INTRASPECIFIC VARIATION IN FRESHWATER FISHES; INSIGHTS INTO TROPHIC RELATIONSHIPS, MORPHOLOGY AND BIOACCUMULATION

by

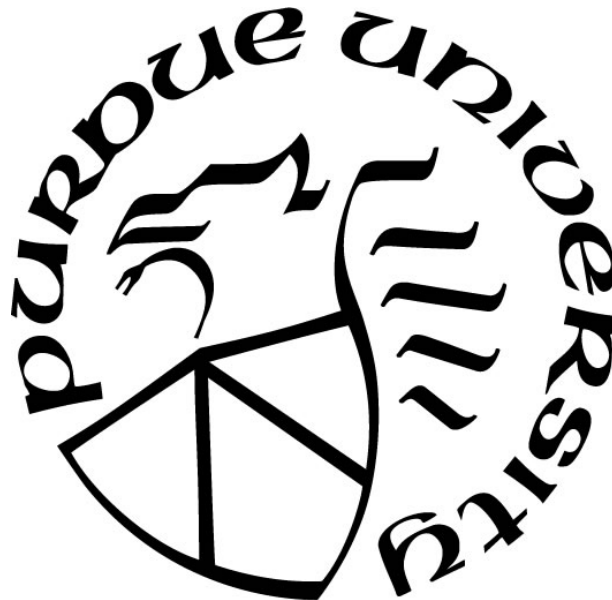**Timothy David Malinich**

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This dissertation is dedicated to the Tuber and the Flipper Sac.

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ABSTRACT

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Individuals within fish populations differ in many traits, such as sex, life-history, habitat residence, diet, and morphology. Such trait differences among individuals (i.e. intra-population variation) may be greater than the differences among populations (i.e. inter-population variation). My dissertation examines intra-population variation, with a focus on trophic relationships and morphology; as well as how variation in these attributes may reflect differences in bioaccumulation of contaminants. The second chapter of my dissertation examines the influence of spatial-temporal variation on the trophic structures of round goby (*Neogobius melanstomus*) and two age classes of yellow perch (*Perca flavescens*) within Saginaw Bay, Lake Huron. Using stable isotope ratios (δ^{13}C, δ^{15}N, δ^2H, δ^{18}O) and stomach contents as trophic indicators, I examined variation of diets. I found that spatial variation had a greater impact on diet indicators than both annual and seasonal variation. This spatial variation could represent a form of compartmentalization within the community of fish residing in Saginaw Bay, and could provide stability to the community. Chapter three of my dissertation examines intra-population variation in yellow perch morphology through a series of mesocosm experiments. My first mesocosm study determined that yellow perch could be experimentally manipulated to display divergent morphologies using simulated habitats, specifically pelagic and littoral habitats. Following this experiment, I focused on specific environmental drivers (structure, prey resources, and predation risk) as possible influences on yellow perch morphology. Within experimental pools, I exposed yellow perch to one of four treatments (an open pool, a structured pool, pools with chironomid prey resources and pools with a perceived, olfactory, predation risk) in the summer of 2015. Following exposure to these treatments I examined the morphological changes in yellow perch in magnitude and direction. I observed that while each treatment induced some difference in morphology, the open and structured treatments

had the greatest magnitude of difference. I repeated the open and structure treatments during the following summer (2016). Again, I found that structure and open morphologies could be induced by my mesocosm treatments, but also observed that shapes differed from the previous year's structure and open treatments. Finally, my fourth chapter examined how variation in trophic niches and morphology may reflect variation in contaminant concentration of fish in their natural environment. In this chapter, I extended my work with yellow perch to also include black crappie (*Pomoxis nigromaculatus*) and examined fish from 5 northern Indiana glacial lakes. Using model inference techniques, I found that variation in mercury was closely associated with not only fish total length, but also stable isotopes (δ^{13}C and δ^{15}N) and morphology. Interestingly, morphology-related variables of

both species were strong predictors of mercury concentration in fish, following total length. Together, the chapters within my dissertation highlight the importance of considering intra-population variation, in which local factors such as habitat conditions and prey availability can influence individual variation in trophic structuring and morphology. These in turn may reflect other attributes of interest, such as the accumulation of contaminants.

