Ecological contributions to organochlorine contributions

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

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

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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 (δ¹³C, δ¹⁵N, and δ³⁴S). 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 (δ¹³C and δ³⁴S), trophic position (δ¹⁵N), and interactions between age and δ¹⁵N, age and species, and δ¹⁵N and δ³⁴S were significant predictors of POP concentration based on PC1 scores, whereas δ¹³C 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

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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 (KOW), 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 log$K_{ow}$ 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

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exposure of an organism, trophic position and habitat use can be characterized through the stable isotopes of nitrogen (δ^{15}N), carbon (δ^{13}C) and sulphur (δ^{34}S; Colborne et al., 2016; Croisetière et al., 2009; Newsome et al., 2007). Stable isotopes of nitrogen (δ^{15}N) increase with trophic position, while δ^{13}C and δ^{34}S both reflect habitat usage based on diet (Croisetière et al. 2009; Colborne et al. 2016). Carbon (δ^{13}C) values are often higher in littoral or nearshore habitat and decrease with distance from shore (Newsome et al. 2007), whereas δ^{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 americana*) 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$_2$SO$_4$ 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 isoctane 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 Na₂SO₄. 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 iso-octane. 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 × 10³ mbar for 48 hours) muscle samples were powdered using a mortar and pestle or dissection scissors. Samples for sulphur (δ³⁴S) were weighed into tin cups for a final mass 5000-6000µg analyzed on a Delta V Plus Thermo Scientific Continuous Flow Mass Spectrometer (Thermo Scientific, Bremen, Germany) coupled to a
Lipids were extracted prior to δ^{13}C and δ^{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 δ^{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 δ^{13}C and δ^{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 δ^{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 δ^{15}N, and within 0.1‰ (NIST 8573, 8542, and 8544) for δ^{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 δ^{13}C and δ^{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 ≤ 0.3‰ for δ^{34}S. Precision as indicated...
by duplicate ($\delta^{34}\text{S}$) and triplicate ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) was within ±0.1‰ (n = 83 for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$; n = 12 for $\delta^{34}\text{S}$) 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-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 $\delta^{13}\text{C}$, $\delta^{34}\text{S}$, and $\delta^{15}\text{N}$ 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, $\alpha$ – hexachlorocyclohexane, $\beta$ – hexachlorocyclohexane, $\gamma$ – hexachlorocyclohexane, 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]/[ΣPCB]*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:

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

where \( p \) is the number of components and \( k \) is the \( k \)th 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 logK\(_{\text{OW}}\) 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 \( \delta^{13}\text{C}, \delta^{15}\text{N}, \delta^{34}\text{S}, \text{age, species, and two-way interactions.} \) 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.
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 $\Sigma$DDT, $\Sigma$CLDn, and $\Sigma$CLBz ($\Sigma$PCB, all samples, 6560.9 ± 779.6 μg/Kg lipid). Percent composition of $\Sigma$PCB 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 $\Sigma$PCB in all individuals (Table 1, Figure 1).

Chlorobenzenes had the lowest concentrations ($\Sigma$CLBz, 24.3 ± 3.0 μg/Kg lipid), and HCB accounted for 99% or more of $\Sigma$CLBz in all species (Table 1). $\Sigma$CLDn had mean concentrations of 138.9 ± 15.8 μg/kg lipid and trans-nonachlor accounted for 50% of the mean $\Sigma$CLDn concentration in all species (Table 1). Over 70% of the proportion of $\Sigma$DDT 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

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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 \( \Sigma PCB \) 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 20\(^{th}\) 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 $\delta^{13}\text{C}$ (littoral or benthic resources) and lower concentrations with low $\delta^{13}\text{C}$ (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 $\log K_{\text{OW}} > 5.6$ (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 $\delta^{13}\text{C}$ 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 ($\delta^{34}\text{S}$) is potentially a useful predictor of legacy POP concentrations in freshwater ecosystems, and highlights the need to understand $\delta^{34}\text{S}$ dynamics within freshwater ecosystems better. Interest in $\delta^{34}\text{S}$ 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 $\delta^{34}\text{S}$ and POP concentration is not clear as dynamics driving differentiation of $\delta^{34}\text{S}$ 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 (δ¹³C, δ³⁴S), and trophic position (δ¹⁵N) 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.

