Cecilia Heuvel ORCID iD: 0000-0003-3999-2919

21-00401

Environmental Toxicology

Influence of Feeding Ecology on Legacy Organochlorine Contaminants in Freshwater Fishes of Lake Erie

Cecilia E. Heuvel[a*], Kenneth G. Drouillard[a], G. Douglas Haffner[a], Yingming Zhao[b], and Aaron T. Fisk[a]

[a] Great Lakes Institute for Environmental Research, University of Windsor, 401 Sunset Ave, Windsor, Ontario N9B 3P4, Canada

[b] Ontario Ministry of Natural Resources and Forestry, Aquatic Research and Monitoring Section, 320 Milo Road, Wheatley, Ontario N0P 2P0, Canada

(Submitted 10 November 2020; Returned for Revision 16 December 2020; Accepted 28 September 2021)

Abstract: Persistent organic pollutants (POPs) in biota are influenced by ecological, physiological, and physicochemical properties, but there is a need for a better understanding

about the interplay of these parameters on POP dynamics and fate. To address this, POPs in three Lake Erie freshwater fishes (freshwater drum, *Aplodinotus grunniens*; walleye, *Sander vitreus*; and white perch, *Morone americana*) with different feeding ecologies were assessed using life history characteristics and three stable isotopes ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$). Lipid normalized POP concentrations were in the range of past studies and were generally similar among the three species when all ages were combined. Principal component analysis (PCA) found the two significant PCs (explaining 59 and 10% of the variation), with all POPs loading significantly onto PC1, indicating a common source of contamination, likely legacy sediment loads. Loadings on both PCs were correlated with POP $\log K_{OW}$. Age, habitat use ($\delta^{13}C$ and $\delta^{34}S$), trophic position ($\delta^{15}N$), and interactions between age and $\delta^{15}N$, age and species, and $\delta^{15}N$ and $\delta^{34}S$ were significant predictors of POP concentration based on PC1 scores, whereas $\delta^{13}C$ and species were significant predictors of PC2 scores. The similar concentrations between the species, yet variation related to the ecology (age and trophic position) across individuals demonstrates the complexity of contaminant dynamics in freshwater fish in a large lake system and the need to consider variation across individuals within species.

Keywords: Stable isotopes, ecology, freshwater fish, contaminants, bioaccumulation, food web

*Address correspondence to heuvelc@uwindsor.ca

Published online XXXX 2021 in Wiley Online Library (www.wileyonlinelibrary.com).

DOI: 10.1002/etc.xxxx

INTRODUCTION

Despite being banned throughout most of the world for several decades, persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), dichloro-diphenyl-trichloroethane (DDT), chlordanes (CLDn), and chlorobenzenes (CLBz) continue to be a source of concern in aquatic systems due to their persistence and toxicity, as well as their propensity to bioaccumulate within food webs, including those of the Laurentian Great Lakes (hereafter Great Lakes; Debruyn and Gobas 2006; MacKay and Arnot 2011). Programs to monitor POP concentrations within compartments (e.g., sediment, biota, etc.) of the Great Lakes have been in place since the mid-1960s (Baumann & Whittle 1988; Marvin et al. 2004). While concentrations have declined over the past fifty years, POPs persist within the environment and result in continued fish consumption advisories for humans (Sadraddini et al. 2011; Kashian et al. 2014). A large body of research has focussed on understanding the bioaccumulation of POPs within food webs and their behaviour throughout the environment (MacKay and Arnot 2011; McLeod et al. 2014). Current knowledge indicates that concentrations of POPs within organisms are strongly influenced by physical-chemical properties (Mackay and Hughes 1984), biological processes (Borgå et al. 2004), and environmental factors (Rasmussen et al. 1990; Paterson et al. 2016).

The octanol-water coefficient (K_{OW}), often logged, is a measure of chemical hydrophobicity and is a strong predictor of how contaminants partition through aquatic food webs, and characterizes the hydrophobicity of the chemical (Hawker and Connell 1988). Since many POPs are hydrophobic, the partitioning of POPs into fish tissues is sensitive to

lipid content such that fatty fish and tissues have a higher potential to achieve greater POP concentrations (Stow et al. 1997). For this reason, POP concentrations are often lipid-normalized to make comparisons of relative differences in chemical potential achieved among species and individuals without the confounding effect of varying lipid content (Kainz and Fisk 2009). POPs with log K_{OW} > 6.5 are almost entirely accumulated from dietary sources in fish, with little to no elimination throughout an individual's lifespan, meaning that any differences in concentrations between individuals or species should be the result of differing feeding ecology (e.g. trophic position, resource use; McLeod et al. 2015; Paterson et al. 2016). Diet is a key exposure pathway for lower logK_{ow} POPs (< 6.5) in aquatic organisms as well but accumulation from water also plays a role (Fisk et al. 1998).

Beyond the influence of the chemical-physical properties, concentrations of POPs in aquatic organisms are influenced by the ecology and physiology of the organism, including age, metabolism (Pastor et al. 1996; Borgå et al. 2004), growth, and foraging behaviour (e.g. diet) that influence both daily chemical exposures and accumulation of chemical over time (Borgå et al. 2004). There is a positive correlation between concentration and trophic position for hydrophobic POPs (log $K_{ow} \approx 5$ or greater), driven by biomagnification, which is the accumulation of higher concentrations of POPs in predators relative to their prey (McIntyre and Beauchamp 2007). This relationship allows trophic position to act as a general predictor of POP concentration (Kidd et al. 2001; Kelly et al. 2007). However, fish occupying similar trophic positions but utilizing different habitats or foraging strategies can have different POP concentrations. For example, species that utilize benthic rather than pelagic resources tend to have higher POP concentrations (Hebert and Haffner 1991; Borgå et al. 2004). In place of collecting stomach contents to estimate diet and quantify POP

exposure of an organism, trophic position and habitat use can be characterized through the stable isotopes of nitrogen ($\delta^{15}N$), carbon ($\delta^{13}C$) and sulphur ($\delta^{34}S$; Colborne et al., 2016; Croisetière et al., 2009; Newsome et al., 2007). Stable isotopes of nitrogen ($\delta^{15}N$) increase with trophic position, while $\delta^{13}C$ and $\delta^{34}S$ both reflect habitat usage based on diet (Croisetière et al. 2009; Colborne et al. 2016). Carbon ($\delta^{13}C$) values are often higher in littoral or nearshore habitat and decrease with distance from shore (Newsome et al. 2007), whereas $\delta^{34}S$ tends to be lower in pelagic habitat than benthic (Croisetière et al. 2009). Thus, stable isotopes provide a set of tracers that are expected to reflect differences in POP concentrations in diet items consumed by different species and individuals within a species (Fisk et al. 2002; Paterson et al. 2016).

Body size is also an important factor that influences POP concentrations in fish (Borgå et al. 2004). Elimination of POPs is diminished in larger bodied fish because their surface area to volume ratio are reduced, resulting in greater accumulation and higher concentrations (Thomann 1989). Body size is correlated to age and therefore relates to the total time of chemical exposures and ability to approach or achieve steady state. At the same time, body size relates to a fish's growth rate as the rate of growth decreases as they approach their maximum size (Paterson, et al. 2007). Since fish are gape limited predators, larger fish generally eat at higher trophic positions and show a tendency to be omnivorous than smaller fish, which may confound or exacerbate the impact of body size on POP concentrations through the process of biomagnification (Borgå et al. 2004; Paterson, et al. 2007).

Lake Erie is a large (25,700 km^2), dynamic and complex ecosystem that is home to over 130 fish species (Hartman 1972; International Joint Commission 2014). Sediment POP concentrations follow a decreasing gradient of concentration from west to east throughout the

lake, but are relatively homogenous within each basin (Oliver and Bourbonniere 1985). Within the lake, freshwater drum (*Aplodinotus grunniens*), walleye (*Sander vitreus*), and white perch (*Morone amerciana*) are all common, widespread, and large bodied (average total length ≥ 200 mm) species with previously observed differences in their feeding ecologies (Bur 1982; Schaeffer and Margraf 1986; Galarowicz et al. 2006).

Freshwater drum larvae and young-of the-year (YOY) typically feed on zooplankton but quickly transition to benthic invertebrates as juveniles (<150 mm total length; Bur 1982). At ages 1-5 (150mm-350mm in total length), freshwater drum generally begin to exploit more pelagic prey, after which they revert to a benthos-based diet for the remainder of their lives (6+ years; Bur, 1982; French and Bur, 1996; Gopalan et al., 1998; Morrison et al., 1997). As YOY until age 3 (total lengths < 200 mm), white perch are generalist omnivores with a preference for zooplankton, after which they begin to become more piscivorous (Schaeffer & Margraf, 1986a; Scott & Crossman, 1998; Stanley & Danie, 1983). Walleye feed on a mixture of zooplankton, benthic invertebrates, and fish until they reach total lengths of ~80mm, after which walleye will transition to a diet consisting solely of fish by the time they are 1 year old (total length ~200mm; Galarowicz et al., 2006; Hartman and Margraf, 1992; Mittelbach and Persson, 1998).

To understand the relative roles of trophic position, habitat use and fish size on POP concentrations, a direct comparison of species with different feeding ecologies in the same system is needed. We used three species of fish that achieve a similar adult size but with different feeding strategies within the western basin of Lake Erie to quantify the relative importance of differing species ecology and chemical hydrophobicity on concentrations of PCBs, DDT, CLDn, and CLBz. The Great Lakes, and particularly Lake Erie, provide a good

system for this type of study, due to the diversity of species, habitats, and contaminants. Trophic position was estimated using $\delta^{15}N$ and habitat use was traced using $\delta^{13}C$ and $\delta^{34}S$; $\delta^{34}S$, a tracer used most often in marine ecosystems but with increasing use in freshwater (Colborne et al. 2016; Heuvel et al. 2019), has been used to examine Hg bioaccumulation but not POPs (Ofukany et al. 2014; Clayden et al. 2017; Willacker et al. 2017). The three species included: a benthivore, freshwater drum (Bur, 1982, 1984); a piscivore, walleye (Mittelbach & Persson, 1998; Scott & Crossman, 1998); and a trophic omnivore, white perch (Stanley & Danie, 1983; Guzzo *et al.*, 2013). Assessment of the contaminant dynamics of these species is lacking, particularly for freshwater drum and white perch, and no research has been conducted to look specifically at how legacy POP concentrations varies with body size of these species.

