Examination of Direct and Indirect Effects of Legacy Industrial Pollution On Organ Growth in Wild Yellow Perch (*Perca flavescens*)

by

Irene Yin Liao

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ABSTRACT

EXAMINATION OF DIRECT AND INDIRECT EFFECTS OF LEGACY INDUSTRIAL POLLUTION ON ORGAN GROWTH IN WILD YELLOW PERCH (*PERCA FLAVESCENS*)

Irene Yin Liao
University of Guelph, 2022

Advisors:
Frederic Laberge
Nicholas Bernier

To identify new bioindicators of legacy industrial pollution in aquatic ecosystems, I investigated the effects of chronic pollution on organ growth in fish. Specifically, I examined which organs are most sensitive to pollution and whether these changes were prompted by the indirect effects of impoverished food webs resulting from chronic pollution or the direct toxic effects of pollutants on cell proliferation. I measured relative organ size, trophic position, and hepatic *cyp1a1* gene expression in wild yellow perch from impacted and reference sites across the Detroit River and Lake St. Clair. Overall, sites with higher levels of contaminants exhibited increased *cyp1a1* expression, food web disruption, lower relative brain size, and higher relative liver size. However, the effects of pollution on organ growth were best explained by disruption of foraging ecology rather than direct toxic effects of contaminant exposure due to significant associations between organ size and trophic position, but not *cyp1a1* expression.

DEDICATION

This thesis is dedicated to my mom. Thank you for letting me choose my own path in life and cooking me congee whenever I was sick.

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LIST OF ABBREVIATIONS

40s	40s ribosomal protein S7
actb	b-actin
AhR	Aryl hydrocarbon receptor
BI	Belle Isle
cyp1a1	Cytochrome P450 family 1 subfamily A polypeptide
GLM	General linear models (or generalized linear model where indicated)
MB	Mitchell's Bay
mRNA	Messenger ribonucleic acid
PAHs	Polycyclie aromatic hydrocarbons
PCBs	Polychlorinated biphenyls
PI	Peche Island
qRT-PCR	Quantitative real-time polymerase chain reaction
TC	Trenton Channel

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1 Chapter 1: Introduction

1.1 Thesis Objectives

In this thesis, I pose the question: how does industrial pollution affect the overall body and organ growth of wild fish? While previous research has investigated the effects of pollution on overall growth in fish, studies examining the effects of industrial pollution on the growth of specific organs and potential organ growth trade-offs are lacking. My thesis attempts to fill this gap by examining how energetically costly organs such as the brain and gut are affected by both the direct effects (i.e. tissue damage or changes in cell proliferation) and indirect effects (i.e. impoverishment of food webs) of industrial pollution on wild fish. Specifically, I sought to evaluate the sublethal responses resulting from chronic exposure to industrial pollutants on key aspects of fish physiology and behaviour in yellow perch (*Perca flavescens*) collected from historically impacted and reference sites along the Lake St. Clair and Detroit River area in southwestern Ontario, Canada. First, I examined a bioindicator of exposure to pollutants by measuring liver detoxification enzyme gene expression. Second, I evaluated foraging ecology using muscle stable isotope signatures of nitrogen and carbon. Lastly, I evaluated the fish growth profiles and the relative sizes of their organs.

1.2 Anthropogenic pollution and its general effects on fish physiology

Pollution from anthropogenic sources in water bodies, especially from the industrial and agriculture sectors, has been a persistent threat in populated regions of Canada. Such pollutants include heavy metals, suspended solids, organic contaminants, and oil, which come from a variety of sources including manufacturing and tankage wastewater, ballast drainage and runoff, industrial spills, and fertilizer runoff (Bahadori, 2013). Studies have shown that concentrations of

these pollutants may remain elevated in surface waters and lake sediments for decades after industrial activity has ceased (Sprague and Vermaire, 2018; Detroit River Canadian Cleanup, 2010), with consequences including alteration of water chemistry and biotic composition of local aquatic ecosystems (Heath, 1995; Mehaffey et al., 2005; Moss, 2008; Vanni et al., 2005), reduced aquatic biodiversity (Dickman et al., 1983; Laine et al., 2014; Lambshead, 1986; Sala et al., 2000), and disruptions in local aquatic food webs (Moss, 2008; Vanni et al., 2005). A substantial body of evidence also suggests that chronic exposure to pollution can affect growth, liver function, reproduction, endocrine and nervous systems, respiratory and cardiovascular systems, and osmoregulation (Heath, 1995; Larsson et al., 1988; Austin, 1998; Scott and Sloman, 2004).

1.3 Pollution and fish organ size

Few studies have taken a close look at the relationship between pollution and organ size. Liver damage and hypertrophy are commonly reported consequences of chronic exposure to pollutants, and are often investigated due to the livers' role in detoxification and bioaccumulation of pollutants. Some mechanisms to explain the hypertrophic effects of toxicants on the liver include glycogen and lipid overload, organelle proliferation, and swelling of the endoplasmic reticulum (Wolf and Wolfe, 2005), and overall increased functional load as a result of pollution (Burklakov et al., 2021; Tenji et al., 2020). In comparison, less work has been done on other, non-detoxifying organs (Agbohessi et al., 2015; Ahmed et al., 2013; Louiz et al., 2018). Some studies indicate that chronic exposure to pollutants, such as PAHs and heavy metals, may result in gonad hypertrophy (Agbohessi et al., 2015) or hypotrophy (Levesque et al. 2003; Scholz and Kluver, 2009). Studies have also shown that chronic pollutant exposure can affect cardiac and

brain morphology in fish (Incardona and Scholz, 2017; Puga et al. 2018), but none have investigated changes in the relative sizes of the heart and brain. Similarly, studies on the effects of various industrial pollutants on the gut primarily focus on digestive performance and the gut microbiome (DeBofsky et al., 2020; Hamilton et al., 2017; Redfern et al., 2021) rather than on gut growth or size. While the liver is a logical target for study due to its role in detoxifying pollutants, investigations into the effects of chronic industrial pollution on other organs is lacking and no studies have examined all five aforementioned organs within the same fish, which could help establish which organs are most sensitive to pollution and if growth trade-offs happen between organs. In particular, while the hypertrophy of the liver is commonly observed in fish from chronically polluted environments, it is unknown if this growth comes at the expense of investment in other organs. As such, I measured the relative size of the brain, gut, liver, heart ventricle, and gonads to assess if and how these organs may be affected by chronic pollution, and if growth of certain organs, such as the liver or energetically expensive organs like the brain or gut (Aiello, 1997), result in growth trade-offs in other organs.

1.4 Toxicity of pollutants and *cyp1a1* gene expression

Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are common industrial organic pollutants that potentially have direct effects on growth in fish. Specifically, studies suggest that PAHs and PCBs can activate the aryl hydrocarbon receptor (AhR) in fish, which has been linked to the inhibition of cell proliferation in a variety of tissues (Kolluri et al., 1999; Su et al., 2014; Yang and Lein, 2010) and thus may lead to hypotrophy of organs in exposed fish, while other studies found that AhR activation can also stimulate cell proliferation instead (Bohnenberger et al., 2001; Yin et al., 2016). Notably, studies previously

observed a positive correlation between PAHs and relative liver size in fish (Sloof et al., 1983; Fabacher and Baumann, 1985; Everaarts et al., 1993; Pinkney et al., 2001) which may be due to promotion of cell proliferation stemming from AhR activation (Marlowe and Puga, 2005; Wojdylo et al., 2016) or due to increased functional load from detoxifying environmental pollutants (Burklakov et al., 2021; Tenji et al., 2020). Conversely, other studies found that AhR activation can lead to apoptosis in the brain (Szychowski et al. 2016; Wojtowicz et al. 2017), heart (Huang et al., 2021; Ren et al., 2020; Zhang et al., 2021), and ovary (Weber and Janz, 2001). Evidently, exposure to various toxicants can have differing effects on individual organs, and may be mediated by the AhR pathway. However, many of the aforementioned studies examined the effects of exposure to toxicants in an acute timeline, and in isolation from other organs. As such, we aimed to explicitly investigate if chronic exposure to environmental toxicants can affect a suite of organs in wild fish, whether or not these toxic effects on cell proliferation act evenly across different organs, or if any effects are focused solely on energetically expensive organs, such as the gut or brain.

Exposure to PAHs and PCBs can activate the cytosolic aryl hydrocarbon receptor (AhR) and induce the activation of the cytochrome p-450 (CYP) 1A subfamily of enzymes, which is involved in the metabolism of foreign chemicals in various animals (Bernhardt, 1996; Bucheli and Fent, 1995; Stegeman and Lech, 1991). Due to its activation by foreign contaminants like industrial pollutants, CYP1A activity is commonly used as a bioindicator for contaminant exposure in fish and is often measured using the ethoxyresorufin-O-deethylase (EROD) assay (Whyte et al. 2000). However, due to sample storage constraints associated with field sampling, instead of EROD activity, we chose to measure liver *cyp1a1* gene expression as a biomarker for

contaminant exposure to assess if direct toxicity contributes to the effects of pollution on wild fish growth and organ size.

1.5 Altered food webs and stable isotopes

The effects of pollution on lower trophic level animals that predatory fish consume, such as aquatic invertebrates, have been extensively studied, showing evidence that pollution disrupts prey-predator relationships (Vanni et al., 2005). Many invertebrate taxa are extremely sensitive to pollutants, and a number of pollutants have lethal effects on various invertebrate species (Abel, 1974; Handy, 1994; Spehar et al., 1978) in addition to non-lethal effects resulting in growth reduction (Liberti et al., 2020), thereby reducing both the quantity and quality of food available to higher trophic level organisms like fish. Limits on energy intake by fish can decrease the potential for investment of resources into growth as the immediate survival and maintenance of routine metabolism takes precedence over long-term investment (Perrin and Sibly, 1993). The impoverishment of the food web can therefore affect fish physiology and growth (Sherwood et al., 2000; Sherwood et al. 2002; Ware and Thomson, 2005). While some evidence exists that indicates relative brain size (Edmunds et al., 2016) and heart ventricle size (Edmunds et al., 2018) could increase with trophic position, no studies examining the potential relationship between trophic position and relative gut, liver, and gonad size exist.

Stable isotopes are commonly used to infer an organism's foraging ecology, with stable nitrogen isotopic signatures ($d^{15}N$) used to estimate trophic positions of consumers relative to organisms more basal in the food web, and stable carbon isotopic signatures ($d^{13}C$) used to infer food source in lakes (Vander Zanden and Rasmussen, 1999). Combining stable nitrogen and carbon isotopic signatures can calculate an isotopic niche width to estimate prey diversity and

diet overlap between communities or species (Jackson et al., 2011). As such, I used muscle ^{15}N and ^{13}C stable isotopes to examine how trophic position and niche width vary between impacted and non-impacted sites to investigate the potential indirect effects of industrial pollution on fish growth and organ size.

1.6 Model animal and study system

I conducted a field study in the Detroit River and Lake St. Clair water system. The Detroit River was one of 42 sites identified as an Area of Concern by the International Joint Commissions' Great Lakes Water Quality Board in 1978. A remedial action plan was initiated in 1986, which identified significant losses in fish habitat due to human activity including construction of bulkheads and industrial dumping (Michigan Department of Natural Resources, 1991). In the 35 years since, numerous rehabilitation projects have been implemented which target habitat restoration and protection across the Detroit River, and notable accomplishments include the restoration of soft shorelines, fish spawning reefs, and Common Tern habitat (Hartig et al. 2018). However, a study by Szalinska et al. (2013) indicated that sediment concentrations of heavy metals and organic contaminants (PAHs, PCBs) did not change between 1999 and 2009.