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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.
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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 log\(K_{ow}\) 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, δ^{13}C, δ^{15}N, δ^{34}S, species, and interactions between age and δ^{15}N, age and species, δ^{15}N and δ^{34}S (Adj. R^2 = 0.40, F_{10,51} = 5.11, p < 0.001). (B) The best fitting GLM for PC2 which included δ^{13}C, species and the interaction between the two variables (Adj. R^2 = 0.21, F_{5,56} = 4.19, p = 0.003).
Table 1: Concentrations of selected POPs, total length, and lipids, and stable isotopes of three fish species from the western basin of Lake Erie.\(^a\)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Freshwater Drum</th>
<th>Walleye</th>
<th>White Perch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age 0</td>
<td>Age 1-5</td>
<td>Age 6+</td>
</tr>
<tr>
<td>n</td>
<td>24</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>Mean Age</td>
<td>0.0 ± 0.0</td>
<td>2.9 ± 0.0</td>
<td>16.2 ± 0.0</td>
</tr>
<tr>
<td>Maximum Age(^b)</td>
<td>0</td>
<td>5</td>
<td>46</td>
</tr>
<tr>
<td>Total Length (mm)</td>
<td>91.3 ± 8.1</td>
<td>227.0 ± 22.8</td>
<td>453.4 ± 26.6</td>
</tr>
<tr>
<td>Lipid Content (%)</td>
<td>0.6 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>3.1 ± 0.7</td>
</tr>
</tbody>
</table>

Stable Isotope Analysis (%):  
\(\delta^{18}N\)  
12.7 ± 0.4  
15.9 ± 0.4  
15.2 ± 0.3  
15.0 ± 0.3  
17.2 ± 0.6  
16.9 ± 0.2  
17.0 ± 0.2  
15.6 ± 0.6  
16.9 ± 0.4  
16.2 ± 0.1  

\(\delta^{13}C\)  
-21.4 ± 0.1  
-22.3 ± 0.5  
-21.7 ± 0.2  
-21.8 ± 0.1  
-21.4 ± 0.2  
-21.5 ± 0.1  
-22.4 ± 0.2  
-22.1 ± 0.2  
-22.3 ± 0.2  

\(\delta^{34}S\)  
2.6 ± 0.1  
1.7 ± 0.5  
3.3 ± 0.2  
3.3 ± 0.1  
3.3 ± 0.2  
1.6 ± 0.1  
3.2 ± 0.2  
2.3 ± 0.1  

Chlorobenzenes (μg/Kg lipid):  
Pentachlorobenzene  
Hexachlorobenzene (HCB)  
\(\Sigma\) Chlorobenzene\(^c\)  
Chlordanes (μg/Kg lipid):  
Oxychlordane  
Trans-chlordane  
Cis-chlordane  
Trans-nonachlor  
Cis-nonchlor  
\(\Sigma\) Chlordanes  
DDT (μg/Kg lipid):  
p,p’DDD  
p,p’DDE  
\(\Sigma\) DDT\(^d\)  
Polychlorinated Biphenyls (PCBs, μg/Kg lipid):  
28/31  
33  
44  
49  