MATERIALS AND METHODS

Sample collection

Freshwater drum, walleye, and white perch across a range of sizes were collected from the western basin of Lake Erie during interagency trawls conducted by the Ohio Department of Natural Resources (ODNR), Ohio United States Geological Survey (Ohio USGS), and Ontario Ministry of Natural Resources and Forestry (OMNRF) in 2016. Samples were put on ice and transported back to the Great Lakes Institute for Environmental Research (GLIER) at the University of Windsor and stored at -20°C in food-grade plastic bags until processed for stable isotope (muscle only) and contaminant analysis. Otoliths harvested from each individual during dissections were cleaned of any adhering tissue and stored dry until they were used to age each fish used in the analysis of this study. The total length and weight of

each fish was measured, and a muscle tissue sample was taken from just below the dorsal fin for stable isotope analysis. For POP analysis, if the fish exceeded a total length of 200mm, the entire fish was chopped into large pieces and homogenized in an industrial meat grinder which was cleaned and rinsed with acetone and hexane between every fish. Fish that had lengths less than 200mm were homogenized in a blender.

POP extraction and quantification

Samples were analyzed to determine concentrations of organochlorine contaminants using the micro-extraction and Florisil clean-up process as described in Daley, et al. (2009). All glassware was cleaned with soap and water and rinsed with hexane and acetone prior to being used in the microextraction and Florisil clean-up procedure. Approximately 1g of homogenized sample was ground in ~10g of anhydrous sodium sulphate in a glass mortar and pestle to dehydrate the tissue, and wet packed into a 30 mL mini-column pre-packed with glass wool, ~2cm anhydrous Na_2SO_4, and 15mL of a 1:1 Hexane(Hex)-Dichloromethane(DCM) (*v*/*v*) extraction mixture. Mortar and pestle were rinsed with 10mL of Hex:DCM that was added to the mini-columns, and spiked with extraction performance recovery standards (35ng of a PCB 34 and 50ng of a BDE-71 [2,3',4',6-Tetrabromodiphenyl ether]). The mini-columns were drained after 2 h, and the eluate transferred to 125mL flat bottom flasks. Approximately 5 mL of isooctane was added to the eluate and evaporated under vacuum to approximately 5 mL. Samples were diluted to 10mL with hexane, and 1mL was used to determine lipid content gravimetrically. Samples were transferred back into flat-bottom flasks and evaporated under vacuum to ~2mL.

For clean-up and fractionation, samples were transferred to glass columns packed with 6g of activated Florisil and 2cm of Na_2SO_4. The first fraction was collected using 50mL of hexane and the second fraction was collected using 50mL of a 15% DCM in hexane (v/v) solution. Samples were evaporated under vacuum to < 1 mL and diluted to a final volume of 1mL with isooctane. All samples were analyzed on an Agilent 6890 series gas chromatography system (Santa Clara, California, USA) equipped with a gas chromatography-electron capture detector (GC-ECD) at the GLIER Chemical Exposure and Bioavailability Laboratory (University of Windsor, Windsor, ON). Concentrations of 41 PCB congeners (see Table 1 for complete list), 1,2,4,5-tetrachlorobenzene (TCB-1), 1,2,3,4-tetrachlorobenzene (TCB-2), hexachlorobenzene (HCB), pentachlorobenzene (QCB), oxychlordane, trans-chlordane, cis-chlordane, trans-nonachlor, cis-nonachlor, p,p'-DDE, p,p'DDD, and p,p'DDT were quantified. Minimum detection levels (MDL) ranged between 0.003 and 0.038 μg/Kg w.w. for all the targeted chemicals.

Every set of 6 samples was randomly selected to prevent bias in concentrations and was extracted simultaneously with a method blank and in-house reference tissue homogenate of Detroit River carp for quality assurance. All POP concentrations quantified in the reference tissue within this study were in compliance with GLIER's organic analytical laboratory quality assurance guidelines (mean ± 2 standard deviations (SD)). Recoveries of the internal standards was 91 ± 1% (mean ± standard error, n = 86) for PCB 34 and 95 ± 1% for BDE-71, and sample concentrations were not recovery corrected.

Age Determination

Otoliths were aged according to the procedure used by the OMNRF Lake Erie Management Unit (LEMU; C. Benoit, personal communications, Feb. 13 2019). Otoliths were cracked along their nucleus and passed through a pure alcohol flame to increase contrast between summer growth and denser winter growth. Open otolith halves were mounted in putty under a dissecting microscope, coated with a light emersion oil, and annular rings were counted under transmitting light and backlit using a fibre optic light pipe. Ages for all otoliths were estimated based on counts of pairs of growth bands (one translucent and one opaque for each year) and were only assigned after agreement on multiple reads.

Otoliths were not available for five walleye, therefore ages for those individuals was estimated from a length-age database of 1600 walleye collected in 2016 by the OMNRF (Y. Zhao, personal communication, Feb. 6 2019). Ages estimated for four walleye were all less than 2 years, a period of life when age classes are relatively distinct by size. One walleye was removed from further analysis because its age was difficult to estimate accurately given its length (530mm) which meant it could fit into a wide range of potential age classes (3 – 7 years).

Stable isotope analysis: sulphur, carbon, and nitrogen

Lyophilized (-48°C and 133×10^3mbar for 48 hours) muscle samples were powdered using a mortar and pestle or dissection scissors. Samples for sulphur ($\delta^{34}S$) were weighed into tin cups for a final mass 5000-6000µg analyzed on a Delta V Plus Thermoscientific Continuous Flow Mass Spectrometer (Thermo Scientific, Bremen, Germany) coupled to a

4010 Elemental Combustion System (Costech Instruments, Valencia, CA, USA) in the GLIER Chemical Tracers Laboratory.

Lipids were extracted prior to $\delta^{13}C$ and $\delta^{15}N$ analysis, using a 2:1 chloroform:methanol mixture (as described by Nawrocki *et al.*, 2016) to remove the bias they can create in $\delta^{13}C$ compared to pure protein (Fry *et al.*, 2003; Boecklen *et al.*, 2011). Briefly, 1.9 mL of a 2:1 chloroform:methanol mixture was added to a ~0.5g subsample of muscle tissue, and placed in a 30°C water bath for 24 hours. Samples were then centrifuged for 4-6 minutes before the 2:1 chloroform:methanol mixture was poured off, and another 1.9 mL of the mixture added. Finally, samples were vortexed for 10 sec, and centrifuged for 4-6 minutes before the chloroform:methanol mixture was removed, air-dried and re-homogenized.

Lipid extracted samples were weighed into tin cups at a mass between 400 and 600µg for $\delta^{13}C$ and $\delta^{15}N$ analysis. Carbon and nitrogen isotopic composition were determined by a Delta V Advantage Thermoscientific Continuous Flow Mass Spectrometer (Thermo Scientific, Bremen, Germany) coupled to a 4010 Elemental Combustion System (Costech Instruments, Valencia, CA, USA) in the Chemical Tracer Lab at GLIER (University of Windsor, Windsor, ON). Instrument accuracy, as measured by NIST standards run during the analysis of samples during this study had values for $\delta^{34}S$ within 0.3‰ (NIST 8554, NIST 8555, and NIST 8529) of certified values, within 0.1‰ (NIST 8573 and NIST 8574) and 0.2‰ (NIST 8547) for $\delta^{15}N$, and within 0.1‰ (NIST 8573, 8542, and 8544) for $\delta^{13}C$. Four laboratory standards (NIST 1577c, tilapia muscle, USGS 40 and Urea (n = 104 for each)), run every 12 samples, had a precision of 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$, and five internal laboratory standards (NIST 1577c, NIST 8529, NIST 8555, tilapia muscle, and USGS 42; n = 18 for each), run every 10 samples, had a precision of $\leq$ 0.3‰ for $\delta^{34}S$. Precision as indicated

by duplicate ($\delta^{34}S$) and triplicate ($\delta^{13}C$ and $\delta^{15}N$) was within ±0.1‰ (n = 83 for $\delta^{13}C$ and $\delta^{15}N$; n = 12 for $\delta^{34}S$) for all isotopes.

Statistical analysis

To account for variation in diet and behaviour that may result from ontogenetic diet shifts with increasing size and age, fish were grouped into 2 or 3 age classes depending on the species and the specific life history traits described above. Freshwater drum were grouped into 3 age classes consisting of a young-of-of-the-year (YOY), Age 1-5, and Age 6+; walleye formed two age classes consisting of a YOY and Age 1+ groups; and white perch were grouped into an Age 0-3 and Age 4+ age classes. These age classes were used to conduct pair-wise comparisons (Kruskal-Wallis test) of $\delta^{13}C$, $\delta^{34}S$, and $\delta^{15}N$ for all individuals and explore differences in resource use among and within species. Dunn's test was used as a post-hoc test to determine which groups had significantly different stable isotope values.

Any POPs with detection rates in samples that were less than 60% were eliminated, which included: 1,2,3,4 – tetrachlorobenzene, 1,2,4,5 – tetrachlorobenzene, pentachlorobenzene, α – hexochlorocyclohexane, β – hexochlorocyclohexane, γ – hexochlorocyclohexane, DDT, mirex, OCS, PCB 17/18, PCB 31/28, PCB169, and PCB 205. These POPs were excluded from further statistical analysis for two reasons: 1) their samples sizes were too small to be representative of all three species (i.e., the majority of detections for each POP was almost solely detected in a single species), and 2) manufacturing such a large amount of data would bias the results of the statistical analyses used (e.g. Principal Component Analysis, general linear regression). Lipid normalized means and standard error (SE) of each of these POPs is included in Table 1 where appropriate, and the distribution of

these concentrations within the sample population is discussed below. POPs that met the criterion of 60% detection were used for further statistical analysis and included hexachlorobenzene (HCBz), 5 chlordanes, 2 DDT metabolites (p,p'DDE, p,p'DDD), and 31 PCB congeners. Prior to statistical analysis, POPs that met the inclusion criteria but were not detected in a sample were replaced with randomly generated concentrations based on a log-normal distribution between 0 and the minimum detection level for that POP.

The relative concentration of individual PCB congeners ($[PCB_i]/[\Sigma PCB]*100$) were compared between species using bar graphs (mean ± SE) to assess POP patterns in fish.

All POP concentrations were lipid normalized to account for the variation caused by varying lipid contents among individuals and species. POP data was lognormally distributed so all concentrations were natural log-transformed to achieve a normal distribution (Shapiro-Wilks test: $p > 0.05$). Due to the large number of individual POPs and the correlations that are commonly found between them (Koslowski et al. 1994; Liu et al. 2010), a Principal Components Analysis (PCA) was carried out on the lipid normalized concentration data of all POPs to reduce the number of dependent variables (i.e. POPs; King and Jackson, 1999; Liu et al., 2010). This approach is common with POP datasets to ensure statistical power (Liu et al. 2010), and also provides insights on the patterns of POPs between the study species and variables. Significant Principal Components (PCs) were identified using the broken stick method (King and Jackson 1999; Peres-Neto et al. 2003), which considers a principal component statistically significant if the variance explained by a particular component is greater than its expected proportion of variance (b_k) in that component; this was calculated using the equation:

$$b_k = \frac{1}{k}\sum_{i=k}^{p}\frac{1}{i} \quad \text{(Eq. 1)},$$

where p is the number of components and k is the kth component.