Yellow perch (*Perca flavescens*) are endemic and abundant in the Detroit River and Lake St. Clair, and serve as a particularly good model for studying food web structure, as they exhibit ontogenetic shifts in foraging ecology throughout their lifetime (Pothoven et al., 2000; Wu and Culver, 1992). Thus, clear deviations in food web structure can be assessed based on stable isotope values in yellow perch and baseline organisms. Additionally, yellow perch in our designated study sites appear to display strong site fidelity (Sullivan and Stepien, 2014),

allowing us to infer that contaminant exposure levels in perch are primarily determined by their capture site, and not influenced by movement between sites.

1.7 Hypothesis and Predictions

I aimed to examine two mechanisms by which chronic exposure to pollution may elicit sublethal effects on organ size and growth in wild fish: 1) through the direct toxic effects of contaminants such as PAHs and PCBs, and 2) through the indirect effects of food web impoverishment. As such, I tested the following hypotheses and predictions:

Hypothesis 1: Exposure to industrial pollution inhibits the growth of non-detoxifying organs via direct toxic effects of pollutants that promote apoptosis and/or inhibit cell proliferation, but leads to liver hypertrophy due to increased functional load in this organ as a result of detoxifying and metabolizing toxicants.

Prediction 1a: Fish from impacted sites will have higher liver gene expression of the detoxifying enzyme CYP1A1, indicating higher levels of exposure to contaminants.

Prediction 1b: Non-detoxifying organs, such as the brain and gut, will be smaller in fish from impacted sites, while the liver will be larger.

Prediction 1c: *cyp1a1* liver gene expression will be negatively correlated with the relative size of energetically expensive organs, such as the brain, and positively correlated with relative liver size.

Hypothesis 2: Industrial pollution affects the growth of organs indirectly via the impoverishment of food webs, which reduces energy available for growth of energetically expensive organs.

Prediction 2a: Fish from impacted sites will have lower trophic position.

Prediction 2b: Fish from impacted sites will have smaller energetically expensive organs, such as the brain and gut, but no changes in liver size.

Prediction 2c: Trophic position will be positively correlated with relative brain and gut size.

2 Chapter 2: Methods

2.1 Field sites & sediment analysis

Yellow perch were caught by boat electrofishing and hoop nets from three locations along the Detroit River (Trenton Channel (TC), Belle Isle (BI), and Peche Island (PI)), and one location in Lake St. Clair (Mitchell's Bay (MB)) (Fig. 1). These sites were selected due to previous classification of the Detroit River as an area of concern by the International Joint Commission Great Lakes Water Quality Board (1978), coupled with previous evidence that the river can be categorized into discrete areas varying in pollution levels, with levels of contamination increasing downstream along the Detroit River (Farwell et al., 2012; Farwell et al., 2013). The highest levels of contamination in the Detroit River have been measured at Trenton Channel (furthest downstream), and lowest levels at Peche Island (furthest upstream). Mitchell's Bay in Lake St. Clair was selected as a reference site due to its location upstream of the Detroit River, with little historical records of industrial pollution. Previous work has demonstrated that sediment concentrations of various contaminants, such as PAHs and heavy metals, have declined over the past three decades in Lake St. Clair, with Mitchell's Bay in particular demonstrating the lowest levels of sediment contamination compared to other parts of the lake (Gewurtz et al. 2007).

To confirm these posited contamination levels, sediment samples were obtained using an Ekman grab sampler and stored in tinfoil-wrapped, acetone-washed Mason jars at -20°C until analysis. Three sediment samples obtained at each site were combined to form a single composite sample representative of each site. Samples were stored for no more than 2 weeks before screening for PAHs, PCBs, and heavy metals was performed by Maxxam Analytics

(Mississauga, ON, Canada). Analytical procedures followed standard United States Environmental Protection Agency (EPA) methods. PAHs were analyzed using EPA method 8270D as described in Ebitson and Gallagher (2011). Briefly, sediment extracts were injected into a temperature-programmed gas chromatograph to separate analytes, which were then detected and identified using an attached mass spectrometer. PCBs were analyzed using EPA method 8082A as described in Zhang et al. (2016). Briefly, sediment extracts were subjected to a sequential sulfuric acid/potassium permanganate cleanup, and samples were then analyzed using a gas chromatograph equipped with a fused-silica capillary column and an electron capture detector. Heavy metals were analyzed using EPA method 6020B as described in Mikulski et al. (2017). Briefly, sediment samples were acid digested, and extracted liquids were then nebulized. The resulting aerosol were than transported into a plasma torch, producing ions entrained in plasma gas. These were then introduced and analyzed according to their mass-to-charge ratios via mass spectrometer.

2.2 Body, organ size, and age measurements

Yellow perch were terminally anesthetized using tricaine methanesulfonate (250 mg/L; Syndel International, Qualicum Beach, BC, Canada). Fish were weighed and then dissected. Livers were excised first, weighed, and immediately wrapped in tinfoil before freezing in liquid nitrogen for storage at -80°C. Livers were used for qPCR analysis. The gut and gonad were excised for immediate weighing followed by disposal. Hearts were excised and stored in 20 mL glass scintillation vials filled with 10 mL of 10% buffered formalin until further dissection and weighing of the ventricle in the lab using a high resolution scale (Accu-124D, Fisher Scientific). The heads of sampled fish were separated from the body using scissors and also stored in

formalin until further dissection, trimming, and weighing of the brain in the lab. Otoliths were also extracted and used for aging perch as per Robillard and Marsden (1996). Briefly, otoliths were broken manually using a blade along the anterior-posterior axis. The posterior half was ground on 400 grit sandpaper to expose the nucleus and was examined under a dissecting microscope at 30-60x magnification. The number of annuli around the nucleus was counted and used as a measure of age. Each otolith was examined by two individuals to confirm the number of annuli counted.

2.3 Stable isotopes analyses & quantification of trophic ecology

Stable isotope analyses were performed at the Great Lakes Institute of Environmental Research (University of Windsor, ON, Canada) in collaboration with Dr. Aaron Fisk as per Mumby et al. (2017). Briefly, approximately 2 g of freeze-dried white muscle was homogenized to a fine powder using a mortar and pestle and bulk (non lipid extracted) powdered samples were weighed into 5 mm x 9 mm tin capsules on a microbalance. Masses of samples were between 400 and 600 ug. Samples were then combusted into CO_2 and N_2 gases with an elemental analyzer (Costech, Valencia, California, USA) and relative abundances of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotopes were determined using a Delta V mass spectrometer (Thermo Finnigan, San Jose, California, USA). Delta notation for $d^{13}C$ and $d^{15}N$ express differences from standard reference materials (Pee Dee Belemnite for carbon and atmospheric nitrogen) as follows:

$$d^{13}C \text{ or } d^{15}N = [(R_{sample}/R_{standard} - 1)] \times 1000$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$ (Fry 1991; Hobson and Clark 1992).

We used nitrogen ratios from yellow perch and forage fish to estimate the trophic position of each yellow perch with the following equation:

$$\text{Trophic position} = [d^{15}N_{\text{yellow perch}} - d^{15}N_{\text{forage fish}})/3.4] + 2$$

The value of 3.4 is the assumed increase in $d^{15}N$ per trophic level, as suggested by Vander Zanden et al. (2000), and a value of 2 was added to compensate for using primary consumers (i.e. forage fish) instead of primary producers (i.e. invertebrates) in the calculations. The $d^{15}N$ predator:forage fish ratio was also calculated to assess if yellow perch occupied a trophic level higher than that of small forage fish by dividing the nitrogen signatures of yellow perch by the average nitrogen signature of forage fish located at each site. A value greater than 1 indicates yellow perch are at a higher trophic level than forage fish (i.e., typical food web), while a value less than 1 indicates yellow perch are at a lower trophic level than forage fish (i.e., atypical food web). The maximum likelihood standard ellipses for isotopic niche width and overlap were calculated using the SIBER package in R software (R-project, Version 3.0.1, R Development Core Team, University of Auckland, New Zealand) to evaluate differences in diet.

2.4 Quantification of gene expression

Quantification of *cyp1a1* liver gene expression followed the same methods as Williams et al. (2017). Briefly, total RNA extraction was performed on perch livers. Frozen liver samples were homogenized in a RNA extraction reagent (TRIzol Reagent, Invitrogen, Carlsbad, CA, USA) using a Precellys Evolution homogenizer (Bertin Instruments, Montigny-le-Bretonneux, France). Extracted total RNA was then quantified using ultraviolet spectrophotometry at 260 nm (Nanodrop 8000; Nandrop Products, Wilmington, DE, USA). A RNA sample of 1 ug was treated with Ambion DNase (Thermo Fisher, Waltham, MA, USA) and used to synthesize cDNA using

Quanta qScript (Quanta Biosciences, Gaithersburg, MD, USA) as per manufacturer instructions. Triplicates of each cDNA sample were amplified by real-time PCR using a CFX96 system (Bio-Rad Laboratories, Hercules, CA, USA) with Bio-Rad SYBR Green Master Mix (Bio-Rad Laboratories) using primers listed in Table 1. The 20 µL reactions comprised 10 µL 2x master mix, 5 µL 10-fold diluted first-strand cDNA template, and 2.5 µL of both forward and reverse primers (0.6 µM). Default cycling conditions were used. The specificity of each PCR product was verified using a melting curve analysis. Each qPCR assay included a negative reverse transcriptase control and a negative template control to ensure the absence of contamination. Standard curves were constructed for each gene using known dilutions of liver cDNA. Input values for each gene were obtained by fitting the average threshold cycle (CT) value to the antilog of the gene-specific standard curve thereby correcting for differences in primer amplification efficiency. To correct for minor variations in template input and transcriptional efficiency, the input values were normalized to the geometric mean of two housekeeping genes 40s ribosomal protein S7 (*40s*) and ß-actin (*actb*). Note that the expression of each housekeeping gene did not differ between sampling sites ($p>0.05$).

2.5 Statistical analysis

Between-site analyses were first conducted to confirm if yellow perch from historically polluted sites (TC, BI, PI) varied in characteristics compared with our reference site (MB). The variables analyzed included: *cyp1a1* liver mRNA expression levels, trophic position, $d^{15}N$ predator:forage fish ratio, and relative brain, gut, liver, heart ventricle, and gonad size. Organ growth curves obtained by graphing $\log_{10}$ organ size against $\log_{10}$ body mass revealed linear relationships for all organs, except the brain. As such, relative organ size was obtained from

residuals of these linear relationships and the quadratic non-linear relationship for the brain including perch of all sites. Residual values for trophic position were similarly obtained from the linear relationship between body mass and trophic position to correct for the expected increase in trophic position with body size. All data violated the assumption of normality when tested by site, as determined using a Shapiro-Wilk test despite log transformation, so a Kruskal-Wallis test was used to assess differences between sites with a statistical threshold of 0.05. When significant, a post-hoc Dunn's multiple comparisons test was conducted determine which sites differed from one another. All aforementioned tests were performed using GraphPad Prism 6.0.0 for Windows (San Diego, California, USA).

General linear models (or a generalized linear model in the case of non-normal data) were then used to determine if age, *cyp1a1* liver mRNA levels, and trophic position explained observed differences in relative organ size between sites. Several models were tested (Appendix A) with age, *cyp1a1* liver mRNA levels, and trophic position as covariates, site as a fixed effect, and site*age, site*cyp1a1, and site*trophic position as interaction terms. These models were followed by pairwise comparisons using the Fisher's least significant difference (LSD) method to assess divergences in relationships between sites. All models were computed using SPSS Statistics for Windows, Version 27.0 (Armonk, New York, USA: IBM Corp).