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<table>
<thead>
<tr>
<th>Variable</th>
<th>Freshwater Drum</th>
<th>Walleye</th>
<th>White Perch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age 0</td>
<td>Age 1+</td>
<td>Age 6+</td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>65.0±191.1±114.7±129.4±66.8±108±100.8±107.1±71.8±93.5±</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>53.1±39.3±72.2±58.8±33.9±82.3±73.9±56.5±45.3±51.7±</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>92.7±65.5±163.3±123.8±136.9±238.6±221.7±204.6±131.6±181.1±</td>
<td>37.0</td>
</tr>
<tr>
<td></td>
<td>129</td>
<td>127.1±146.7±225.3±184.3±114.8±187.4±172.9±136.7±240.1±181.0±</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>149</td>
<td>390.1±411.6±597.8±509.3±541.9±617.5±602.4±366.8±363.1±365.2±</td>
<td>122.4</td>
</tr>
<tr>
<td></td>
<td>151/82</td>
<td>1189.9±245.1±210.1±391.2±180.5±226.2±217.0±179.3±149.4±166.5±</td>
<td>107.2</td>
</tr>
<tr>
<td></td>
<td>153</td>
<td>826.5±958.8±1086.1±975.9±864.7±891.3±886.0±529.7±658.4±589.9±</td>
<td>147.7</td>
</tr>
<tr>
<td></td>
<td>156/171</td>
<td>102.2±113.5±156.9±134.2±110.6±120.7±118.7±74.4±91.0±81.5±</td>
<td>23.8</td>
</tr>
<tr>
<td></td>
<td>158</td>
<td>57.4±62.3±70.9±66.0±64.8±67.0±67.2±47.5±40.2±39.8±40.0±</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
<td>170</td>
<td>194.7±277.8±302.7±276.3±207.0±217.2±215.1±109.3±149.6±126.6±</td>
<td>141.5</td>
</tr>
<tr>
<td></td>
<td>177</td>
<td>59.1±36.9±83.4±65.0±150.6±144.7±145.9±66.1±82.6±73.2±</td>
<td>26.1</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>542.4±715.6±854.9±758.2±592.6±614.0±609.7±301.6±424.5±354.2±</td>
<td>180.4</td>
</tr>
<tr>
<td></td>
<td>183</td>
<td>199.6±223.8±267.6±242.4±210.8±208.2±208.7±103.7±135.2±116.8±</td>
<td>33.8</td>
</tr>
<tr>
<td></td>
<td>185</td>
<td>448.9±392.3±583.8±459.1±418.5±403.3±403.6±227.5±275.3±295.4±</td>
<td>103.3</td>
</tr>
<tr>
<td></td>
<td>191</td>
<td>8.5±11.4±10.7±7.5±8.7±8.5±5.8±4.5±5.0±</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>194</td>
<td>99.1±190.7±221.1±190.6±98.9±104.3±103.2±54.9±80.5±65.8±</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>195/208</td>
<td>63.1±74.9±116.1±94.3±57.2±54.8±55.3±32.7±41.2±36.3±</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>199</td>
<td>136.2±130.6±227.0±181.9±153.1±142.8±145.0±76.7±94.8±84.4±</td>
<td>33.8</td>
</tr>
<tr>
<td></td>
<td>205</td>
<td>20.4±</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>206</td>
<td>42.3±73.8±111.5±88.0±36.1±36.4±36.3±23.0±27.9±25.1±</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>209</td>
<td>27.1±45.0±87.3±59.9±21.2±20.2±20.4±13.8±15.9±14.7±</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>309</td>
<td>6518.9±6300.1±7266.3±7266.3±9490.0±9497.5±4710.6±7266.3±1417.2±1531.4±2181.4±1229.1±</td>
<td></td>
</tr>
</tbody>
</table>

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

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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.

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

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

ΣDDT: mean sum of p,p′DDD, p,p′DDE, and p,p′DDT


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. 

<table>
<thead>
<tr>
<th>Variable</th>
<th>Effect Size (Coefficient)</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>-3.8</td>
<td>-3.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>$\delta^{13}$C (%)</td>
<td>-2.4</td>
<td>-3.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>$\delta^{15}$N (%)</td>
<td>-2.9</td>
<td>-3.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>$\delta^{34}$S (%)</td>
<td>-14.8</td>
<td>-3.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Species (White Perch)</td>
<td>0.8</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Species (Walleye)</td>
<td>1.2</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Age $\times$ $\delta^{15}$N</td>
<td>0.2</td>
<td>3.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Age $\times$ Species (White)</td>
<td>-0.7</td>
<td>-1.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Effect Size (Coefficient)</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\delta^{13}$C (%)</td>
<td>1.0</td>
<td>2.5</td>
<td>0.02</td>
</tr>
<tr>
<td>Species (White Perch)</td>
<td>-14.6</td>
<td>-1.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Species (Walleye)</td>
<td>-31.4</td>
<td>-2.1</td>
<td>0.04</td>
</tr>
<tr>
<td>$\delta^{13}$C × Species (White Perch)</td>
<td>-0.6</td>
<td>-0.8</td>
<td>0.4</td>
</tr>
<tr>
<td>$\delta^{13}$C × Species (Walleye)</td>
<td>-1.4</td>
<td>-2.0</td>
<td>0.04</td>
</tr>
</tbody>
</table>

$^a$ The best-fit model comparing PC2 scores and environmental data included $\delta^{13}$C, and species (Adj. $R^2 = 0.21$, $F_{3,56} = 4.19$, $p = 0.003$) 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.