Any individual POP that had a ranked squared loading > b_k was considered significant for that PC (Peres-Neto et al. 2003). Linear regressions were run on the significant loadings from each significant PC and each POP's corresponding $\log K_{OW}$ to assess the extent of the influence chemical properties had on POP concentrations.

Stepwise regression was conducted on the scores from PC1 and PC2 to parameterize initial generalized linear models (GLM) that selected the most influential variables explaining lipid-normalized concentrations of POPs measured in this study. Independent variables included in the regression were $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, age, species, and two-way interactions. A correlation analysis of all the variables indicated that total length and age were highly correlated (Pearson's correlation coefficient = 0.65), so total length was removed for consideration in the GLM as result because age was considered a better indicator of length of exposure. Each model was further refined by removing statistically non-significant interactions or main effect terms, one at a time until all terms included within the model were either significant or included in an interaction. All model iterations were compared, and the best model was considered to be the one that had the lowest Akaike information criterion (AIC), or if similar, the model with the fewest terms.

Residuals of each model (GLMs and linear regression models) were checked against the assumptions of normality through leverage, residual vs fitted plots, scale-location, and qq

plots; no model violated regression assumptions. All statistical analyses were conducted in R version 3.5.2).

RESULTS

Biological variables

A total of 63 fish ranging in age from < 1 to 46 years old were collected. Freshwater drum had the largest mean age (9.3 ± 2.2 years, mean ± SE,), while walleye had intermediate ages (4.2 ± 0.9), and white perch were the youngest (2.4 ± 0.5 years; Table 1).

Pairwise comparisons of lipid contents and stable isotopes of the three species indicated significant differences among species for all variables (Kruskal-Wallis test; lipid content: $\chi^2_{(2)} = 6.1$, $p = 0.05$; $\delta^{13}C$: $\chi^2_{(2)} = 8.3$, $p = 0.02$; $\delta^{34}S$: $\chi^2_{(2)} = 17.3$, $p < 0.001$; $\delta^{15}N$: $\chi^2_{(2)} = 18.5$, $p < 0.001$; Table 1). Post hoc tests (Dunn's test) revealed that lipid content was lower in walleye than white perch ($p = 0.01$), but all other species combinations had similar lipid contents ($p > 0.04$). Freshwater drum $\delta^{13}C$ did not differ from either of the other two species ($p > 0.03$), but walleye had lower $\delta^{13}C$ than white perch ($p = 0.002$). Walleye $\delta^{34}S$ was higher than both freshwater drum ($p < 0.001$) and white perch ($p = 0.02$) which were similar ($p = 0.07$). Trophic position as measured by $\delta^{15}N$ was lower in freshwater drum than both walleye ($p < 0.001$) and white perch ($p = 0.02$).

Within species, pairwise comparisons showed no significant differences between age classes for any variable except $\delta^{15}N$ (Lipid content: $\chi^2 < 3.1$, $p > 0.2$; $\delta^{13}C$: $\chi^2 < 3.1$, $p > 0.2$; $\delta^{34}S$: $\chi^2 < 2.3$, $p > 0.2$). Trophic position ($\delta^{15}N$) had significant differences between age groups in freshwater drum ($\chi^2_{(2)} = 9.4$, $p = 0.01$) but not walleye ($\chi^2_{(1)} = 0.9$, $p = 0.3$) or white

perch ($\chi^2_{(1)}$ = 3.75, p = 0.05). YOY freshwater drum had lower $\delta^{15}N$ than either of the age classes (p < 0.01 for both; Table 1).

Common baseline species (benthic invertebrates, seston, Dreissenidae spp.) and prey fish showed significant ranges in stable isotopes within the study system ($\delta^{13}C$: -29.7 to -20.8‰; $\delta^{15}N$: 7.2 to 16.2‰; $\delta^{34}S$: -1.4 to 5.4‰), and encompassed the values measured in the study species, indicating that the fish were likely feeding within the study system (*data not shown*).

Composition of POPs:

Sum PCB concentrations exceeded all other POPs including ΣDDT, ΣCLDn, and ΣCLBz (ΣPCB, all samples, 6560.9 ± 779.6 μg/Kg lipid). Percent composition of ΣPCB for the majority of PCB congeners did not differ significantly among species (Figure 1). Overall, 7 PCB congeners (IUPAC #: 101, 110, 138, 149, 153, 180, and 187) of the 41 measured accounted for ~60% of the ΣPCB in all individuals (Table 1, Figure 1).

Chlorobenzenes had the lowest concentrations (ΣCLBz, 24.3 ± 3.0 μg/Kg lipid), and HCB accounted for 99% or more of ΣCLBz in all species (Table 1). ΣCLDn had mean concentrations of 138.9 ± 15.8 μg/kg lipid and trans-nonachlor accounted for 50% of the mean ΣCLDn concentration in all species (Table 1). Over 70% of the proportion of ΣDDT was p,p'DDE (480.6 ± 56.5 μg/Kg lipid)., with p,p'DDD making up the bulk of the remaining concentration; p,p'DDT was only detected in small quantities in 33% of individuals measured and predominantly in walleye and freshwater drum that were older than 4 years of age (Table 1).

Principal component analyses

Both PC1 and PC2 were significant in the PCA and explained 59.2% and 10.3% of the variance within the data respectively (Figure 2). All POPs loaded significantly onto PC1, indicating a general index of POP contamination which was used to assess the general contributions of environmental data on POP concentrations (Taylor et al. 1991). On PC2, all POP's loaded significantly except for p,p'DDD, cis-nonachlor, PCB 52, 180 and 177. Loadings on both significant PCs were strongly correlated with chemical log K_{OW} (linear regression; PC1: $R^2 = 0.40$, $F_{1,36} = 25.6$, $p < 0.001$; PC2: $R^2 = 0.71$, $F_{1,31} = 80.8$, $p < 0.001$; Figure 3). Young-of-the-year walleye and age 1-5 freshwater drum showed the most variation in scores, while the other age classes generally showed less variation (Figure 2). Young-of the year freshwater drum and age 0-3 white perch showed very little overlap in PCA scores (Figure 2).

General Linear Models (GLMs)

The general linear model performed on scores from PC1 that performed best indicated that POP concentrations were significantly related to $\delta^{34}S$, age, $\delta^{15}N$, and $\delta^{13}C$ after interactions between age and $\delta^{15}N$, age and species, and $\delta^{15}N$ and $\delta^{34}S$ were taken into account (GLM: Adj. $R^2 = 0.40$, $F_{10,51} = 5.11$, $p < 0.001$; Table 2, Figure 4A). A GLM conducted on log-transformed lipid-normalized PCB 153 concentrations had the same results as the GLM on PC1 scores (*data not shown*), indicating that the GLM accurately reflects POP dynamics within the system for these three species.

The best fitting model for PC2 indicated that the variation explained by this PC was driven largely by $\delta^{13}C$, species after the interaction between the two variables was accounted for (Adj $R^2 = 0.21$, $F_{5,56} = 4.19$, $p = 0.003$; Table 3, Figure 4B).

DISCUSSION

Concentrations and patterns of POPs in three freshwater fish species were similar in Lake Erie, despite differences in stable isotopes and ages. These POP concentrations and patterns are similar to other fish species from this lake (De Vault et al. 1996b; Morrison et al. 1998; Carlson et al. 2010; Sadraddini et al. 2011), which has a history of high loadings from industry and agriculture sources (Oliver and Bourbonniere 1985). When all individuals were combined, several ecological, including trophic position and habitat use based on $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$, and biological, age, factors were found to significantly influence POP concentrations. Given this, the lack of difference in POP concentration between the species may be the result of higher trophic positions in the walleye and white perch matched by the older ages of the freshwater drum. Food web magnification, or biomagnification, and age are established drivers of concentrations in fish (Borgå et al. 2004). Stronger loadings for more hydrophobic POPs in the principal component analysis support what is known about POP uptake by organisms within food webs. This study demonstrates the importance of considering ecology and biological factors and variation within species when assessing POPs in freshwater fish.

POP patterns were similar among and within species and may be a result of chemical and physical characteristics of the western basin of Lake Erie. This basin is shallow and well mixed, meaning that exposure to POPs among species and individuals is likely similar and

that differences we might expect to see due to variation in habitat utilisation are minimal (Oliver and Bourbonniere 1985; Heuvel et al. 2019). Indeed, the relative composition of ΣPCB was similar within and among species and PC1 showed concentrations of all POPs were strongly correlated with each other, indicating a common source of exposure for all individuals (Taylor et al. 1991), as fish generally have limited biotransformation capacity to change the relative proportions of POPs (Kwon et al. 2006). POP sources are likely a combination of the Detroit River, which has deposited significant amounts of contaminated sediments within the western basin, and legacy contamination from industry in the mid to late 20th century (Oliver and Bourbonniere 1985). Within Lake Erie, sediment/water fugacity ratios are on the order of 10 (Gewurtz and Diamond 2003; Debruyn and Gobas 2006) owing to sediments acting as the main repository of legacy loadings to the system and sediments presently reverting from a sink to source of POPs.

Differences in POP hydrophobicity as measured by log K_{OW} had an impact on POP concentrations and patterns within individuals but this was not affected by species. Hydrophobicity can be an important driver of bioaccumulation for these POPs as implied by the dependence of tissue lipid content on bioaccumulation potential (Fisk et al. 1998; Paterson et al. 2007). Although all the POPs loaded onto PC1, regardless of their log K_{OW}, the negative correlation between PC1 POP loadings with log K_{OW} implies hydrophobicity does influence dynamic and that non-steady state bioaccumulation kinetics predominate (Paterson et al. 2007). This suggests that there has been insufficient exposure time across the age range of fish sampled to resolve differences in bioaccumulation potential as should be evident if steady state had been achieved (Paterson et al. 2007).

Contaminant concentrations were similar for all species based on the results from the GLM, despite different reported feeding ecology and habitat use from literature (Bur 1982; Schaeffer and Margraf 1986; Mittelbach and Persson 1998), and also variation found here for trophic position and habitat use based on stable isotope. As fish have been shown to have limited to no capacity for biotransformation of the POPs measured in this study (Kwon et al. 2006), variation in feeding ecology of individuals was more important than differences between species in explaining differences in POP concentrations. Indeed, freshwater drum and white perch both undergo periods of time in which they are piscivorous like walleye, while also consuming a variety of other prey types (Bur 1982; Schaeffer and Margraf 1986; Heuvel et al. 2019). Additionally, due to the shallow depth (~7m) of Lake Erie's western basin, divisions between different habitats (and prey with different POP loads) are potentially not as distinct as they are in deeper regions of Lake Erie (Schindler and Scheuerell 2002).