3 Chapter 3: Results

3.1 Site Characteristics

3.1.1 Sediment Analysis

Composite samples of sediments from the four sites included in this study were analyzed for their content in common industrial contaminants. Sum concentrations (ug/g) by category of contaminants are used to facilitate comparisons between sites. Mitchell's Bay, our reference site located upstream of the Detroit River in Lake St. Clair, had the lowest sum concentrations of metals and non-detectable levels of PAHs and PCBs (Table 2). Of our three impacted sites located along the Detroit River, Trenton Channel had the highest sum concentrations of PAHs and PCBs, while Peche Island had the highest sum concentration of heavy metals. Belle Isle had the lowest sum concentration of heavy metals and second highest levels of PAHs of the three impacted sites (Table 2). These contamination levels appear to form a gradient of increasing sum concentrations of contaminants as you go from Lake St. Clair (Mitchell's Bay) to the mouth of the Detroit River (Peche Island and Belle Isle) to the site furthest downstream on the Detroit River (Trenton Channel). A more detailed breakdown of compounds detected in sediments from each site can be found in Appendices B, C, and D.

3.1.2 Contaminant Exposure

The mRNA levels of *cyp1a1* were measured in the liver of yellow perch from each site to estimate contaminant exposure experienced by individuals. A Kruskal-Wallis test found that there was a significant difference in liver *cyp1a1* expression between sites ($H(138) = 22.87$, $p < 0.0001$), with fish from Mitchell's Bay showing the lowest mean levels of *cyp1a1* expression and fish from Trenton Channel showing the highest (Fig. 2). A post hoc Dunn's multiple

comparisons test found that fish from Mitchell's Bay had significantly lower levels of *cyp1a1* expression than fish from Peche Island ($p = 0.01$) and Trenton Channel ($p < 0.001$), but not Belle Isle ($p = 0.06$). Overall, the data showed that gene expression of a liver detoxification enzyme generally paralleled the contamination gradient found in the sediments of our four test sites.

3.1.3 Growth

Environmental contamination could influence the overall growth of perch. We examined this possibility by assessing the relationships between age and body mass between sites (Fig. 3). Our generalized linear model found a significant interaction effect between age and site on the mass of perch ($\chi^2(8) = 16.6$, $p = 0.03$), with post hoc pairwise comparisons indicating that fish from Peche Island attain a higher mass than fish from Mitchell's Bay at ages 2 ($p = 0.02$), 3 ($p = 0.001$) and 4 years ($p < 0.001$). Fish from Belle Isle also have a higher mass than fish from Mitchell's Bay, but only at age 4 years ($p = 0.03$). Overall, it appears that perch from Peche Island grow faster from the second year of life onwards compared to perch sampled from our reference site and a similar trend is found later in life in perch from Belle Isle, but no difference in body growth is found between perch from Trenton Channel and perch from our reference site.

3.1.4 Trophic Ecology

Stable isotopes of nitrogen were used to assess the trophic position of perch at the different sites. There was a significant difference in trophic position between sites ($H(140) = 93.00$, $p < 0.0001$; Figure 4A). A post hoc Dunn's multiple comparisons test showed that perch from Trenton Channel had significantly lower trophic positions than perch from the other three sites ($p < 0.0001$), while Belle Isle perch also had significantly higher trophic positions than

Mitchell's Bay ($p < 0.001$). Mitchell's Bay and Peche Island perch showed no significant differences in trophic position ($p > 0.05$).

The d15N predator:forage fish ratio was also analyzed to assess if perch occupied a typical trophic level above that of small forage fish at our four test sites. This measure differed between sites ($H(140) = 102.5$, $p < 0.0001$; Figure 4B) and a post hoc Dunn's test showed that perch from Trenton Channel had a significantly lower median d15N ratio than perch at the other three sites ($p < 0.001$). Additionally, Trenton Channel was the only site to have a median d15N ratio lower than 1. In contrast, we found that Belle Isle perch had the highest median trophic position and d15N predator:forage fish ratio of all four sites.

Isotopic niche width and overlap were also analyzed to assess differences in diet, whereby smaller isotopic niche widths indicate a more specialized diet, and overlap between isotopic niches indicate similarity in diets. Of our four sites, we found that Mitchell's Bay had the smallest isotopic niche width, and Peche Island had the largest (Table 3). Both Peche Island and Belle Isle's isotopic niches overlapped with Mitchell's Bay, while Trenton Channel's did not overlap with Mitchell's Bay's (Table 4; Fig. 5).

3.2 Relative Organ Size

Previous literature suggests that environmental contamination could influence relative organ size in various ways depending on the organ. We examined these possibilities by first assessing between-site differences in relative organ size, followed by a general linear model approach (or generalized linear model if data did not meet the assumptions of parametric statistics) to determine which factors contributed to differences between sites. Preliminary models assessed age and *cyp1a1* expression as covariates due to between-site differences in these

factors, but neither age, *cyp1a1* expression levels, nor interaction terms including these factors (age*site, CYP1A1*site) had any significant effects on relative organ sizes. As such, our final models for relative organ sizes only examined trophic position, site, and the interaction of trophic position with site.

3.2.1 Brain

We found a significant difference in relative brain size between sites ($H(138) = 53.1$, $p < 0.0001$; Fig. 6A), with a post hoc Dunn's multiple comparisons test showing Trenton Channel perch had significantly smaller brains than the other three sites ($p < 0.001$). We assessed this between-site difference in relative brain size using a GLM that included trophic position as a covariate due to previous observations of a positive relationship between trophic position and relative brain size in fishes (Kondoh, 2010; Edmunds et al. 2016). Our model found that relative brain size was positively associated with relative trophic position ($\eta_p^2 = 0.254$, $F(1) = 43.9$, $p < 0.001$), site ($\eta_p^2 = 0.10$, $F(3) = 5.0$, $p = 0.003$), and the interaction between site and trophic position was statistically significant ($\eta_p^2 = 0.16$, $F(3) = 8.2$, $p < 0.001$). Pairwise comparisons between sites showed that Mitchell's Bay and Peche Island have significantly different relationships between relative trophic position and relative brain size. This divergence in relationships between sites is further supported through site-specific linear regressions of trophic position versus relative brain size, which showed Mitchell's Bay having the most positive slope of all four sites, while Peche Island had the least positive slope (Fig. 6B).

3.2.2 Gut

We found a significant difference in relative gut size between sites ($H(141) = 40.70$, $p < 0.0001$; Fig. 7), with a post hoc Dunn's multiple comparisons test showing Mitchell's Bay perch

had significantly smaller guts than the other three sites ($p < 0.01$). We assessed this between-site difference in relative gut size using a GLM, and found that relative gut size was positively affected by trophic position ($\eta_p^2 = 0.089$, $F(1) = 13.0$, $p < 0.001$) and site ($\eta_p^2 = 0.10$, $F(3) = 5.0$, $p = 0.003$), but not the interaction of site and trophic position ($\eta_p^2 = 0.27$, $F(3) = 1.2$, $p = 0.303$).

3.2.3 Liver

We found a significant difference in relative liver size between sites ($H(141) = 17.76$, $p = 0.0005$; Fig. 8). A post hoc Dunn's multiple comparisons test showed that Mitchell's Bay perch had significantly smaller livers than Peche Island and Trenton Channel ($p < 0.05$). We assessed this between-site difference in relative liver size using a generalized linear model, and found that it was affected only by site ($\chi^2(3) = 9.2$, $p = 0.03$), but not by trophic position ($\chi^2(1) = 3.7$, $p = 0.055$) or the interaction of site and trophic position ($\chi^2(3) = 2.1$, $p = 0.558$).

3.2.4 Heart Ventricle

We found a significant difference in relative heart ventricle size between sites ($H(138) = 13.77$, $p = 0.003$; Fig. 9A), with a post hoc Dunn's multiple comparisons test showing that Peche Island perch had significantly larger heart ventricles than Mitchell's Bay and Trenton Channel ($p < 0.05$). We assessed this between-site difference in relative heart ventricle size using a GLM, and found a significant effect of site ($\eta_p^2 = 0.075$, $F(3) = 3.5$, $p = 0.02$) and the interaction between site and trophic position ($\eta_p^2 = 0.073$, $F(3) = 3.4$, $p = 0.02$). Pairwise comparisons between sites showed that Mitchell's Bay's relationship between trophic position and relative ventricle size differed significantly from the remaining three sites ($p < 0.04$). Linear regressions of trophic position versus relative heart ventricle size showed that relationships at Trenton

Channel and Peche Island have a more positive slope than at Mitchell's Bay, and this relationship is negative at Belle Isle (Fig 9B).

3.2.5 Ovary

Upon dissection, we discovered that our samples were 80% females, 4% males, and 16% with underdeveloped and unmeasurable gonads. As such, we conducted all further analyses on the ovaries alone due to the large sex bias in our samples, and will refer solely to the ovaries throughout the rest of this document. We found no significant differences in relative ovary size between sites ($H(121) = 6.5$, $p = 0.09$; Fig. 10).

4 Chapter 4: Discussion

While several studies have evaluated the effects of industrial pollution on overall growth in fish (Feist et al., 2005; Henshel et al., 2006; Koeller and Parsons, 1977; Maceina and Sammons, 2019; Pavlov et al., 2014; Resier et al., 2004; Rypel and Bayne, 2010), few have examined the relationship between pollution and organ growth. We attempted to fill this gap by examining how chronic industrial pollution affects organ growth in wild fish via the direct toxic effects of pollutants (i.e. tissue damage and inhibition of cell proliferation in non-detoxifying organs) and/or the indirect effects of impoverished food webs (i.e. reduction in energy availability). Additionally, we sought to determine which organs are most sensitive to these effects. We expected to find hypertrophy of the liver due to its role as a detoxifying organ, with previous studies crediting this hepatic growth to increased functional load (Buklalove et al., 2021; Tenji et al., 2020) as well as increased glycogen & lipid loading, organelle proliferation, and swelling of the endoplasmic reticulum as a result of exposure to toxicants (Wolf and Wolfe, 2005). We suspected that this hepatic growth would come at the expense of other organs, specifically energetically expensive organs like the gut or brain, should chronic pollution reduce energy intake via impoverishment of food webs. We additionally expected that all non-detoxifying organs may experience hypotrophy due to the apoptotic effects resulting from activation of the AhR pathway seen in these tissues (Huang et al., 2021; Ren et al., 2020; Szychowski et al. 2016; Weber and Janz, 2001; Wojtowicz et al. 2017; Zhang et al., 2021).

