ww) of composite fish samples (points) plotted across sites and species collected from the Wolastoq | SJR. Boxplots are labeled by letter and display different elements. Within the boxplots, lower case letters represent significant differences between sites within Smallmouth Bass and Yellow Perch separately, and capital letters represent significant differences between species at the NF (as determined by Tukey posthoc test ($p < 0.05$)).

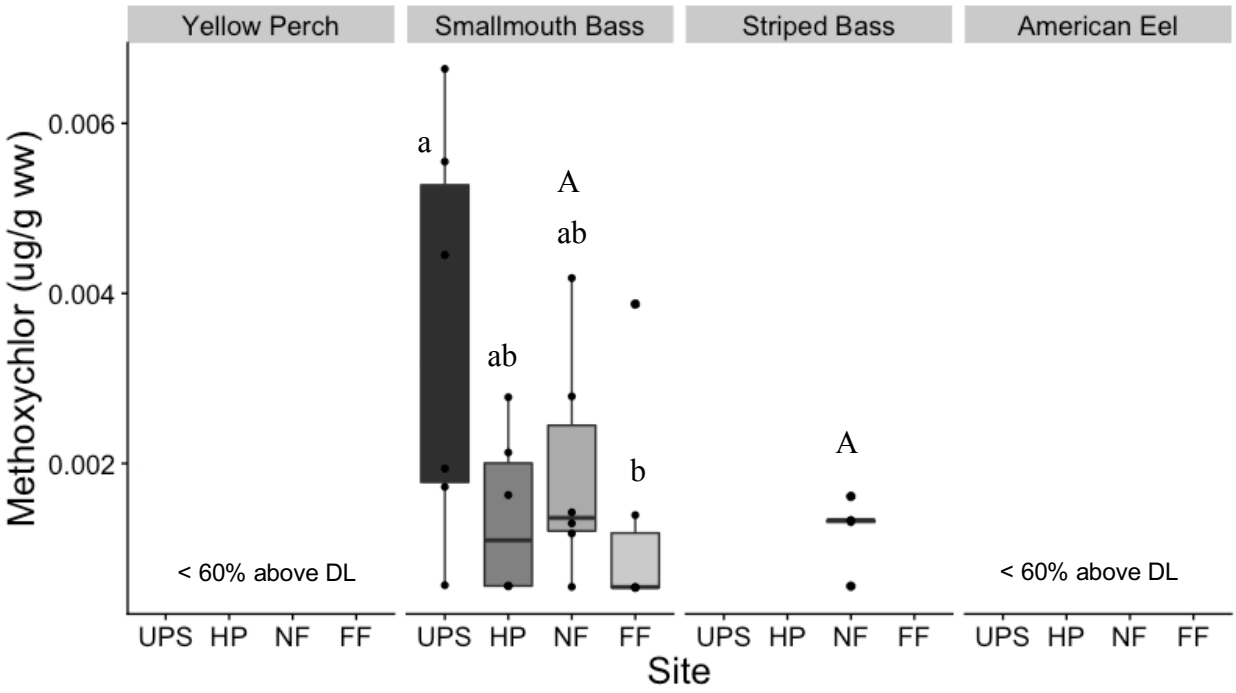


Figure S15. Methoxychlor concentrations (µg/g ww) of composite fish samples (points) plotted across sites and species collected from the Wolastoq | SJR. Lower case letters represent significant differences between sites within Smallmouth Bass and capital letters represent significant differences between Smallmouth Bass and Striped Bass at the NF site ($p < 0.05$ for Mann-Whitney-Wilcoxon).