Age, trophic position ($\delta^{15}N$), and habitat use ($\delta^{13}C$, $\delta^{34}S$), were all significant predictors of POP logged lipid normalized concentrations after interactions between age and $\delta^{15}N$, age and species, and $\delta^{15}N$ and $\delta^{34}S$ had been accounted for, demonstrating the complexity of processes driving POP concentrations in fish. While each variable on its own has an influence on POP concentrations, none of them are completely independent of each other and are all correlated to some degree (Borgå et al. 2004), as seen in this study with the inclusion of several interaction terms in the best fit model. All three variables were equally important in predicting logged lipid normalized concentrations as indicated by their similar t-values in the GLM.

Age played an important role in contaminant dynamics within this study, corroborating past research that the length of exposure time to POPs is one of the main drivers of

increasing concentrations. This is seen in the presence of age as a significant main effect in the GLM as well as in two of the three interaction terms. Species and differences in life history characteristics (e.g. growth rate) can also play a role in determining the importance of age as a predicting variable of POP concentrations as seen here in the presence of an interaction between age and species (Borgå et al. 2005). Indeed, lipid normalized POP concentrations for white perch and walleye both showed a correlation with age (Pearson's correlation coefficient) and logged lipid normalized PCB 153 concentrations in this study.

Trophic position as measured by $\delta^{15}N$ demonstrates the complexity of teasing apart the influences different factors have on POP concentrations as $\delta^{15}N$ was present in two of the three interactions in the final GLM model. Trophic position typically increases with age, body size, and gape width in fish, thus it can be difficult to tease apart the direct contribution of $\delta^{15}N$ to POP concentrations due to its interactions with other variables (Borgå et al. 2004). The cause for the interaction between $\delta^{15}N$ and $\delta^{34}S$ within the GLM is less clear, although it could be attributed to different processes of each nutrient cycle dominating under different conditions (e.g. anoxic vs. oxic; (Peterson and Howarth 1987; Leggett et al. 2000). Overall, $\delta^{15}N$ was positively correlated with POP concentrations when all other variables and interactions were taken into account, which is consistent with a large body of research within the Great Lakes and globally (Cabana and Rasmussen 1994; Kiriluk et al. 1995; Lopes et al. 2011). These results show that teasing apart the main effects of variables on POP concentrations can be difficult due to the interactions and collinearity of said variables.

Habitat use as measured by $\delta^{13}C$ of individual fish was found to be an important factor driving POP concentrations, although given the similarity of $\delta^{13}C$ among species, likely driven by the homogeneity of the study system, this is likely more determined by individual

diet rather than differences in feeding ecology among species. The trend of higher POP concentration with high $\delta^{13}C$ (littoral or benthic resources) and lower concentrations with low $\delta^{13}C$ (pelagic resources) is consistent with a study on cyprinids in the St Clair and Detroit River, where differences in lipid normalized concentrations of POPs in philopatric species were related to using different habitats (benthic vs. pelagic) when the contaminant had log $K_{OW} > 5.6$ (Hebert and Haffner 1991; McLeod et al. 2014). Similar POP concentration patterns among species within this study are probably related to a lack of variation in habitat $\delta^{13}C$ as observed in benthic invertebrates, seston, and prey fish (*data not shown*) caused by mixing within the western basin and the commonality of sediment/water fugacity ratios exceeding equilibrium predictions for legacy contaminants as discussed above.

This study shows that sulphur ($\delta^{34}S$) is potentially a useful predictor of legacy POP concentrations in freshwater ecosystems, and highlights the need to understand $\delta^{34}S$ dynamics within freshwater ecosystems better. Interest in $\delta^{34}S$ as a tracer of habitat use in freshwater food web dynamics has only recently developed (Colborne et al. 2016; Heuvel et al. 2019), and has only been applied in a couple of studies looking at mercury trends in aquatic biota (Ofukany et al. 2014; Willacker et al. 2017) and never for POPs. Here, the link between $\delta^{34}S$ and POP concentration is not clear as dynamics driving differentiation of $\delta^{34}S$ in freshwater ecosystems are not yet well understood, but is likely related to differences in sulphur cycling throughout different habitats (Peterson and Howarth 1987; Croisetière et al. 2009; Colborne et al. 2016)

The concentrations of POPs observed in the three species, walleye, white perch and freshwater drum, are similar to other fish species reported for the western basin of Lake Erie

in the 1980s and 1990s (Baumann and Whittle 1988; Koslowski et al. 1994; De Vault et al. 1996; Russell et al. 1999; Morrison et al. 2002). This is consistent with temporal trends of POP concentrations in fish in the Great Lakes which have found that the rate of decline in concentration has slowed or stopped for many POPs in the past few decades (De Vault et al. 1996; Morrison, Frank A.P.C. Gobas, et al. 1998; Carlson et al. 2010; Sadraddini et al. 2011). Morrison et al., (2002) found similar lipid normalized PCB 138 concentrations in walleye collected in western Lake Erie during 1994 and 1995 (~750 μg/kg lipid) to those here (802.6 μg/kg lipid). De Vault et al., (1996) observed higher concentrations of ΣDDT, oxychlordane, cis-chlordane, trans-chlordane, cis- and trans-nonachlor in Lake Erie walleye, but also observed significant decreases in the concentrations of the same chemicals over a twenty-year time period. Concentrations of wet weight ΣPCB for freshwater drum in this study (93.4 ± 14.9 ug/Kg w.w.) were half those reported by Sadraddini et al. (2011; 236.2 ± 290.4 ug/Kg w.w.) whose values were averaged over a 30-year period, but are likely comparable considering the large standard deviation observed in their study. White perch lipid normalized POP concentrations (p',p-DDE, HCB, PCB 52, 87, 101,138, 153 and 180) from the nearby Detroit River in the 1980s were higher than those in our study by 200-400 μg/kg lipid (Russell et al. 1999), consistent with higher concentrations in this system.

CONCLUSIONS

Despite overall similar concentrations and patterns of POPs between species, we found that POP concentrations and patterns of individuals of three species of freshwater fish with different feeding strategies is governed by similar environmental, biological and physiochemical factors. Age, habitat use ($\delta^{13}C$, $\delta^{34}S$), and trophic position ($\delta^{15}N$) all played

important roles in influencing bioaccumulation, but interactions between these variables highlight the complexity of the mechanisms driving POP uptake and elimination.

Supporting Information—The Supporting Information are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

Acknowledgment—The present study was funded by NSERC Discovery awarded to A.T. Fisk. We would like to thank K. Johnston and the Chemical Tracers Lab for running samples for stable isotope analysis, and N. Ismail and the Chemical Exposure and Bioavailability Lab for assistance with the contaminant analysis. Thanks also to the contributions of the Ohio Department of Natural Resources, Ontario Ministry of Natural Resources, and U.S. Geological survey for their assistance collecting samples. Finally we would like to thank three anonymous reviewers for their comments which helped improve this manuscript.

Disclaimer—The data and interpretations disclosed within are the views of the authors and not influenced by any affiliation with industry.

Data availability statement—Data, associated metadata, and calculation tools are available from the corresponding author (heuvelc@uwindsor.ca).

Author contributions statement—D.H., A.F., Y.Z., and C.H. conceived and designed the study. C.H. collected the samples, performed the chemical and statistical analysis, and wrote the manuscript. K.D. and A.F. assisted with statistical analysis and interpretation, and assisted with writing the manuscript. Y.Z. and D.H. assisted with sample collections, and provided editorial assistance.

REFERENCES

Baumann PC, Whittle MD. 1988. The status of selected organics in the Laurentian Great Lakes: An overview of DDT, PCBs, dioxins, furans, and aromatic hydrocarbons. Aquat Toxicol. 11(3–4):241–257. doi:10.1016/0166-445X(88)90077-X.

Borgå K, Fisk AT, Hargrave B, Hoekstra PF, Swackhamer D, Muir DCG. 2005. Bioaccumulation factors for PCBs revisited. Environ Sci Technol. 39(12):4523–4532. doi:10.1021/es050376i.

Borgå K, Fisk AT, Hoekstra PEF, Muir DCG, Borgå Katrine, Fisk AT, Hoekstra PEF, Muir DCG. 2004. Biological and chemical factors of importance in the bioaccumulation and trophic transfer of persistent organochlorine contaminants in Arctic marine food webs. Environ Toxicol Chem. 23(10):2367–2385. doi:10.1897/03-518.

Bur MT. 1982. Food of freshwater drum in western Lake Erie. J Great Lakes Res. 8(4):672–675. doi:10.1016/S0380-1330(82)72007-6.

Cabana G, Rasmussen JB. 1994. Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. Lett to Nat. 372(6503):255–257. doi:10.1038/372255a0.

Carlson DL, De Vault DS, Swackhamer DL. 2010. On the rate of decline of persistent organic contaminants in lake trout (Salvelinus namaycush) from the great lakes, 1970-2003. Environ Sci Technol. 44(6):2004–2010. doi:10.1021/es903191u.

Clayden MG, Lescord GL, Kidd KA, Wang X, Muir DCG, O'Driscoll NJ. 2017. Using sulfur stable isotopes to assess mercury bioaccumulation and biomagnification in temperate lake food webs. Environ Toxicol Chem. 36(3):661–670. doi:10.1002/etc.3615.

Colborne SF, Rush SA, Paterson G, Johnson TB, Lantry BF, Fisk AT. 2016. Estimates of lake trout (*Salvelinus namaycush*) diet in Lake Ontario using two and three isotope mixing models. J Great Lakes Res. 42(3):695–702. doi:10.1016/j.jglr.2016.03.010.

Croisetière L, Hare L, Tessier A, Cabana G. 2009. Sulphur stable isotopes can distinguish trophic dependence on sediments and plankton in boreal lakes. Freshw Biol. 54(5):1006–1015. doi:10.1111/j.1365-2427.2008.02147.x.

Debruyn AMH, Gobas FAPC. 2006. A bioenergetic biomagnification model for the animal kingdom. Environ Sci Technol. 40(5):1581–1587. doi:10.1021/es051800i.

Fisk AT, Norstrom RJ, Cymbalisty CD, Muir DCG. 1998. Dietary accumulation and depuration of hydrophobic organochlorine: Bioaccumulation paramters and their relationship with the octanol/water partition coefficient. Environ Toxicol Chem. 17(5):951–961.

Fisk AT, Tittlemier SA, Pranschke JL, Norstrom RJ. 2002. Using anthropogenic contaminants and stable isotopes to assess the feeding ecology of Greenland sharks. Ecology. 83(8):2162–2172. doi: 10.1890/0012-9658(2002)083[2162:UACASI]2.0.CO;2

French JRP, Bur MT. 1996. The effect of zebra mussel consumption on growth of freshwater drum in Lake Erie. J Freshw Ecol. 11(3):283–289. doi:10.1080/02705060.1996.9664450.