Our initial characterization of field sites found that differences in sediment contaminant levels were matched by differences in perch hepatic *cyp1a1* expression levels, trophic position, and relative organ size, with most noticeable differences occurring between Trenton Channel,

our most polluted site, and Mitchell's Bay, our reference site. We found that Mitchell's Bay had the lowest levels of heavy metals, PAHs, and PCBs across all four sites, while Trenton Channel had the highest levels of PAHs and PCBs, and Peche Island the highest level of heavy metals. Belle Isle's contaminant levels fell between both of these sites. Liver *cyp1a1* expression in yellow perch matched these between site differences in sediment contaminant levels, with the highest expression levels seen at Trenton Channel and Peche Island, and the lowest seen at Mitchell's Bay, suggesting that fish contaminant exposure mirrored environmental contaminant levels in the sediments. These between site differences were also seen in foraging behaviour, with the lowest trophic positions being observed at Trenton Channel, suggesting a relationship between impoverishment of the food web and observed environmental contaminant levels. Between site differences were further observed in relative brain, gut, liver, and heart ventricle size. The smallest brains were seen at Trenton Channel, while the smallest guts were seen at Mitchell's Bay. Unsurprisingly, the largest livers were seen at Peche Island and Trenton Channel, which matches previous observations of liver hypertrophy in response to contaminant exposure (Agbohessi et al., 2015; Ahmed et al., 2013; Louiz et al., 2018). Curiously, heart ventricle size was largest at Peche Island, a site impacted by heavy metal contamination of sediments. However, upon closer examination of the factors that could explain observed differences in relative organ size between sites, we found that contaminant exposure had no significant effects on any observed differences in the relative size of the brain, gut, heart ventricle, or ovary. This offers no support for our first hypothesis that changes in the growth of non-detoxifying organs may be due to the direct toxic effects of pollutants through stimulation of apoptosis and/or inhibition of cell proliferation. Conversely, we found evidence that trophic

position was associated with the growth of the brain, gut, and heart ventricle, but not the liver or ovaries. In particular, we found that the effects of trophic position on brain growth seemed to vary according to pollution levels by site, with a stronger positive relationship being seen at Mitchell's Bay, our reference site, compared to our impacted sites. In contrast, we found that the effects of trophic position on gut growth was similar across all four sites, despite differences in pollution levels. Overall, this offers only partial support for our second hypothesis that impoverishment of food web via chronic pollutant exposure could indirectly affect growth of organs that are energetically expensive to maintain.

4.1 Liver *cyp1a1* expression as a bioindicator of contaminant exposure

The cytochrome p-450 (CYP) 1A subfamily of enzymes is involved in the metabolization of foreign chemicals and endogenous molecules in various animals (Bernhardt, 1996), and can be induced by the binding of various environmental contaminants to a cytosolic aryl hydrocarbon receptor (AhR) (Bucheli and Fent, 1995; Stegeman and Lech, 1991). Due to its activation by xenobiotics (and subsequent role in detoxifying said foreign contaminants), CYP1A activity is commonly used as a biomarker for contaminant exposure in fish and is often measured using the EROD assay (Whyte et al., 2000). However, tissue storage limitations are an important constraint to reliably measure EROD activity in samples. For example, storing frozen liver samples for only 24 hours can result in a decline of up to 35% in EROD activity levels in rainbow trout (Forlin and Andersson, 1985). Due to sample storage constraints associated with field sampling, we chose to measure liver *cyp1a1* gene expression as a biomarker of contaminant exposure instead of EROD activity. Expression of *cyp1a1* precedes the induction of CYP1A and contributes to increase its catalytic activity. This method has been proposed previously (Hahn

and Stegeman, 1994) and is increasing in usage to examine contaminant exposure in fish (Huang et al., 2014; McClain et al., 2009; Sorrentino et al., 2005). Additionally, *cyp1a1* gene expression is not subject to inhibition by certain contaminants (e.g. PCB156) like EROD activity (Gooch et al., 1989; Rice and Schlenk, 1995; Stien et al. 1997).

We found that perch of Trenton Channel and Peche Island both had significantly higher levels of hepatic *cyp1a1* gene expression compared to Mitchell's Bay, our reference site. Trenton Channel and Peche Island had the highest sum concentrations of PAHs and heavy metals of all four study sites, respectively, indicating that *cyp1a1* gene expression was a suitable measure of contaminant exposure in this study. Belle Isle hepatic *cyp1a1* gene expression was not significantly different than in Mitchell's Bay perch, despite having the second highest sum concentrations of PAHs and heavy metals measured in sediment, but the difference was close to the statistical threshold ($p = 0.06$). Overall, hepatic *cyp1a1* expression was generally higher at sites with higher levels of sediment contaminant levels, which was in line with our predictions.

4.2 Stable isotopes indicate altered foraging behaviour at Trenton Channel

Stable nitrogen isotopic signatures ($d^{15}N$) are commonly used to infer trophic positions of consumers relative to organisms more basal in the food web, while stable carbon isotopic signatures ($d^{13}C$) are used to infer food source in lakes (Vander Zanden and Rasmussen, 1999). Combined, the two can be used to calculate an isotopic niche width to estimate prey diversity and diet overlap between communities or species (Jackson et al., 2011). We used these measures to compare foraging behaviours between fish from our impacted and reference sites.

We found that yellow perch from Trenton Channel, our most contaminated site, exhibited significantly lower trophic levels compared to perch from the three other sites. The adult yellow perch from Trenton Channel also occupy a lower trophic position than forage fish at this site, which is not seen at our other sites. This indicates a potential disruption of the food web at Trenton Channel, an observation further supported by the limited overlap between isotopic niche widths of Trenton Channel and the other sites. These results suggest that Trenton Channel's perch are primarily feeding on different prey. During dissections, we observed that almost all perch from Trenton Channel had their entire digestive tracts packed with undigested snail shells, something that was only rarely observed in perch captured at other sites. Such extreme reliance on snail prey might explain the observed differences in perch trophic position and isotopic niche width between Trenton Channel and other sites considering that snails have low nitrogen isotopic signatures and rarely occur in the typical diet of yellow perch (Duncan et al., 2011; Elrod et al., 1981; Keast, 1977; Kidd et al., 1995).

Interestingly, we also found that yellow perch from Mitchell's Bay had a lower trophic position than those from Belle Isle, and exhibited no difference in trophic position with perch from Peche Island. This could potentially be explained by yellow perch at Mitchell's Bay experiencing more competition compared to the other sites, which can be inferred from the small isotopic niche width observed there, indicating a narrower selection of prey available and a more specialized diet. In support of this idea, compression of the isotopic niche width under intense interspecific competition has been observed in Arctic charr (Sandlund et al., 2016). Previous studies have also suggested that yellow perch may experience increased competition from the non-native white perch (*Morone americana*) in Lake St. Clair (Henderson and Nepszy, 1989).

This increased competition and diet specialization may prevent yellow perch from Mitchell's Bay from ascending to higher trophic positions in their food web relative to perch from Belle Isle.

4.3 Differences in body growth

We found that fish from our reference site grow at a slower rate than fish from Peche Island, starting at age 2 years and above, and fish from Belle Isle, starting at age 4 years and above. This difference in growth rates could potentially be explained by differences in diets at each site. Previous work on the common roach (*Rutilus rutilus*), has suggested that a larger isotopic niche width and generalist diet can confer growth benefits in spite of variations in prey availability (Hayden et al., 2014). Based on their isotopic niche widths, we can infer that fish from Mitchell's Bay had the least varied and most specialized diet of all studied sites, while fish from Peche Island and Belle Isle have a more varied and generalist diet. Higher competition could limit the diversity and quantity of prey available to perch at Mitchell's Bay, leading to a slower rate of growth. Furthermore, a report by the State of Michigan Department of Natural Resources (Thomas and Haas, 2004) indicated that yellow perch in Lake St. Clair (i.e. Mitchell's Bay) were eating progressively fewer *Hexagenia* spp. in their diet, which has been previously linked with reduced growth rates in this species (Tyson and Knight, 2001).

Interestingly, there is no notable difference in growth between fish from Mitchell's Bay and fish from Trenton Channel, despite Trenton Channel being our most contaminated site, and having an isotopic niche width that lacks any overlap with Mitchell's Bay (i.e. indicating a completely different diet). However, research in other species has found that contaminant exposure could both increase and slow growth rate (or not affect growth rate at all) depending on

the species, age, and type of contaminant (Feist et al., 2005; Henshel et al., 2006; Maceina and Sammons, 2018; Pavlov et al., 2014). In yellow perch specifically, Maceina and Sammons (2018) found that perch growth rates were similar across PCB-contaminated and reference sites, while Eastwood and Couture (2002) found that perch from metal-contaminated lakes experience lower growth rates. Evidently, the control of somatic growth is complex and differential growth rates seen across our sites could be due to more factors than simply diet composition or contaminant exposure.

4.4 Differences in relative organ size

GLM models indicated that hepatic *cyp1a1* gene expression levels were not a significant predictor of relative organ size in yellow perch. However, we found that trophic position influenced the relative size of several organs, including the brain, gut, and heart ventricle. This could suggest that differences in relative organ size between sites are primarily driven by the indirect effects of pollution on food webs rather than the direct effects of contaminant exposure.

4.4.1 Relative brain size

Of our four sites, we found that fish from Trenton Channel had the smallest relative brain size. Fish from Trenton Channel also have the lowest trophic position of all four sites, and we also found evidence that yellow perch at this site are occupying a lower trophic position than smaller forage fish, indicating disruption in the typical food chain (Luek et al. 2010; Sherwood et al. 2002). Typically, perch will experience ontogenetic diet shifts and begin eating progressively larger prey as they grow (Sherwood et al. 2002) allowing for maintenance of growth efficiency (Iles and Rasmussen, 2005). However, the lack of a diet shift as perch grow would result in an energy bottleneck that severely reduces the efficiency of food energy conversion, thus reducing

the amount of energy available for growth (Iles and Rasmussen, 2005; Sherwood et al. 2002), especially for a metabolically expensive organ like the brain (Aiello, 1970). Existing evidence also suggests that relative brain size increases with trophic position in order to maintain the cognitive ability needed to successfully hunt larger organisms (Edmunds et al. 2016). Our data appear to support this idea as perch from Trenton Channel occupy a lower trophic position than perch from any other site and exhibit the smallest relative brain size. The absence of a diet shift to larger prey in perch from Trenton Channel suggests that the lack of larger prey in a diet does not require and/or can not maintain larger relative brain sizes in this species.

We also found a site-specific relationship between relative trophic position and relative brain size. While all four sites demonstrated a positive trend between relative trophic position and relative brain size, Mitchell's Bay exhibited the most positive slope of all four sites, suggesting that relative brain size increases more with relative trophic position at Mitchell's Bay than at other sites. This is potentially an effect of their more specialized diet, as increased competition at this site may place pressure on yellow perch to invest in cognitive ability in order to successfully obtain food. In contrast, Peche Island perch had the least positive slope between relative trophic position and relative brain size of all four sites, as well as the largest isotopic niche width, and consequently, most generalist diet. The increased prey diversity and assumed lesser competition at this site could contribute to the weaker relationship between relative trophic position and relative brain size. In addition, perch from Peche Island exhibit a significantly faster rate of body growth than perch from Mitchell's Bay, suggesting that perch from Peche Island are prioritizing growth of other organs or overall body growth over brain growth.

4.4.2 Relative gut size

Of our four sites, we found that perch from Mitchell's Bay had the smallest relative gut size. Contrary to our predictions, this pattern does not match that of relative brain size and suggests that metabolically expensive organs are not affected the same way by chronic pollution, and that organ-specific patterns exist. Our GLM analysis found that the relative gut size trends positively with trophic position in a similar fashion across all four sites. Oddly enough, however, perch from Trenton Channel had larger guts than perch from Mitchell's Bay and similar sized guts to Peche Island and Belle Isle despite having a significantly lower trophic position. This suggests that diet composition may be playing a bigger role in gut size than trophic position alone. While our study lacks diet composition data, we did observe that the majority of perch at Trenton Channel had guts filled with snail shells, but rarely observed snail shells in the guts of perch at other other sites. This could suggest that perch at Trenton Channel may be investing more in gut growth due, in part, to this difference in diet resulting in similar relative gut size to perch from Peche Island and Belle Isle despite having a lower trophic position. Mitchell's Bay's perch smaller gut size also mirrors its smaller isotopic niche width (i.e. specialized diet), further suggesting that diet composition is playing a role in determining relative gut size. Perhaps the primary prey type eaten by perch at Mitchell's Bay is smaller and does not require a larger gut to digest compared to the varied prey types eaten by perch at Peche Island and Belle Isle. This falls in line with previous research observing changes in gut length as a result of diet composition and food quality in a number of fish species. For example, prickleback fishes increase gut length in response to low-protein diets (German and Horn, 2006) while Eurasian perch (*Perca fluviatilis*) increase gut length in response to less digestible food in their diets (Olsson et al., 2007). Silver

carp (*Hypophthalmichthys molitrix*) also lengthen their guts in response to more plankton in their diet, while some cichlids demonstrate an inverse relationship between gut length and algal nutrient quality (Wagner et al., 2009).