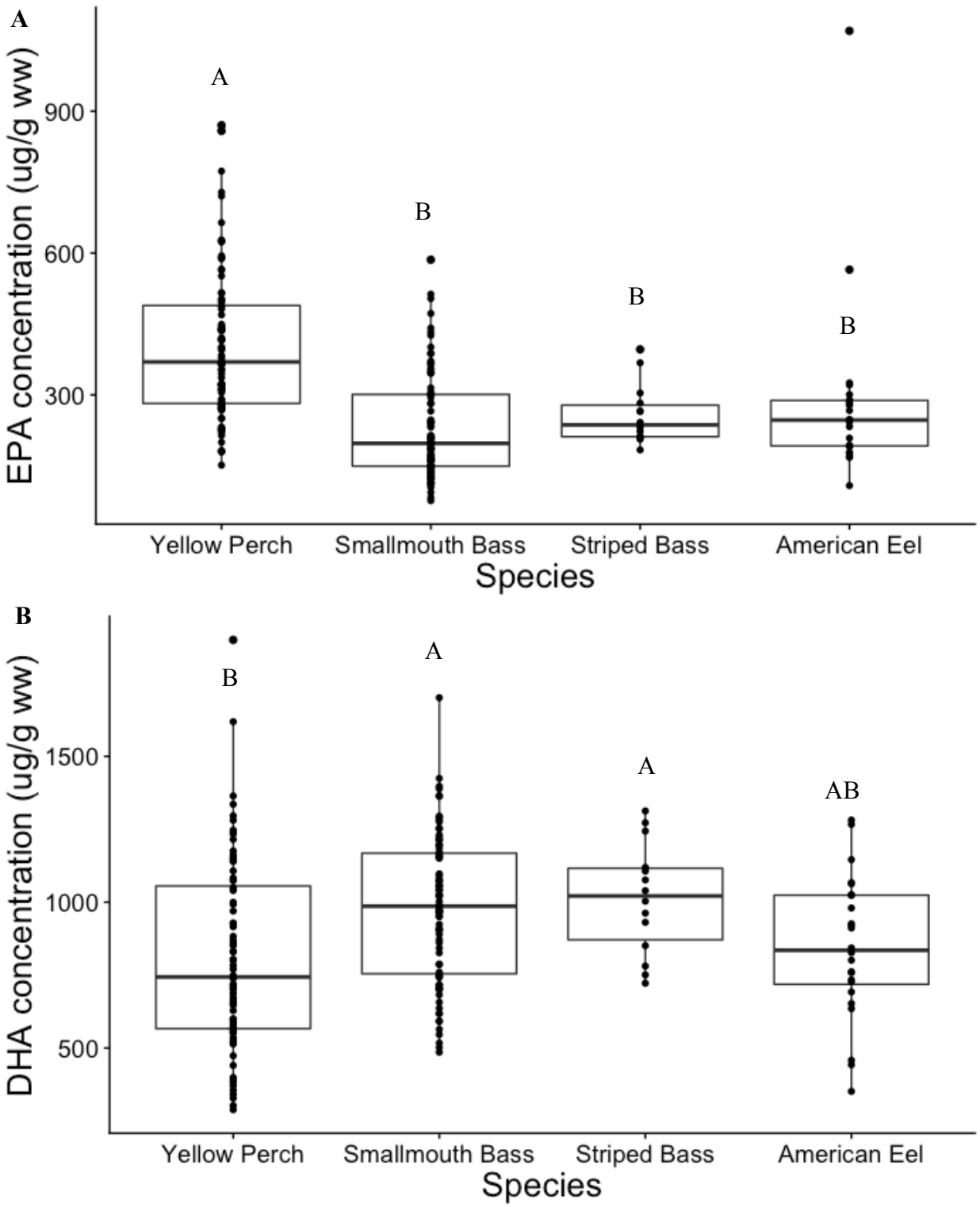


Figure S16. Boxplots showing A) EPA and B) DHA concentrations (µg/g ww) among species, where American Eel and Striped Bass were collected from the NF and Smallmouth Bass and Yellow Perch are pooled across all sites from the Wolastoq | SJR. Letters represent significant differences between species, as determined by Tukey posthoc test ($p < 0.05$).