Galarowicz TL, Adams JA, Wahl DH. 2006. The influence of prey availability on ontogenetic diet shifts of a juvenile piscivore. Can J Fish Aquat Sci. 63(8):1722–1733. doi:10.1139/f06-073.

Gewurtz SB, Diamond ML. 2003. Distribution and burdens of bioaccumulative contaminants in the Lake Erie food web: A review. Environ Rev. 11(3):141–160. doi:10.1139/a03-014.

Gopalan G, Culver D a, Wu L, Trauben BK. 1998. Effects of recent ecosystem changes on the recruitment of young-of-the-year fish in western Lake Erie. Can J Fish Aquat Sci. 55(12):2572–2579. doi:10.1139/f98-130.

Hartman KJKJ, Margraf FJ. 1992. Effects of prey and predator abundances on prey consumption and growth of walleyes in western Lake Erie. Trans Am Fish Soc. 121(2):245–260. doi:10.1577/1548-8659(1992)121<0245.

Hartman WL. 1972. Lake Erie: Effects of exploitation, environmental changes and new species on the fishery resources. J Fish Res Board Canada. 29(6):899–912. doi: 10.1139/f72-133

Hawker DW, Connell DW. 1988. Octanol-water partition coefficients of polychlorinated biphenyl congeners. Environ Sci Technol. 22(4):382–387. doi:10.1021/es00169a004.

Hebert CE, Haffner GD. 1991. Habitat partitioning and contaminant exposure in cyprinids. Can J Fish Aquat Sci. 48(2):261–266. doi:10.1139/f91-036.

Heuvel CE, Haffner GD, Zhao Y, Colborne SF, Despenic A, Fisk AT. 2019. The influence of body size and season on the feeding ecology of three freshwater fishes with different diets in Lake Erie. J Great Lakes Res. 45(4):795–804. doi:10.1016/j.jglr.2019.05.001.

International Joint Commission. 2014. A balanced diet for Lake Erie: Reducing phosphorus loadings and harmful algal blooms. Report of the Lake Erie Ecosystem Priority. http://www.ijc.org/en_/leep/report.

Kainz MJ, Fisk AT. 2009. Integrating lipids and contaminants in aquatic ecology and ecotoxicology. In: Arts MT, Kainz MJ, Brett MT, editors. Lipids in aquatic ecosystems. New

York: Springer Science+Business Media, LLC. p. 93–113.

Kelly BC, Ikonomou MG, Blair JD, Morin AE, Gobas FAPC. 2007. Food web-specific biomagnification of persistent organic pollutants. Science (80-). 317(5835):236–239. doi:10.1126/science.1138275.

Kidd KA, Bootsma HA, Hesslein RH, Muir DCG, Hecky RE. 2001. Biomagnification of DDT through the benthic and pelagic food webs of Lake Malawi, East Africa: Importance of trophic level and carbon source. Environ Sci Technol. 35(1):14–20. doi:10.1021/es001119a.

King JR, Jackson DA. 1999. Variable selection in large environmental data sets using principal components analysis. Environmetrics. 10(1):67–77. doi:10.1002/(sici)1099-095x(199901/02)10:1<67::aid-env336>3.0.co;2-0.

Kiriluk RM, Servos MR, Whittle DM, Cabana G, Rasmussen JB. 1995. Using ratios of stable nitrogen and carbon isotopes to characterize the biomagnification of DDE, mirex, and PCB in a Lake Ontario pelagic food web. Can J Fish Aquat Sci. 52:2660–2674. doi:10.1139/f95-855.

Koslowski SE, Metcalfe CD, Lazar R, Haffner GD. 1994. The distribution of 42 PCBs, including three coplanar congeners, in the food web of the Western Basin of Lake Erie. J Great Lakes Res. 20(1):260–270. doi:10.1016/S0380-1330(94)71145-X.

Kwon T-D, Fisher SW, Kim GW, Hwang H, Kim J-E. 2006. Trophic transfer and biotransformation of polychlorinated biphenyls in zebra mussel, round goby, and smallmouth bass in Lake Erie, USA. Environ Toxicol Chem. 25(4):1068–1078. doi:10.1897/05-180R.1.

Leggett MF, Johannsson O, Hesslein RH, Dixon DG, Taylor WD, Servos MR. 2000. Influence of inorganic nitrogen cycling on the $\delta^{15}N$ of Lake Ontario biota. Can J Fish Aquat Sci. 57(7):1489–1496. doi:10.1139/f00-085.

Liu J, Haffner GD, Drouillard KG. 2010. The influence of diet on the assimilation efficiency of 47 polychlorinated biphenyl congeners in Japanese koi (Cyprinus carpio). Environ Toxicol Chem. 29(2):401–409. doi:10.1002/etc.47.

Lopes C, Perga ME, Peretti A, Roger MC, Persat H, Babut M. 2011. Is PCBs concentration variability between and within freshwater fish species explained by their contamination pathways? Chemosphere. 85(3):502–508. doi:10.1016/j.chemosphere.2011.08.011.

MacKay D, Arnot JA. 2011. The application of fugacity and activity to simulating the environmental fate of organic contaminants. J Chem Eng Data. 56(4):1348–1355. doi:10.1021/je101158y.

Mackay D, Hughes AI. 1984. Three-parameter equation describing the uptake of organic compounds by fish. Environ Sci Technol. 18(6):439–444. doi:10.1021/es00124a009.

McIntyre JK, Beauchamp DA. 2007. Age and trophic position dominate bioaccumulation of mercury and organochlorines in the food web of Lake Washington. Sci Total Environ. 372(2–3):571–584. doi:10.1016/j.scitotenv.2006.10.035.

McLeod AM, Paterson G, Drouillard KG, Haffner GD. 2014. Ecological factors contributing to variability of persistent organic pollutant bioaccumulation within forage fish communities of the Detroit River, Ontario, Canada. Environ Toxicol Chem. 33(8):1825–1831. doi:10.1002/etc.2606.

McLeod AM, Paterson G, Drouillard KG, Haffner GD. 2015. PCB food web dynamics quantify nutrient and energy flow in aquatic ecosystems. Environ Sci Technol. 49(21):12832–12839. doi:10.1021/acs.est.5b03978.

Mittelbach GG, Persson L. 1998. The ontogeny of piscivory and its ecological consequences. Can J Fish Aquat Sci. 55(6):1454–1465. doi:10.1139/cjfas-55-6-1454.

Morrison HA, Gobas Frank A P C, Lazar R, Whittle DM, Haffner GD. 1998. Development and verification of a benthic/pelagic food web bioaccumulation model for PCB congeners in Western Lake Erie. Environ Sci Technol. 31(11):3267–3273. doi:10.1021/es970265m.

Morrison HA, Gobas Frank A.P.C., Lazar R, Whittle DM, Haffner GD. 1998. Projected changes to the trophodynamics of PCBS in the western Lake Erie ecosystem attributed to the presence of zebra mussels (Dreissena polymorpha). Environ Sci Technol. 32(24):3862–3867. doi:10.1021/es980434i.

Morrison HA, Whittle DM, Haffner GD. 2002. A comparison of the transport and fate of polychlorinated biphenyl congeners in three Great Lakes food webs. Environ Toxicol Chem. 21(4):683–692. doi: 10.1002/etc.5620210401

Morrison TW, Lynch WE, Dabrowski K. 1997. Predation on zebra mussels by freshwater drum and yellow perch in western Lake Erie. J Great Lakes Res. 23(2):177–189. doi:10.1016/S0380-1330(97)70895-5.

Newsome SD, del Rio CM, Bearhop S, Phillips DL. 2007. A niche for isotope ecology. Front Ecol Environ. 5(8):429–436. doi:10.1890/060150.01.

Ofukany AFA, Wassenaar LI, Bond AL, Hobson KA. 2014. Defining fish community structure in Lake Winnipeg using stable isotopes ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$): Implications for monitoring ecological responses and trophodynamics of mercury & other trace elements. Sci Total Environ. 497–498:239–249. doi:10.1016/j.scitotenv.2014.07.125.

Oliver BG, Bourbonniere RA. 1985. Chlorinated contaminants in surficial sediments of Lakes Huron, St. Clair, and Erie: Implications regarding sources along the St. Clair and Detroit Rivers. J Great Lakes Res. 11(3):366–372. doi:10.1016/S0380-1330(85)71780-7.

Pastor D, Boix J, Fernández V, Albaigés J. 1996. Bioaccumulation of organochlorinated contaminants in three estuarine fish species (*Mullus barbatus*, *Mugil cephalus* and *Dicentrarcus labrax*). Mar Pollut Bull. 32(3):257–262. doi:10.1016/0025-326X(95)00166-K.

Paterson G, Drouillard Kenneth G., Haffner GD. 2007. PCB elimination by yellow perch (*Perca flavescens*) during an annual temperature cycle. Environ Sci Technol. 41(3):824–829. doi:10.1021/es060266r.

Paterson G, Drouillard Ken G, Leadley TA, Haffner GD. 2007. Long-term polychlorinated biphenyl elimination by three size classes of yellow perch (Perca flavescens). Can J Fish Aquat Sci. 64(9):1222–1233. doi:10.1139/f07-088.

Paterson G, Ryder M, Drouillard KG, Haffner GD. 2016. Contrasting PCB bioaccumulation patterns among Lake Huron lake trout reflect basin-specific ecology. Environ Toxicol Chem. 35(1):65–73. doi:10.1002/etc.3179.

Peres-Neto PR, Jackson DA, Somers KM. 2003. Giving meaningful interpretation to ordination axes: Assessing loading significance in principal component analysis. Ecology. 84(9):2347–

2363. doi:10.1890/00-0634.

Peterson BJ, Howarth RW. 1987. Sulfur, carbon, and nitrogen isotopes used to trace organic matter flow in the salt-marsh estuaries of Sapelo Island, Georgia. Limnol Oceanogr. 32(6):1195–1213. doi:10.4319/lo.1987.32.6.1195.

Rasmussen JB, Rowan D, Lean DRS, Carey JH. 1990. Food chain structure in Ontario lakes determines PCB levels in lake trout (<ital>Salvelinus namaycush</ital>) and other pelagic fish. Can J Fish Aquat Sci. 47(Moriaty 1988):2030–2038. doi:10.1139/f90-227.

Russell RW, Gobas FAPC, Haffner GD. 1999. Role of chemical and ecological factors in trophic transfer of organic chemicals in aquatic food webs. Environ Toxicol Chem. 18(6):1250–1257. doi:10.1897/1551-5028(1999)018<1250:ROCAEF>2.3.CO;2.