4.4.3 Relative liver size

We found similar patterns for relative liver size and hepatic *cyp1a1* gene expression levels between our study sites, with perch from Peche Island and Trenton Channel having the largest relative liver size and *cyp1a1* expression. This appears to initially support the idea that liver hypertrophy is linked with the direct effects of contaminant exposure as documented in previous studies (Fletcher et al. 1982; Slooff et al. 1983; Tenji et al. 2020) but further analysis using GLM indicated that hepatic *cyp1a1* gene expression levels do not significantly predict variation in liver size within sites. We also found no relationship between trophic position and relative liver size, indicating that these observed differences in relative liver size between sites are not due to the impoverishment of local food webs at our impacted sites. These results could suggest two things. First, measuring hepatic *cyp1a1* gene expression levels may be sufficient for measuring general levels of exposure to environmental toxicants, but may not be sufficient in measuring the activation of the AhR pathway and the subsequent processes associated with this activation. As such, measuring EROD or overall CYP enzyme activity may be more appropriate in future studies to examine a finer relationship between toxicant exposure, AhR activation, and liver growth. Second, it may also suggest that the observed liver hypertrophy is not solely due to the direct effects of the AhR activation, and may instead be caused by other mechanisms such as overloading of glycogen and lipids in the liver, organelle proliferation, or swelling of the endoplasmic reticulum as a result of toxicant exposure (Wolf and Wolfe, 2005).

4.4.4 Relative heart ventricle size

We found that fish from Peche Island had the largest relative heart ventricle size out of the four sites we studied. It is unclear why Peche Island perch exhibited this difference. Notably, Peche Island has the largest sum concentration of heavy metals of all sites, but there is little to no previous literature suggesting that metal pollution affects overall heart size. There is some work examining how heart morphology and function is adversely affected during early development (e.g. partial to complete atrioventricular blockage and irregular arrhythmia) by other, oil-based contaminants and how overall function may be impacted by various water pollutants (Incardona and Scholz, 2017), but this does little to explain the observed heart ventricle hypertrophy seen at Peche Island. Curiously enough, we also found that all sites except for Belle Isle exhibited a positive correlation between relative trophic position and relative heart ventricle size. Again, it is unclear why fish from Belle Isle are exhibiting this unique pattern. It is interesting to note that Belle Isle was the only site where trace amounts of DDT metabolites were detected. Previous work has shown that DDT can accumulate in the heart of Atlantic salmon (Premdas and Anderson, 1963), and one study has found that DDT exposure slows heart rate in early-stage zebrafish (Ton et al., 2006). This relationship warrants further investigation of the potential deleterious effects of chronic exposure to DDT metabolites on heart growth.

4.4.5 Relative ovary size

At all four sites, approximately 80% of our samples were found to be female. Of the few males we were able to collect, all were small and with underdeveloped testes, demonstrating a clear bias in the sex ratio of collected fish. We found no significant differences in relative ovary size between sites. This is likely due to fish being sampled after the reproductive season

(Dabrowski et al. 1996), as there was no need to invest energy in ovary growth at the time of sampling. Previous studies have also found biased sex ratios, particularly in older perch (age 4+), but in favour of males as opposed to our observed sex ratio in favour of females (Schaeffer et al., 2000). These researchers suggested the increase in male to female sex ratio observed in older perch was likely due to greater energy depletion in females, thus leading only males to survive at older ages. This runs counter to what we observed, as our only males were young in age, suggesting that our biased sex ratio was not due to differential lifetime energy usage between males and females. An alternative explanation is that site fidelity in yellow perch may be sex-specific, with only females maintaining high site fidelity and males moving to different locations post reproductive season. This potentially violates our initial assumption that site fidelity in perch was universal between sexes, which could limit the conclusions made from our study to female perch only.

4.5 Conclusion

We performed our study at the Detroit River, which has been defined as an area of concern by the International Joint Commission (1987) and a target of consistent remedial efforts over the past three and a half decades. Performing our study here allowed us to investigate if and how legacy industrial pollution affects fish organ growth, and if the decades long remedial efforts have been beneficial to fish on a physiological level. The Detroit River Remedial Action Plan Stage 2 Report (Detroit River Canadian Cleanup, 2010) reported that the Detroit River still experienced many issues resulting from the legacy industrial pollution, including the occurrence of tumours and deformities in fish, benthos degradation, and degradation of fish, phytoplankton, and zooplankton populations. However, the Detroit River Canadian Cleanup's most recent report

in 2021 indicated that some of these issues have been resolved, including the occurrence of fish deformities and benthos degradation (Detroit River Canadian Cleanup, 2021). The issue of degraded fish, phytoplankton, and zooplankton populations still persists, which is in line with our study's observations. In particular, the decrease in fish deformities and tumours suggests that the direct toxic effects of chronic pollutant exposure have been resolved and/or reduced by remedial efforts, which matches our observation that contaminant exposure as measured by hepatic *cyp1a1* expression levels did not directly affect perch organ growth via AhR pathway activation. Furthermore, the continued degradation of fish, phytoplankton, and zooplankton populations lends support to our hypothesis that the effects of legacy industrial pollution are still persisting via the indirect effects of impoverished food webs, despite current contaminant levels being lower than historical records. Future remedial efforts should therefore incorporate restoration of fish and invertebrate biodiversity into their action plans in order to combat these persistent indirect effects of chronic pollution.

FIGURES AND TABLES

Table 1. Nucleotide sequences of yellow perch primers used for qRT-PCR.

Gene	Accession no.	Sequence (5'-3')	Efficiency (%)
40s	EU073713	F: CCGCACAAAGAATAAGCAGA R: CGGATCCTCTTACCAACGAT	100%
actb	AY332493.2	F: GGAGGTACCACCATGTACCC R: CTCTGGTGGGGCAATAATCT	100%
cyp1a1	XM_028573152.1	F: TTCTCGAGGCCTTCATCTTT R: CACAGGTGTCTTTGGGAATG	100%

40s, 40s ribosomal protein S7; *actb*, b-actin; *cyp1a1*, cytochrome P450 family 1 subfamily A polypeptide 1; F, forward; NA, not available at this time; R, reverse

Table 2. Sum concentrations of contaminants found in sediments sampled at the four test sites of Detroit River and Lake St. Claire. ND: not detected, PAHs: polycyclic aromatic hydrocarbons, PCBs: polychlorinated biphenyls.

Sum concentration (ug/g)	Mitchell's Bay	Peche Island	Bell Isle	Trenton Channel
Metals	36 177	134 094	110 995	67 648
PAHs	ND	0.523	1.206	2.946
PCBs	ND	ND	ND	0.072

Table 3. Standard ellipses area of the isotopic niche widths of our four test sites as determined through Stable Isotope Bayesian Ellipses in R (SIBER) using $d^{15}N$ and $d^{13}C$ stable isotope ratios. Abbreviations: BI: Belle Isle, MB: Mitchell's Bay, PI: Peche Island, TC: Trenton Channel.

Site	Standard Ellipse Area
MB	1.967
PI	4.722
BI	3.527
TC	3.125

Table 4. Proportion of overlapping area to non-overlapping area between isotopic niche widths of our four sites as determined through Stable Isotope Bayesian Ellipses in R (SIBER) using $d^{15}N$ and $d^{13}C$ stable isotope ratios. Abbreviations: BI: Belle Isle, MB: Mitchell's Bay, PI: Peche Island, TC: Trenton Channel.

	MB	PI	BI	TC
MB	-	0.262	0.095	0.000
PI	-	-	0.362	0.106
BI	-	-	-	0.069
TC	-	-	-	-

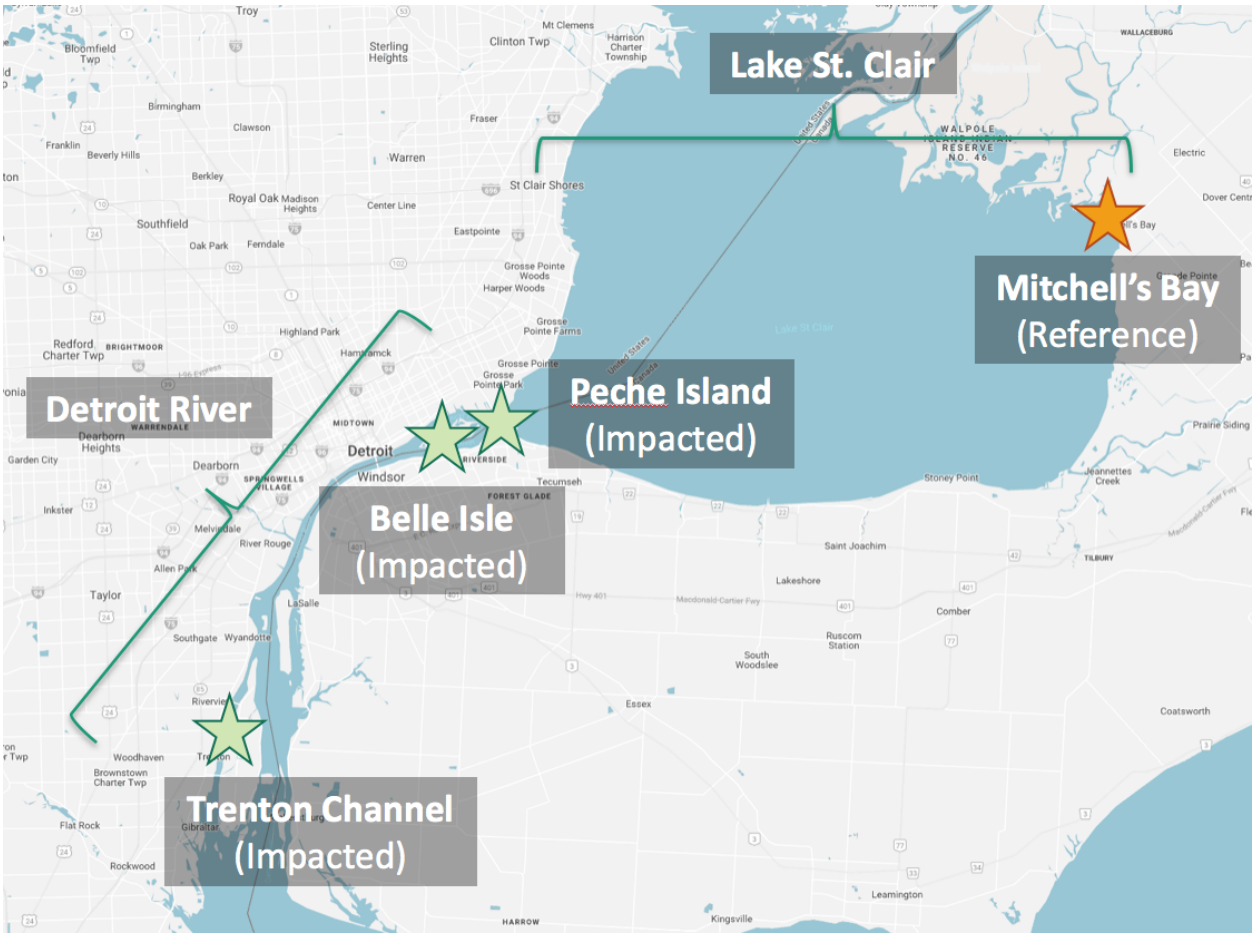

Figure 1. Location of our study sites along the Detroit River and Lake St. Clair. Our reference site, Mitchell's Bay (GPS coordinates: 42.46938649659893, -82.4238789245851), is located in Lake St. Clair and marked with an orange star symbol. Our impacted sites along the Detroit River, Peche Island (GPS coordinates: 42.34944613215649, -82.92955795153985), Belle Isle (GPS coordinates: 42.33459452273901, -82.97417034022823), and Trenton Channel (GPS coordinates: 42.17662574597135, -83.15782387019004), are marked by green star symbols.