Sadraddini S, Ekram Azim M, Shimoda Y, Mahmood M, Bhavsar SP, Backus SM, Arhonditsis GB. 2011. Temporal PCB and mercury trends in Lake Erie fish communities: A dynamic linear modeling analysis. Ecotoxicol Environ Saf. 74(8):2203–2214. doi:10.1016/j.ecoenv.2011.07.031.

Schaeffer JS, Margraf FJ. 1986. Food of white perch (*Morone americana*) and potential for competition with yellow perch (*Perca flavescens*) in Lake Erie. Ohio J Sci. 86(1):26–29.

Schindler DE, Scheuerell MD. 2002. Habitat coupling in lake ecosystems. Oikos. 98(2):177–189.

Scott WB, Crossman EJ. 1998. Freshwater fishes of Canada. 5th ed. Oakville: Galt House Publications Ltd.

Stanley JG, Danie DS. 1983. Species profiles: Life histories and environmental requirements of

coastal fishes and invertebrates (North Atlantic) - white perch. Lafayette. http://www.osti.gov/energycitations/product.biblio.jsp?osti_id=5273596.

Stow C a, Jackson LJ, Amrhein JF. 1997. An examination of the PCB:lipid relationship among individual fish. Can J Fish Aquat Sci. 54(1996):1031–1038. doi:10.1139/cjfas-54-5-1031.

Taylor WD, Carey JH, Lean DRS, McQueen DJ. 1991. Organochlorine concentrations in the plankton of lakes in southern Ontario and their relationship to plankton biomass. Can J Fish Aquat Sci. 48:1960–1966.

Thomann R V. 1989. Bioaccumulation model of organic chemical distribution in aquatic food chains. Environ Sci Technol. 23(6):699–707. doi:10.1021/es00064a008.

De Vault DS, Hesselberg R, Rodgers PW, Feist TJ. 1996. Contaminant trends in lake trout and walleye from the Laurentian Great Lakes. J Great Lakes Res. 22(4):884–895. doi:10.1016/S0380-1330(96)71009-2.

Willacker JJ, Eagles-Smith CA, Ackerman JT. 2017. Mercury Bioaccumulation in Estuarine Fishes: Novel Insights from Sulfur Stable Isotopes. Environ Sci Technol. 51(4):2131–2139. doi:10.1021/acs.est.6b05325.

Figure 1: Mean percent composition of ΣPCB for each PCB congener measured in (A) freshwater drum (n = 23), (B) walleye (n = 24), and (C) white perch (n = 14) collected in western Lake Erie. Error bars are standard error from the mean.

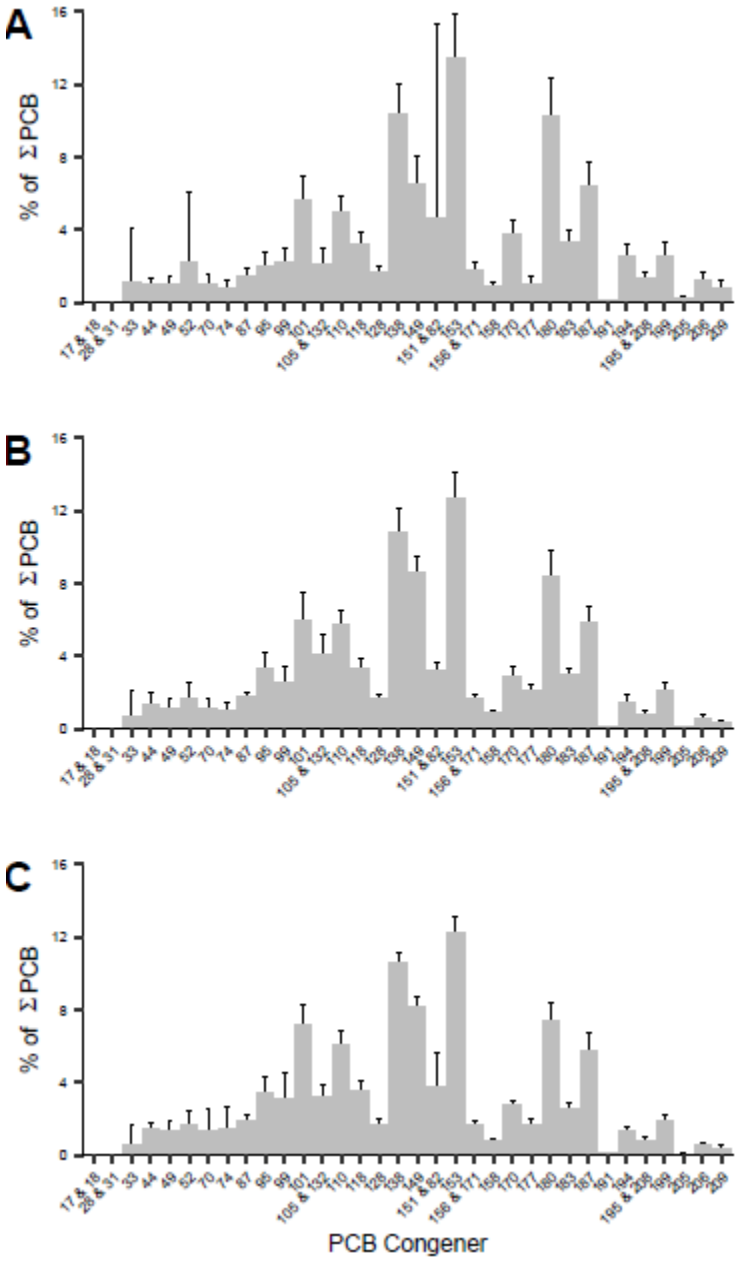


Figure 2: Principal component analyses for all contaminants measured that were detected in more than 60% of individuals. Both PC1 and PC2 were significant based on the broken stick criterion.

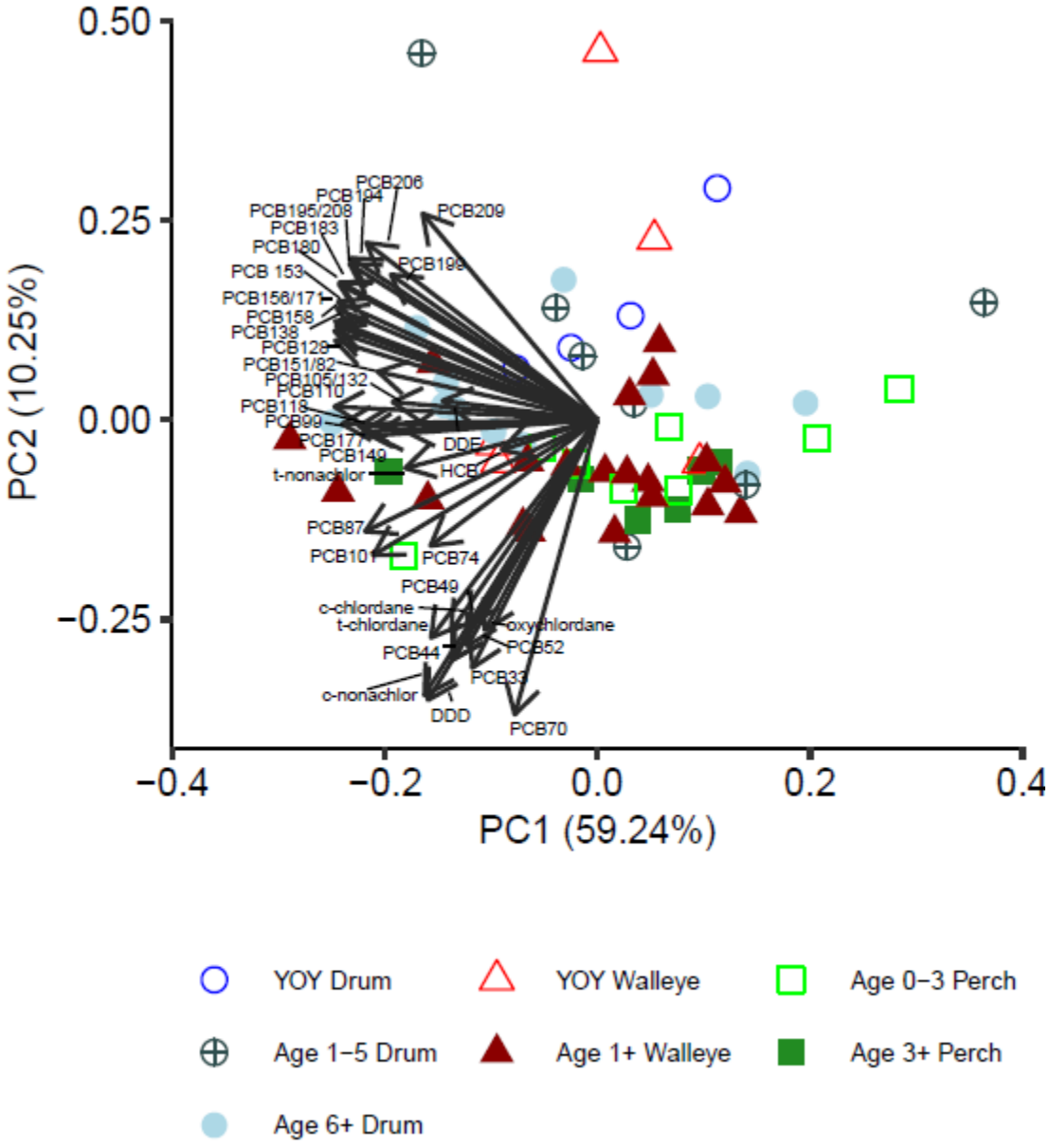


Figure 3: Relationship between loadings on (A) PC1 and (B) PC2 and contaminant logK_{OW} as shown through linear regression (PC1: $R^2 = 0.40$, $F_{1,36} = 25.6$, $p < 0.001$; PC2: $R^2 = 0.71$, $F_{1,31} = 80.8$, $p < 0.001$). Only loadings that were significant on each PC were included in the linear regressions.