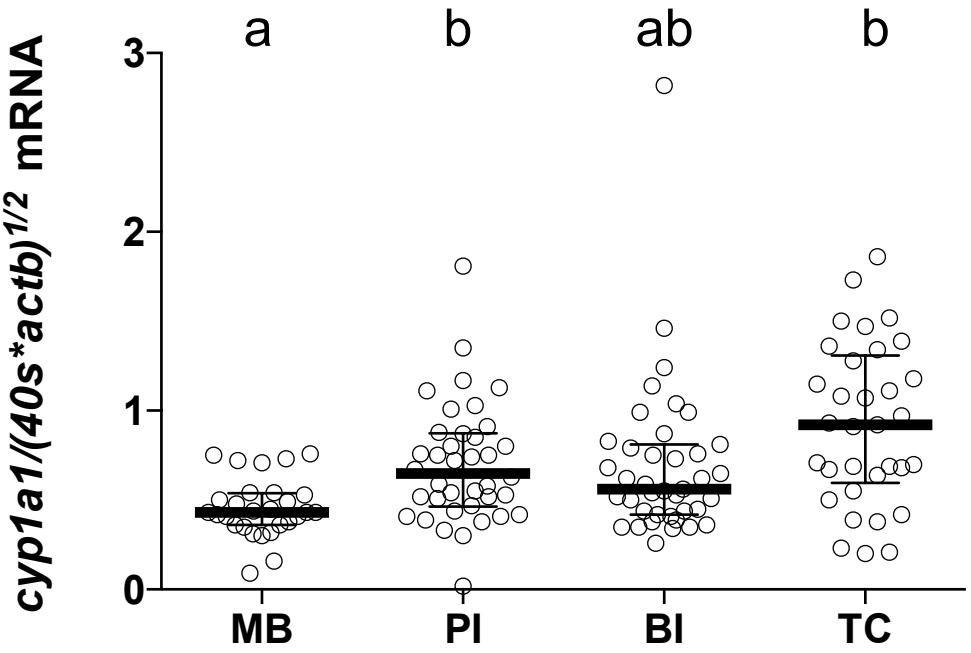

Figure 2. Median liver *cyp1a1* expression of in yellow perch (*Perca flavescens)* across four test sites (n_{MB} = 28, n_{PI} = 38, n_{BI} = 39, n_{TC} = 33). Gene expression was quantified using qPCR. Values are normalized to the geometric mean of *40s* and *actb*. Bars represent median +/- interquartile range. Letters represent significant differences between sites obtained by Dunn's post hoc test. Abbreviations: BI: Belle Isle, MB: Mitchell's Bay, PI: Peche Island, TC: Trenton Channel.

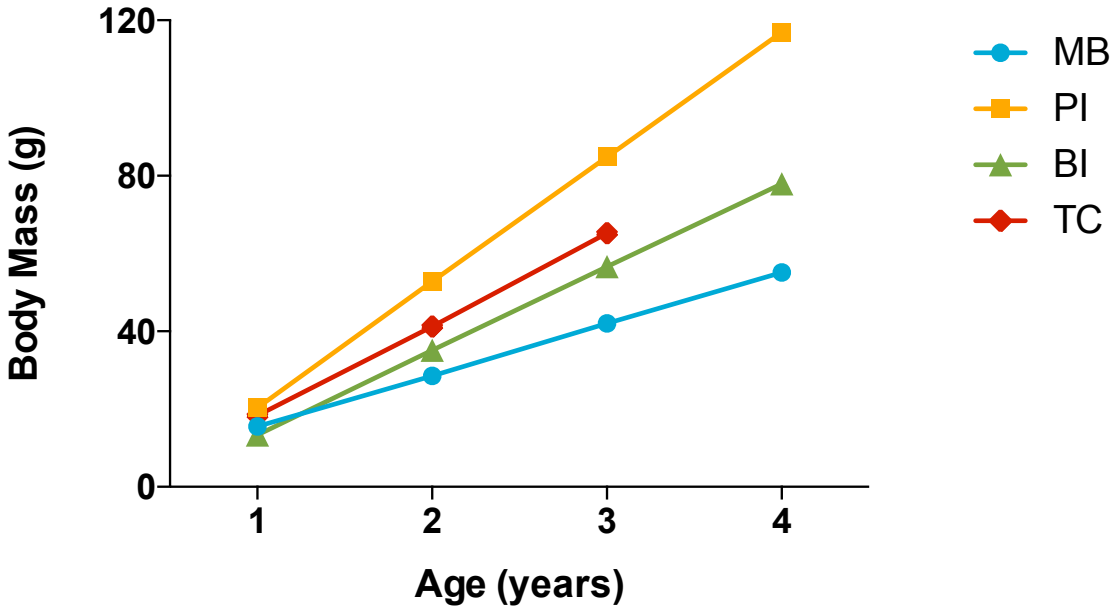

Figure 3. Growth curves of yellow perch (*Perca flavescens)* collected from our four test sites. Age was determined by counting the rings on bisected otoliths. Lines of best fit were determined by a linear regression for each site. A significant divergence between the growth curves for Mitchell's Bay and Peche Island is seen from age 2 years onwards, and between Mitchell's Bay and Belle Isle at age 4. Abbreviations: BI: Belle Isle, MB: Mitchell's Bay, PI: Peche Island, TC: Trenton Channel.

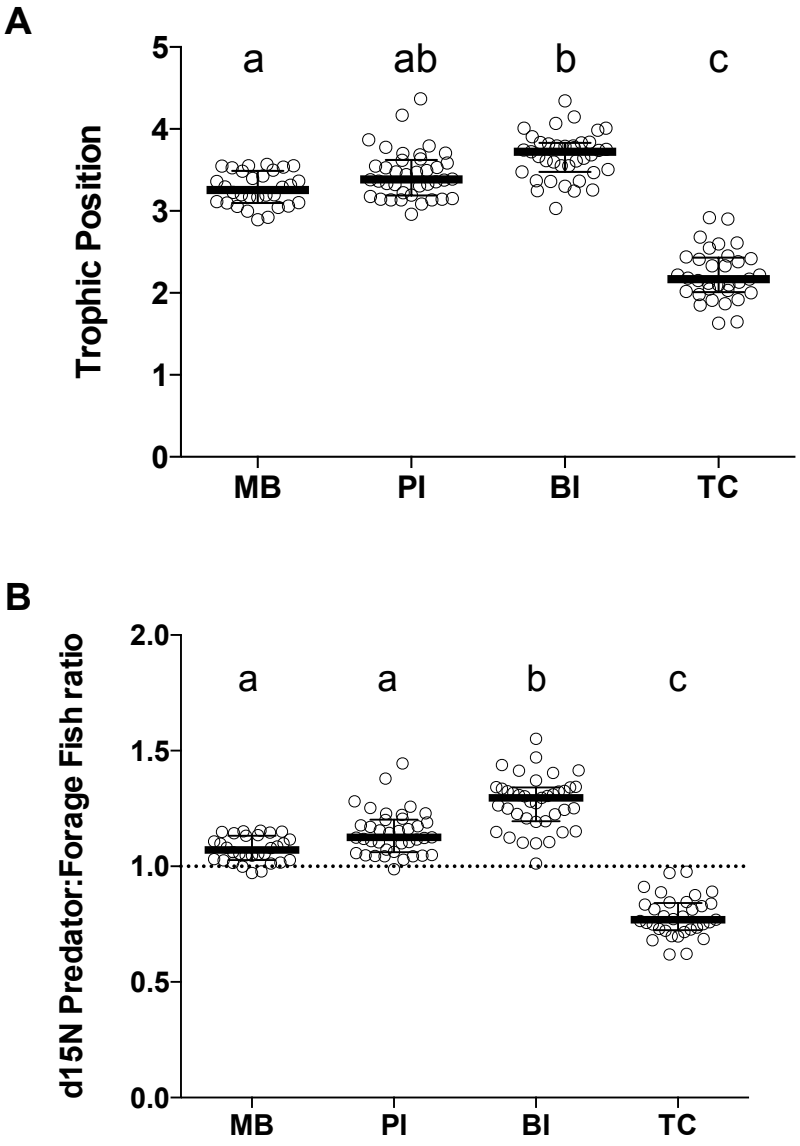

Figure 4. Yellow perch (*Perca flavescens*) trophic ecology at four sites in the Detroit River and Lake St. Clair. A) Median trophic position (n_{MB} = 30, n_{PI} = 38, n_{BI} = 39, n_{TC} = 33). Trophic position was calculated using nitrogen stable isotope ratios of yellow perch and forage fish according to the following equation: 3 + [(d15N perch – d15N forage fish)/3.4]. B) Median d15N predator:forage fish ratio (n_{MB} = 30, n_{PI} = 38, n_{BI} = 39, n_{TC} = 33). A d15N ratio higher than 1 indicates yellow perch are at a higher trophic position than forage fish (typical of an average food web), while a d15N ratio lower than 1 indicates yellow perch are at a lower trophic position than forage fish (atypical of an average food web). Bars represent median and +/- interquartile range. Letters represent significant differences between sites obtained using a Dunn's multiple comparisons test. Abbreviations: BI: Belle Isle, MB: Mitchell's Bay, PI: Peche Island, TC: Trenton Channel.