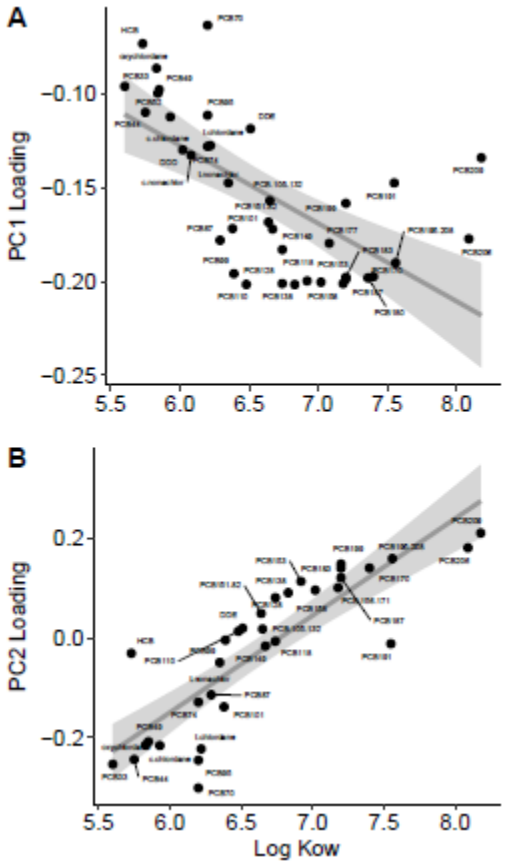
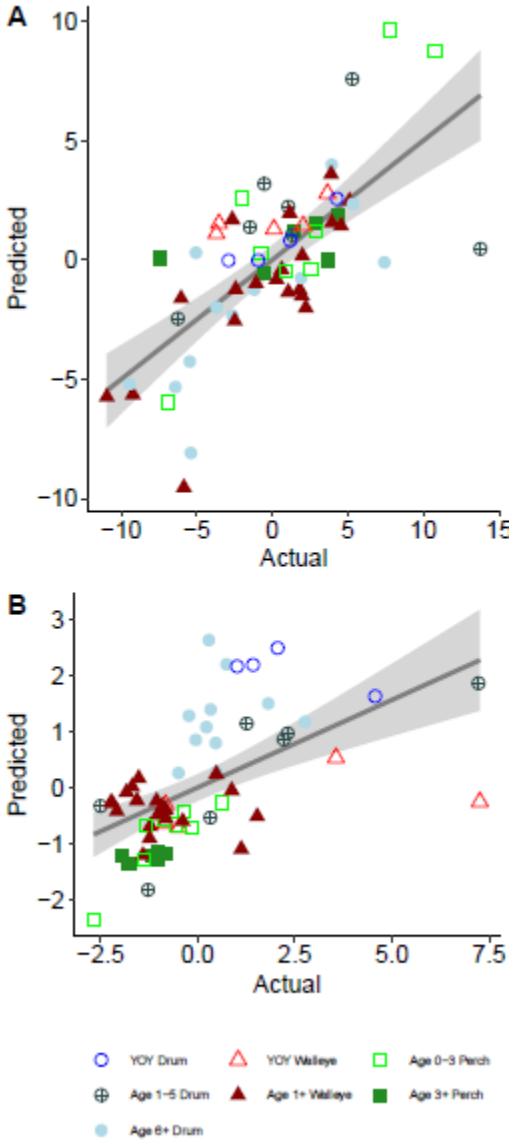


Figure 4: Actual vs. predicted scores for (A) PC1 and (B) PC2. (A) Shows the fit of a GLM on PC 1 scores that included age, $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, species, and interactions between age and $\delta^{15}N$, age and species, $\delta^{15}N$ and $\delta^{34}S$ (Adj. $R^2 = 0.40$, $F_{10,51} = 5.11$, $p < 0.001$). (B) The best fitting GLM for PC2 which included $\delta^{13}C$, species and the interaction between the two variables (Adj. $R^2 = 0.21$, $F_{5,56} = 4.19$, $p = 0.003$).



Tables

Table 1: Concentrations of selected POPs, total length, and lipids, and stable isotopes of three fish species from the western basin of Lake Erie.[a]

	Freshwater Drum				Walleye			White Perch		
Variable	Age 0	Age 1-5	Age 6+	Mean	Age 0	Age 1+	Mean	Age 0-3	Age 4+	Mean
n	4	7	12	23	5	19	24	8	6	14
Mean Age	0.0 ± 0.0	2.9 ± 0.6	16.2 ± 3.0	9.3 ± 2.2	0.6 ± 0.2	5.1 ± 1	4.2 ± 0.9	1.0 ± 0.3	4.3 ± 0.6	2.4 ± 0.5
Maximum Age[b]	0	5	46	46	0	16	16	3	6	6
Total Length (mm)	91.3 ± 8.1	227.0 ± 22.8	453.4 ± 26.6	321.5 ± 34.4	177 ± 13.6	460.8 ± 31.3	404.0 ± 34.1	118.3 ± 18.9	271.3 ± 23.1	183.9 ± 25.3
Lipid Content (%)	0.6 ± 0.1	2.0 ± 1.0	3.1 ± 0.7	2.3 ± 0.5	1.0 ± 0.2	1.5 ± 0.3	1.4 ± 0.2	1.7 ± 0.3	4.5 ± 0.6	2.9 ± 0.5
Stable Isotope Analysis (‰):										
$\delta^{15}N$	12.7 ± 0.4	15.9 ± 0.4	15.2 ± 0.4	15.0 ± 0.3	17.2 ± 0.6	16.9 ± 0.2	17.0 ± 0.2	15.6 ± 0.6	16.9 ± 0.1	16.2 ± 0.4
$\delta^{13}C$	-21.4 ± 0.1	-22.3 ± 0.5	-21.7 ± 0.3	-21.8 ± 0.2	-21.8 ± 0.1	-21.4 ± 0.2	-21.5 ± 0.1	-22.4 ± 0.4	-22.1 ± 0.2	-22.3 ± 0.2
$\delta^{34}S$	2.6 ± 0.3	1.7 ± 0.6	2.0 ± 0.4	2.0 ± 0.3	3.3 ± 0.3	3.3 ± 0.2	3.3 ± 0.1	1.6 ± 0.7	3.2 ± 0.3	2.3 ± 0.5
Chlorobenzenes (µg/Kg lipid):										
Pentachlorobenzene	ND	ND	ND	ND	ND	ND	ND	ND	2.2 ± 0.5	ND
Hexachlorobenzene (HCB)	12.6 ± 2.6	19.4 ± 5.9	21.7 ± 5.4	19.7 ± 3.4	15.6 ± 2.8	20.1 ± 2.6	19.1 ± 2.1	19.5 ± 6.7	20.6 ± 1.6	19.9 ± 4.0
Σ Chlorobenzene[c]	32.8 ± 21.2	19.9 ± 5.7	21.2 ± 5.4	22.8 ± 4.7	22.4 ± 3.3	25.9 ± 4.5	25.2 ± 3.6	29.0 ± 15.4	20.2 ± 6.8	25.2 ± 9.1
Chlordanes (µg/Kg lipid):										
Oxychlordane	ND	11.4 ± 1.4	11.7 ± 2.0	11.6 ± 1.3	7.5 ± 0.6	12.9 ± 1.4	12.1 ± 1.3	17.8 ± 6.6	14.4 ± 4.6	16.1 ± 4.0
Trans-chlordane	ND	8.7 ± 1.3	20.7 ± 6.2	17.6 ± 4.0	20.3 ± 4.3	15.6 ± 2.2	16.2 ± 1.9	12.1 ± 5.9	14.8 ± 3.8	13.4 ± 3.6
Cis-chlordane	43.5 ± 22.1	18.3 ± 4.5	49.0 ± 14.0	38.7 ± 8.5	44.9 ± 4.0	45.0 ± 8.4	45.0 ± 6.9	48.1 ± 14.0	49.7 ± 11.8	48.8 ± 9.1
Trans-nonachlor	21.6 ± 9.5	140.7 ± 88.9	122.9 ± 35.1	119.2 ± 33.5	98.0 ± 50.2	76.8 ± 16.9	81.1 ± 16.4	71.4 ± 26.6	83.2 ± 26.7	76.5 ± 18.4
Cis-nonchlor	17.9 ± 6.1	21.1 ± 4.8	45.2 ± 6.5	34.4 ± 4.6	32.6 ± 9.8	49.0 ± 12.5	46.2 ± 10.4	35.4 ± 14.4	46.5 ± 13.3	40.2 ± 9.8
Σ Chlordanes	50.5 ± 16.5	62.1 ± 15.7	165.8 ± 29.9	114.2 ± 19.8	103.3 ± 34.8	164.7 ± 35.5	152.4 ± 29.4	142.8 ± 49.8	171.9 ± 45.9	155.3 ± 33.6
DDT (µg/Kg lipid):										
p,p'DDD	47.5 ± 18.1	48.6 ± 8.2	143.7 ± 46.6	98.0 ± 26.2	89.9 ± 23.6	108.1 ± 15.5	105.1 ± 13.2	108.2 ± 32.7	141.5 ± 33.0	122.5 ± 23.0
p,p'DDE	198.7 ± 66.9	287.6 ± 118.3	410.5 ± 92.7	336.2 ± 61.8	371.2 ± 93.5	423.3 ± 110.2	412.9 ± 89.4	328.1 ± 135.9	444.2 ± 183.7	372.7 ± 105.9
Σ DDT[d]	246.1 ± 84.9	337.1 ± 120.6	564.3 ± 128.1	439.8 ± 80.4	443.1 ± 108.7	539.5 ± 121.5	520.2 ± 99	441.7 ± 162.4	523.6 ± 210.1	476.8 ± 124.6
Polychlorinated Biphenyls (PCBs, µg/Kg lipid):										
28/31	ND	ND	28.0 ± 9.3	ND	ND	ND	ND	75.4 ± 36.2	22.4 ± 9.5	51.3 ± 21.1
33	ND	164.4 ± 94.8	9.8 ± 3.1	52.0 ± 27.9	15.9 ± 0.2	35.5 ± 10.7	33.2 ± 9.0	22.1 ± 7.2	24.8 ± 14.4	23.4 ± 7.4
44	63.6 ± 24.8	31.1 ± 7.1	86.6 ± 21.0	67.3 ± 12.9	47.6 ± 12.0	96.3 ± 18.4	87.8 ± 15.5	65.3 ± 23.3	56.8 ± 20.0	61.4 ± 15.1
49	44.2 ± 14.1	41.3 ± 11.4	83.3 ± 20.1	63.8 ± 11.8	46.4 ± 12.9	82.9 ± 18.7	76.6 ± 15.5	78.9 ± 34.2	52.9 ± 10.8	68.9 ± 20.5

	Freshwater Drum				Walleye			White Perch		
Variable	Age 0	Age 1-5	Age 6+	Mean	Age 0	Age 1+	Mean	Age 0-3	Age 4+	Mean
52	65.0 ± 24.2	191.1 ± 125.4	114.7 ± 32.3	129.4 ± 39.9	66.8 ± 20.8	108 ± 21.4	100.8 ± 17.9	107.1 ± 52.5	71.8 ± 31.3	93.5 ± 32.9
70	53.1 ± 7.6	39.3 ± 9.6	72.2 ± 16.3	58.8 ± 9.4	33.9 ± 12.9	82.3 ± 15.3	73.9 ± 13.1	56.5 ± 24.6	45.3 ± 14.7	51.7 ± 15.0
74	44.6 ± 7.0	38.5 ± 14.7	72.1 ± 24.0	58.6 ± 13.9	62.3 ± 12.5	63.5 ± 12.7	63.3 ± 10.5	85.1 ± 41.7	65.8 ± 31.9	76.8 ± 26.6
87	75.6 ± 24.3	82.4 ± 28.1	127.6 ± 29.7	104.8 ± 18.3	94.5 ± 23.9	131.2 ± 33.5	125.1 ± 27.7	94.9 ± 43.3	84.6 ± 28.9	90.5 ± 26.8
95	92.7 ± 37.0	65.5 ± 11.9	163.3 ± 33.8	123.8 ± 21.1	136.9 ± 36.9	239.6 ± 49.4	221.7 ± 41.2	204.6 ± 71.5	131.6 ± 33.6	168.1 ± 40.6
99	127.1 ± 34.4	146.7 ± 64.9	225.3 ± 83.3	184.3 ± 47.7	114.8 ± 29.2	187.4 ± 45.4	172.9 ± 37.0	136.7 ± 56	240.1 ± 157.4	181.0 ± 72.5
101	282.4 ± 91.1	326.9 ± 122.6	494.2 ± 118.4	406.5 ± 74.1	244.4 ± 88.6	442.0 ± 103.9	402.5 ± 85.8	318.3 ± 108.7	375.5 ± 159.7	342.8 ± 88.9
105/132	116.6 ± 54.8	94.4 ± 32.2	192.2 ± 44.6	149.3 ± 27.7	305.5 ± 50.0	286.6 ± 79.2	289.7 ± 64.9	156.4 ± 50.9	137.5 ± 52.2	148.3 ± 35.4
110	325.2 ± 78.9	314.3 ± 134.6	412.2 ± 102.7	367.2 ± 67.1	311.5 ± 72.5	414.1 ± 99.1	393.6 ± 80.4	287.9 ± 110.4	264.6 ± 98.5	277.9 ± 73.2
118	185.2 ± 42.1	258.5 ± 96.5	245.1 ± 56.5	237.8 ± 40.2	170.1 ± 35.5	223.4 ± 46.1	212.7 ± 37.5	182.1 ± 85.5	175.9 ± 75.7	179.4 ± 56.5
128	83.8 ± 18.4	119.0 ± 55.5	132.1 ± 30.2	119.7 ± 22.7	119.2 ± 27.9	121.1 ± 31.3	120.7 ± 25.4	79.3 ± 28.7	93.2 ± 43.5	85.3 ± 23.8
138	621.6 ± 128.2	686.6 ± 297.6	791.8 ± 184.1	730.2 ± 129.6	744.2 ± 157.8	782 ± 208.6	774.5 ± 168.5	454.1 ± 152.2	542.7 ± 237.8	492.1 ± 128.8
149	390.1 ± 122.4	411.6 ± 165.6	597.8 ± 149.7	509.3 ± 94.4	541.9 ± 116.3	617.5 ± 154.6	602.4 ± 125.0	366.8 ± 122.9	363.1 ± 138	365.2 ± 88.3
151/82	1189.9 ± 1071.2	245.1 ± 168.7	210.1 ± 62.3	391.2 ± 191.6	180.5 ± 36.8	226.2 ± 51.1	217.0 ± 41.4	179.3 ± 61.9	149.4 ± 53.1	166.5 ± 40.8
153	826.8 ± 147.7	958 ± 440.2	1036.1 ± 237.3	975.9 ± 177.6	864.7 ± 189.8	891.3 ± 224.2	886.0 ± 181.7	529.7 ± 178.8	658.4 ± 308.3	584.9 ± 160.7
156/171	102.2 ± 23.8	113.5 ± 61.3	156.9 ± 34.7	134.2 ± 25.8	110.6 ± 23.5	120.7 ± 32.5	118.7 ± 26.2	74.4 ± 25.0	91.0 ± 42.5	81.5 ± 22.2
158	57.4 ± 16.5	62.3 ± 27.9	70.9 ± 16.6	66.0 ± 12.0	64.8 ± 14.3	67.8 ± 17.7	67.2 ± 14.3	40.2 ± 14.8	39.8 ± 16.1	40.0 ± 10.5
170	194.7 ± 41.5	277.8 ± 133.2	302.7 ± 64.1	276.3 ± 51.5	207.0 ± 43.4	217.2 ± 59.1	215.1 ± 47.7	109.3 ± 31.7	149.6 ± 70.5	126.6 ± 34.1
177	59.1 ± 26.1	36.9 ± 9.7	83.4 ± 20.6	65.0 ± 12.4	150.6 ± 29.7	144.7 ± 36.6	145.9 ± 29.7	66.1 ± 17.7	82.6 ± 36.4	73.2 ± 17.9
180	542.4 ± 104.9	715.6 ± 344.3	854.9 ± 180.4	758.2 ± 138.6	592.6 ± 124.7	614.0 ± 164.8	609.7 ± 133.1	301.6 ± 89.8	424.5 ± 214.0	354.2 ± 101.6
183	199.6 ± 33.8	223.8 ± 106.3	267.6 ± 58.6	242.4 ± 43.6	210.8 ± 44.7	208.2 ± 53.2	208.7 ± 43.1	103 ± 28.8	135.2 ± 62.7	116.8 ± 30.4
187	448.8 ± 103.3	382.3 ± 148.0	507.3 ± 117.0	459.1 ± 76	418 ± 84.8	401.3 ± 97.0	404.6 ± 78.8	227.5 ± 60.2	275.6 ± 119.4	248.1 ± 59.2
191	ND	8.5 ± 4.7	11.4 ± 2.2	10.7 ± 1.7	7.5 ± 1.9	8.7 ± 1.9	8.5 ± 1.6	5.8 ± 1.8	4.5 ± 1.5	5.0 ± 1.1
194	99.1 ± 16.7	190.7 ± 100.3	221.1 ± 47.0	190.6 ± 38.8	98.9 ± 21.8	104.3 ± 28.2	103.2 ± 22.8	54.9 ± 16.4	80.5 ± 43.3	65.8 ± 20.1
195/208	63.1 ± 13.5	74.9 ± 36.5	116.1 ± 25.5	94.3 ± 17.6	57.2 ± 12.8	54.8 ± 14.3	55.3 ± 11.7	32.7 ± 9.3	41.2 ± 19.8	36.3 ± 9.6
199	136.2 ± 33.8	130.6 ± 54.8	227.0 ± 52.6	181.9 ± 33.2	153.1 ± 32.1	142.8 ± 35.2	145.0 ± 28.5	76.7 ± 21.8	94.8 ± 44.4	84.4 ± 21.9
205	ND	ND	20.4 ± 6.3	ND	ND	ND	ND	ND	ND	ND
206	42.3 ± 6.6	73.8 ± 36.2	111.5 ± 24.1	88.0 ± 17.2	36.1 ± 8.9	36.4 ± 9.4	36.3 ± 7.6	23.0 ± 7.0	27.9 ± 13.8	25.1 ± 6.8
209	27.1 ± 3.7	45.0 ± 19.9	78.3 ± 18.5	59.9 ± 12.0	21.2 ± 6.7	20.2 ± 4.7	20.4 ± 3.9	13.8 ± 3.5	15.9 ± 8.0	14.7 ± 3.8
Σ PCB[e]	6518.9 ± 1338.8	6300.1 ± 2635.6	8079.1 ± 1830.5	7266.3 ± 1235.4	6062.5 ± 1242.5	7169.4 ± 1741.4	6948.0 ± 1407.2	4497.5 ± 1531.4	4994.7 ± 2181.4	4710.6 ± 1229.1

[a] Concentrations of selected OCs [mean ± 1 SE, µg/kg lipid], total length (mm), age (years), lipid content (%), and stable isotopes (‰) of three fish species (freshwater drum, walleye and white perch) collected in Lake Erie in 2019. Concentrations of OCs are

only reported if > 60% of individuals in the group had concentrations of the contaminant above instrument MDL, and means omit individuals which had concentrations below the MDL. All measurements are recorded as mean ± 1 SE unless otherwise specified.

[b] Maximum age is the maximum age recorded for individuals in each size class/group.

[c] ΣCLBz: mean sum of chlorobenzene concentrations consisting of 1,2,3,4 TeCB, 1,2,4,5 TeCB, QCB and HCB.

[d] ΣDDT: mean sum of p,p'DDD, p,p'DDE, and p,p'DDT

[e] ΣPCB: mean sum of PCB concentrations consisting of congeners 17, 18, 28, 31, 33, 44, 49, 52, 70, 74, 82, 87, 95, 99, 101, 105, 110, 118, 128, 132, 138, 149, 151, 153, 156, 158, 169, 170, 171, 177, 180, 183, 187, 191, 194, 195, 199, 205, 206, 208, and 209.

MDL = minimum detection level, ND = non-detected, indicates the OC was detected in fewer than 60% of the samples.

Table 2: Coefficients of variables included in GLM including two-way interactions for PC1 of the PCA for POP data for three species of fish from the western basin of Lake Erie. [a]

Variable	Effect Size (Coefficient)	t-value	p-value
Age (years)	-3.8	-3.7	< 0.001
$\delta^{13}C$ (‰)	-2.4	-3.7	< 0.001
$\delta^{15}N$ (‰)	-2.9	-3.7	< 0.001
$\delta^{34}S$ (‰)	-14.8	-3.8	< 0.001
Species (White Perch)	0.8	0.4	0.7
Species (Walleye)	1.2	0.5	0.6
Age × $\delta^{15}N$	0.2	3.5	0.001
Age × Species (White	-0.7	-1.2	0.2

Perch)			
Age × Species (Walleye)	-0.7	-3.0	0.004
$\delta^{15}N \times \delta^{34}S$	0.9	3.9	< 0.001

[a] The best-fit model comparing PC1 scores and environmental data included age, species, and stable isotopes of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ (Adj. $R^2 = 0.40$, $F_{10,51} = 5.11$, $p < 0.001$) The model uses freshwater drum as the base species and included significant 2-way interactions. A variable was considered significant in the model if it had a p-value less than 0.05.

Table 3: Coefficients of variables included in GLM including two-way interactions for PC2 of the PCA or POP data for three species of fish from the western basin of Lake Erie.[a]

Variable	Effect Size (Coefficient)	t-value	p-value
$\delta^{13}C$ (‰)	1.0	2.5	0.02
Species (White Perch)	-14.6	-1.0	0.3
Species (Walleye)	-31.4	-2.1	0.04
$\delta^{13}C \times$ Species (White Perch)	-0.6	-0.8	0.4
$\delta^{13}C \times$ Species (Walleye)	-1.4	-2.0	0.04

[a] The best-fit model comparing PC2 scores and environmental data included $\delta^{13}C$, and species (Adj. $R^2 = 0.21$, $F_{5,56} = 4.19$, $p = 0.003$) The model uses freshwater drum as the base species and included significant 2-way interactions. A variable was considered significant in the model if it had a p-value less than 0.05.