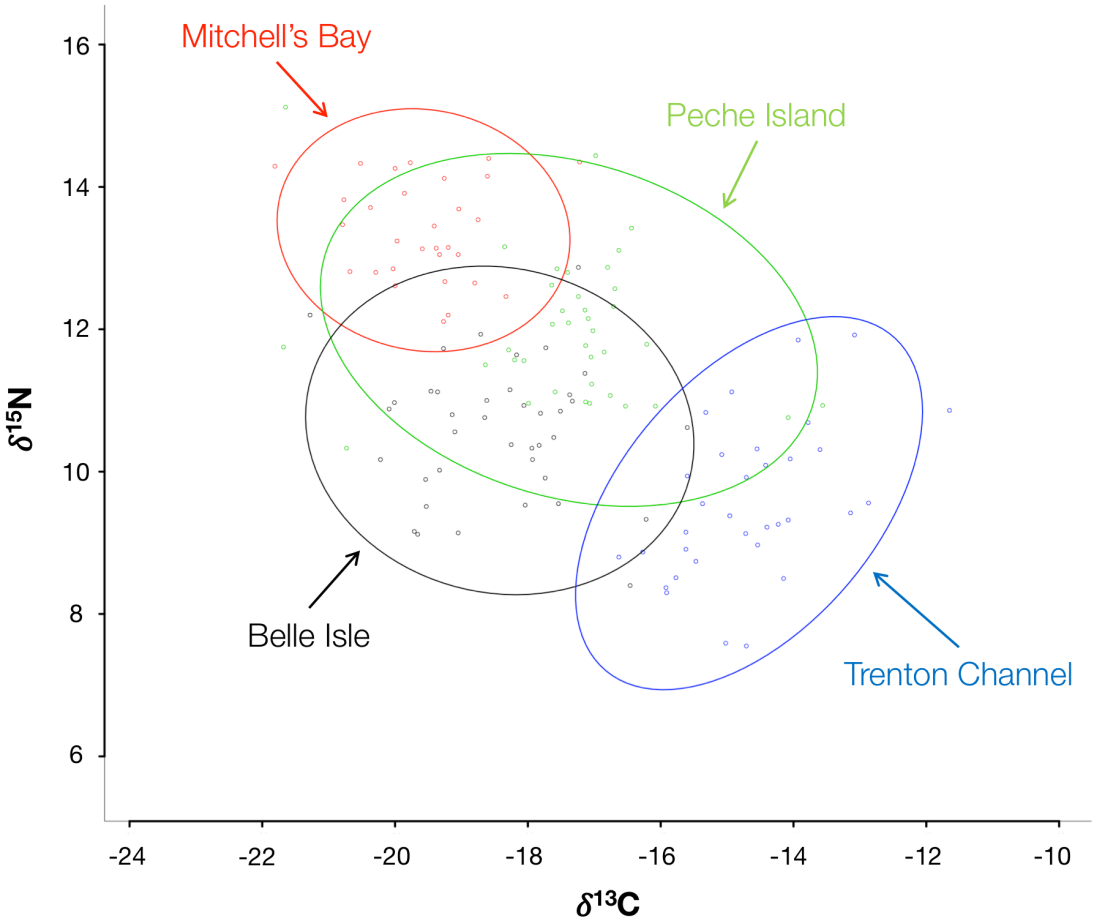

Figure 5. Visual representation of yellow perch (*Perca flavescens)* isotopic niche widths based on $d^{15}N$ and $d^{13}C$ from our four test sites. Maximum likelihood standard ellipses representing niche width were determined using the Stable Isotope Bayesian Ellipses in R (SIBER) statistical package.

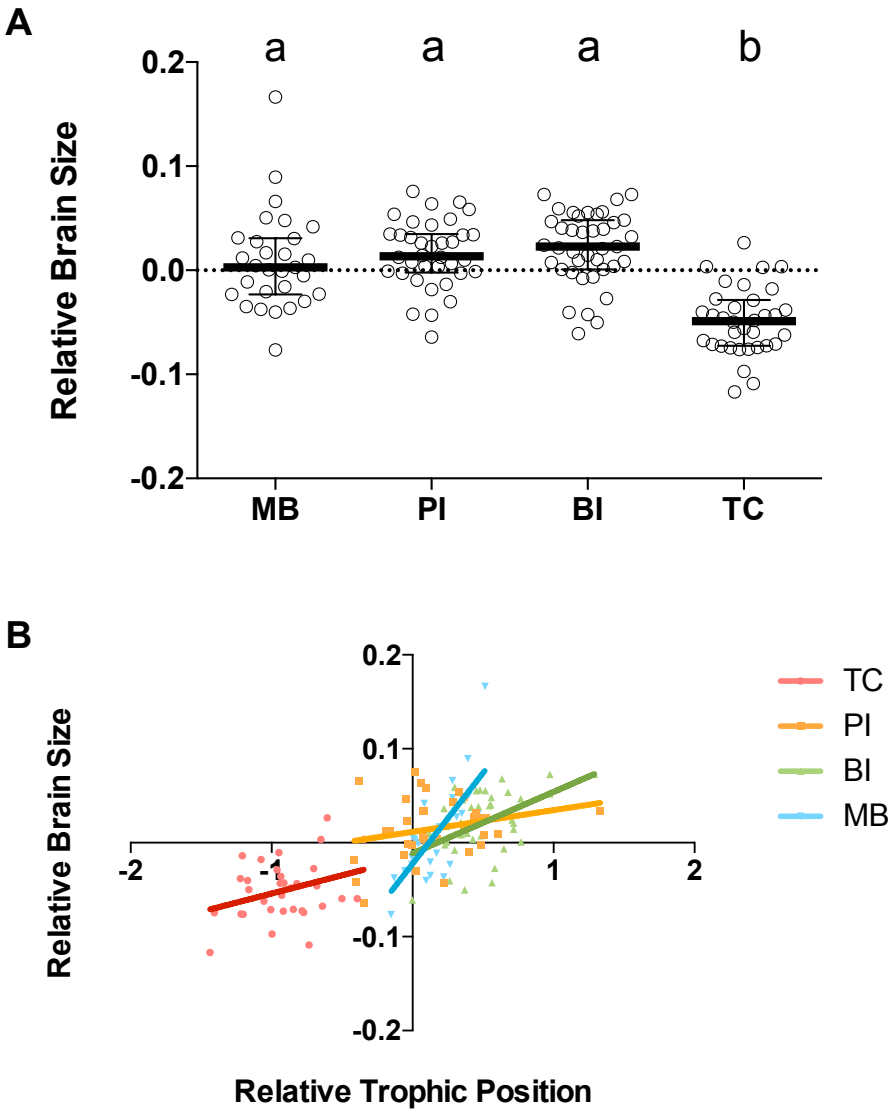

Figure 6. Between site differences and the effect of trophic position on yellow perch (*Perca flavescens*) relative brain size. A) Relative brain size of yellow perch across four sites in the Detroit River and Lake St. Clair (n_{MB} = 29, n_{PI} = 36, n_{BI} = 39, n_{TC} = 34). Relative brain size was obtained from residuals of a quadratic relationship between log-transformed body mass and log-transformed brain mass data including perch of all sites. Bars represent median +/- interquartile range. Letters represent significant differences between sites obtained using a Dunn's multiple comparisons test. B) A visualization of the relationship between relative trophic position and relative brain size of yellow perch from our four test sites. Lines of best fit for each site ere obtained by linear regression. Abbreviations: BI: Belle Isle, MB: Mitchell's Bay, PI: Peche Island, TC: Trenton Channel.

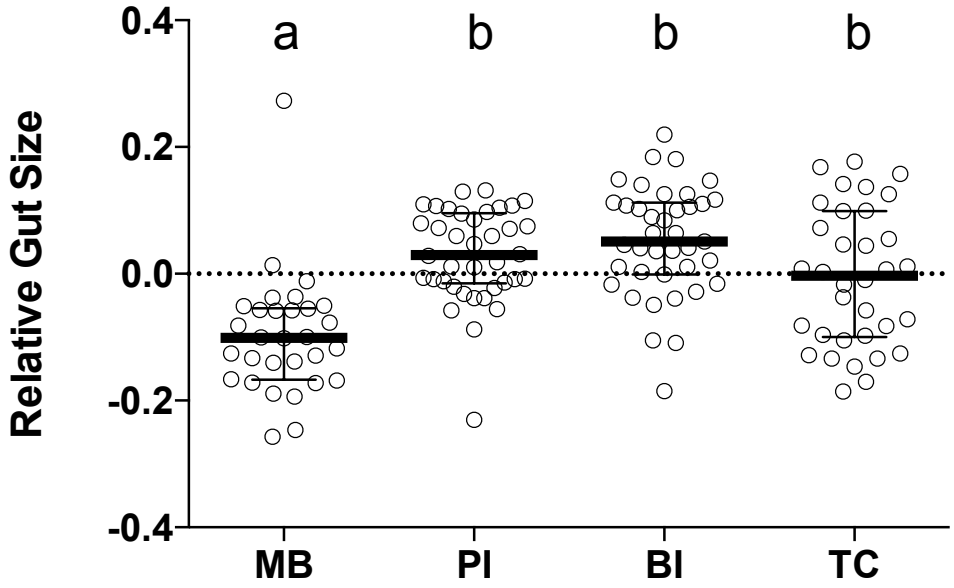

Figure 7. Median relative gut size of yellow perch (*Perca flavescens*) across four sites in the Detroit River and Lake St. Clair (n_{MB} = 30, n_{PI} = 38, n_{BI} = 39, n_{TC} = 34). Relative gut size was obtained from residuals of a linear relationship between log-transformed body mass and log-transformed gut mass data including perch of all sites. Bars represent median +/- interquartile range. Letters represent significant differences between sites obtained using a Dunn's multiple comparisons test. Abbreviations: BI: Belle Isle, MB: Mitchell's Bay, PI: Peche Island, TC: Trenton Channel.

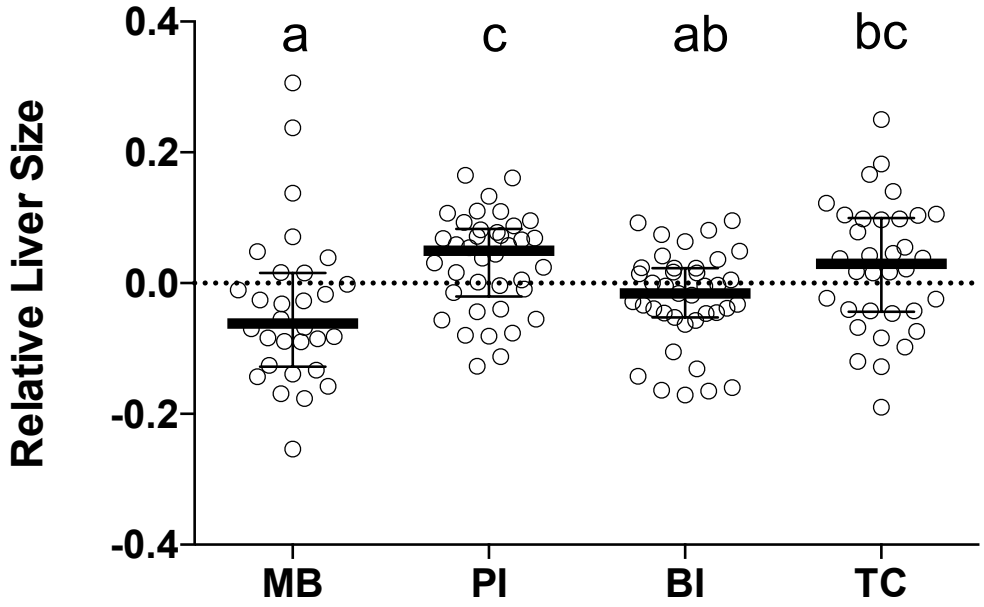

Figure 8. Median relative liver size of yellow perch (*Perca flavescens*) across four sites in the Detroit River and Lake St. Clair (n_{MB} = 30, n_{PI} = 38, n_{BI} = 39, n_{TC} = 34). Relative liver size was obtained from residuals of a linear relationship between log-transformed body mass and log-transformed liver mass data including perch of all sites. Bars represent median +/- interquartile range. Letters represent significant differences between sites obtained using a Dunn's multiple comparisons test. Abbreviations: BI: Belle Isle, MB: Mitchell's Bay, PI: Peche Island, TC: Trenton Channel.

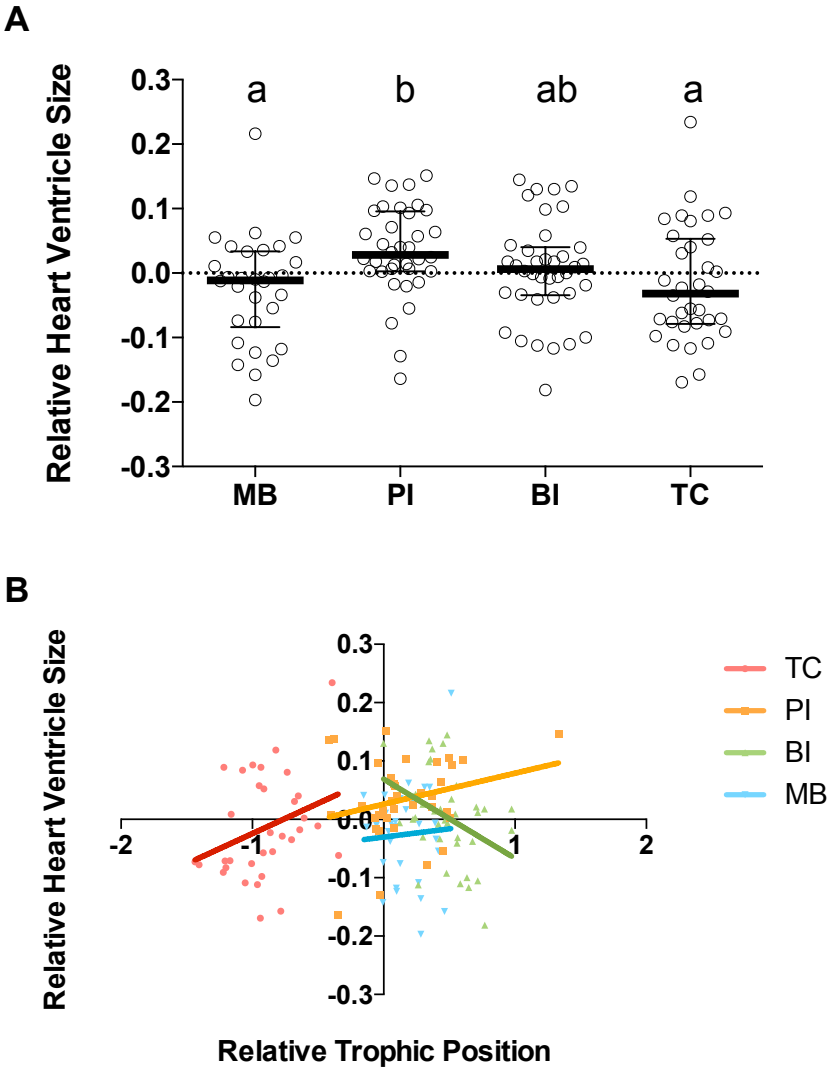

Figure 9. Data representing between site differences in relative heart ventricle size and the relationship between trophic position and relative heart ventricle size. A) Median relative heart ventricle size of yellow perch (*Perca flavescens*) across four sites in the Detroit River and Lake St. Clair (n_{MB} = 30, n_{PI} = 36, n_{BI} = 38, n_{TC} = 34). Relative gut size was obtained from residuals of a linear relationship between log-transformed body mass and log-transformed gut mass data including perch of all sites. Bars represent median +/- interquartile range. Letters represent significant differences between sites obtained using a Dunn's multiple comparisons test. B) A visualization of the relationship between relative trophic position and relative heart ventricle size of yellow perch from our four test sites. Lines represent lines of best fit for each site as determined by a linear regression. Abbreviations: BI: Belle Isle, MB: Mitchell's Bay, PI: Peche Island, TC: Trenton Channel.

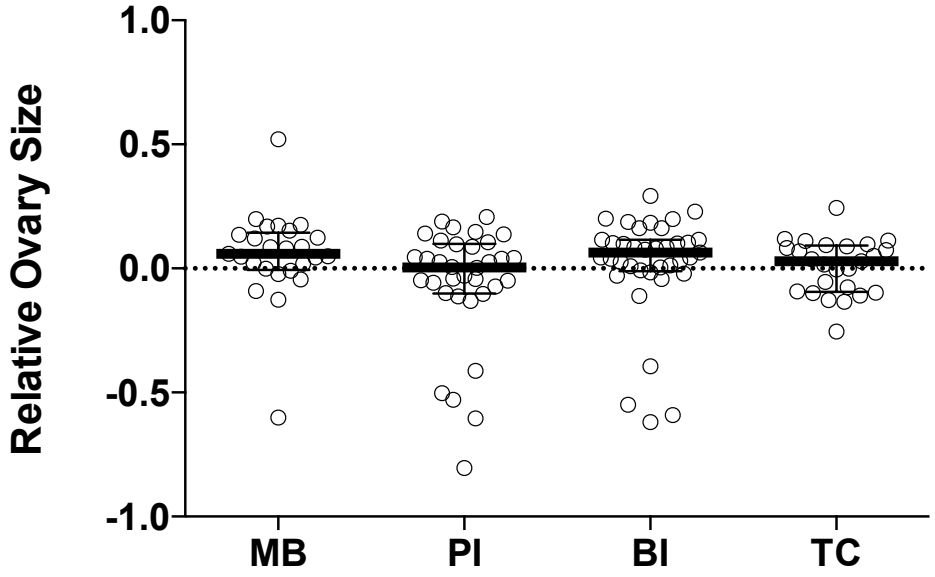

Figure 10. Median relative ovary size of yellow perch (*Perca flavescens*) across four sites in the Detroit River and Lake St. Clair (n_{MB} = 25, n_{PI} = 34, n_{BI} = 37, n_{TC} = 25). Relative ovary size was obtained from residuals of a linear relationship between log-transformed body mass and log-transformed ovary mass data including perch of all sites. Bars represent median +/- interquartile range.

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APPENDICES

Appendix A. List of preliminary general linear models tested to determine statistically significant relationships between covariates and relative organ size. Abbreviations: ros: relative organ size, cyp: *cyp1a1* liver mRNA levels, tp: trophic position.

1. ros = site + tp + cyp + age + tp*site + cyp*site
2. ros = site + tp + cyp + tp*site + cyp*site
3. ros = site + cyp + cyp*site
4. ros = site + tp + age + tp*site + tp*age

Appendix B. Extended list of elements detected by atomic spectroscopy in sediment samples (ug/g). Abbreviations: MB: Mitchell's Bay, TC: Trenton Channel, PI: Peche Island, BI: Belle Isle, RDL: reportable detection limit.

	MB	**TC**	**PI**	**BI**	**RDL**
Metals					
Acid Extractable Aluminum (Al)	1700.00	6400	14000	8300	50
Acid Extractable Antimony (Sb)		0.26	0.45	0.27	0.20
Acid Extractable Arsenic (As)		3.1	5.0	4.5	1.0
Acid Extractable Barium (Ba)	8.00	33	75	40	0.50
Acid Extractable Beryllium (Be)		0.38	0.66	0.46	0.20
Acid Extractable Bismuth (Bi)					1.0
Acid Extractable Boron (B)		6.2	13	9.4	5.0
Acid Extractable Cadmium (Cd)		0.48	0.48	0.39	0.10
Acid Extractable Calcium (Ca)	22000.00	30000	69000	57000	50
Acid Extractable Chromium (Cr)	4.5	18	24	18	1.0
Acid Extractable Cobalt (Co)	1.3	6.3	9.3	7.4	0.10
Acid Extractable Copper (Cu)	1.6	20	25	21	0.50
Acid Extractable Iron (Fe)	3900	17000	24000	18000	50
Acid Extractable Lead (Pb)	1.5	19	19	18	1.0
Acid Extractable Magnesium (Mg)	7400	12000	23000	25000	50
Acid Extractable Manganese (Mn)	81	230	500	350	1.0
Acid Extractable Molybdenum (Mo)		0.90	1.6	1.4	0.50
Acid Extractable Nickel (Ni)	2.9	20	29	22	0.50
Acid Extractable Phosphorus (P)	760	550	760	420	50
Acid Extractable Potassium (K)	210	1100	2300	1500	200
Acid Extractable Selenium (Se)			0.79		0.50
Acid Extractable Silver (Ag)					0.20
Acid Extractable Sodium (Na)	69	110	140	140	50
Acid Extractable Strontium (Sr)	21	33	73	50	1.0
Acid Extractable Thallium (Tl)		0.15	0.23	0.18	0.050
Acid Extractable Tin (Sn)		1.7		1.4	1.0
Acid Extractable Uranium (U)	0.27	0.65	1.1	0.95	0.050
Acid Extractable Vanadium (V)	8.8	18	30	24	5.0
Acid Extractable Zinc (Zn)	7.2	77	87	66	5.0
Acid Extractable Mercury (Hg)		0.11	0.31	0.18	0.050

Appendix C. Extended list of polyaromatic hydrocarbons detected by gas chromatography-mass spectrometry in sediment samples (ug/g). Abbreviations: MB: Mitchell's Bay, TC: Trenton Channel, PI: Peche Island, BI: Belle Isle, RDL: reportable detection limit.

	MB	**RDL**	**TC**	**PI**	**BI**	**RDL**
Polyaromatic Hydrocarbons						
Acenaphthene	ND	0.0050	0.022	ND	0.015	0.010
Acenaphthylene	ND	0.0050	0.038	ND	ND	0.010
Anthracene	ND	0.0050	0.084	ND	0.030	0.010
Benzo(a)anthracene	ND	0.0050	0.22	0.034	0.095	0.010
Benzo(a)pyrene	ND	0.0050	0.24	0.038	0.086	0.010
Benzo(b/j)fluoranthene	ND	0.0050	0.34	0.062	0.11	0.010
Benzo(g,h,i)perylene	ND	0.0050	0.20	0.037	0.052	0.010
Benzo(k)fluoranthene	ND	0.0050	0.12	0.019	0.042	0.010
Chrysene	ND	0.0050	0.23	0.042	0.094	0.010
Dibenz(a,h)anthracene	ND	0.0050	0.043	ND	0.012	0.010
Fluoranthene	ND	0.0050	0.45	0.090	0.24	0.010
Fluorene	ND	0.0050	0.037	ND	0.013	0.010
Indeno(1,2,3-cd)pyrene	ND	0.0050	0.19	0.032	0.057	0.010
1-Methylnaphthalene	ND	0.0050	0.018	ND	ND	0.010
2-Methylnaphthalene	ND	0.0050	0.063	0.036	ND	0.010
Naphthalene	ND	0.0050	0.031	ND	ND	0.010
Phenanthrene	ND	0.0050	0.22	0.051	0.11	0.010
Pyrene	ND	0.0050	0.40	0.082	0.25	0.010
Surrogate Recovery (%)						
D10-Anthracene	101		99	102	101	
D14-Terphenyl (FS)	102		97	100	92	
D8-Acenaphthylene	95		95	95	94	

Appendix D. Extended list of polychlorinated biphenyls detected by gas chromatography-mass spectrometry in sediment samples (ug/g). Abbreviations: MB: Mitchell's Bay, TC: Trenton Channel, PI: Peche Island, BI: Belle Isle, RDL: reportable detection limit.

	MB	**RDL**	**TC**	**RDL**	**PI**	**RDL**	**BI**	**RDL**
PCBs								
Aroclor 1016	ND	0.010	ND	0.020	ND	0.030	ND	0.020
Aroclor 1221	ND	0.010	ND	0.020	ND	0.030	ND	0.020
Aroclor 1232	ND	0.010	ND	0.020	ND	0.030	ND	0.020
Aroclor 1242	ND	0.010	ND	0.020	ND	0.030	ND	0.020
Aroclor 1248	ND	0.010	0.047	0.020	ND	0.030	ND	0.020
Aroclor 1254	ND	0.010	ND	0.020	ND	0.030	ND	0.020
Aroclor 1260	ND	0.010	0.024	0.020	ND	0.030	ND	0.020
Aroclor 1262	ND	0.010	ND	0.020	ND	0.030	ND	0.020
Aroclor 1268	ND	0.010	ND	0.020	ND	0.030	ND	0.020
Total PCB	ND	0.010	0.072	0.020	ND	0.030	ND	0.020
Surrogate Recovery (%)								
Decachlorobiphenyl	83		81		81		81	