CHAPTER 1.   INTRODUCTION

1.1   Introduction

Populations of fish are often distinguished by the relatedness of individuals to one another and the geographic location they inhabit. Populations are useful designations for comparing one group of fish to another and for designing management and monitoring schemes targeting recreational or commercial fisheries. Key population attributes such as growth, fecundity, diet, morphology and behavior are often summarized as equal distributions around a mean value or trait. However, such measures of central tendency may not always be true reflections of the individuals within the population (Dall et al. 2012). Individuals may have different traits such as specialization for specific foraging behaviors (Toscano et al. 2016), dissimilar habitat utilization (Harrison et al. 2016), and even different morphologies (Svanbäck and Eklöv 2004). Individual traits may also interact with one another and compound the variation among individuals, for example habitat residence of an individual may not only influence the individual's morphology (West-Eberhard 2003) but could also influence the diet available to fish (Marklund et al. 2017). Furthermore, trait variation among individuals within a population, may be greater than variation among different populations (Bolnick et al. 2003). Below, I present a series of 3 chapters that examine the presence and drivers of individual variation in populations, with a focus on differences in trophic pathways and morphology and how these traits may influence variation in mercury concentration among individuals.

Aquatic systems are composed of multiple habitats (i.e. littoral, benthic, pelagic, nearshore, offshore etc.) and individuals of a population are influenced by the types of habitat they forage or reside within. Individuals among these different spaces may consume different prey items due to realized prey availability (van Baalen et al. 2001) which could differ across spatial scales (Jennings et al. 1997, Vizzini and Mazzola 2006). Further, the prey supported in these locations may vary due to spatially influenced sources of production, (i.e. allochthonus river effluent or autochthonus pelagic production), as well as temporal effects such as changes in primary production through succession (Tallberg et al. 1999). Such differences in energy pathways could create variation in trophic structuring

within the population. Further, this variation in trophic structuring could be present amongst multiple fish species and across different age-classes within a species, and may be an important influence on the stability of fish communities (McMeans et al. 2016).

In chapter two of this dissertation, I examined the spatial context of trophic pathways using stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$, δ^2H, $\delta^{18}O$) and stomach contents to compare the long and short-term foraging patterns among four spatially proximate sites in Saginaw Bay, Lake Huron. These patterns were examined within two species of freshwater fish, round goby (*Neobobius melanostomus*) and yellow perch (*Perca flavescens*), as well as two age-classes of yellow perch: age-0 and age-1. I found that the stable isotopes and stomach contents of both species, and both age-classes in yellow perch, differed spatially, with a minor influence from seasonal and annual effects. Fish with spatially distinct isotope ratio values were likely foraging specifically on prey items from these sites, since stable isotopes are incorporated into fish tissue from the prey consumed over a temporal period of weeks to months (Layman et al. 2012). In all three fish groups, the variation associated with spatial factors in stable isotope ratios and stomach contents, was often greater than both annual and seasonal factors. This spatial variation in trophic structuring may be beneficial to Saginaw Bay populations and the broader fish community, by reducing the reliance on any one particular production pathway.

Habitats may influence the production pathways and diets of the fish that reside within them and may also have other influences that contribute to variation among individuals. For example, differences among habitats may contribute to morphological variation. Among aquatic systems several fish species have been identified as morphologically diverse, often falling along a continuum from more fusiform to more deep-bodied morphologies (Aguirre and Bell 2012, Faulks et al. 2015, Turgeon et al. 2016, Esin et al. 2018). Several processes may contribute to morphological variation, including phenotypic plasticity, the expression of different phenotypes by the same individual genotype (Pigliucci 2003, West-Eberhard 2003). In addition to plasticity, populations exposed to new or stressful environments may express 'hidden' genes (i.e. cryptic gene expression), a possible mechanism for producing variation in morphology in response to novel or stressful environments (Mcguigan et al. 2011). Opposite of having a large degree of variation in shape, populations may have little variation if canalization of a particular

shape is advantageous (Waddington 1942, Van Buskirk and Steiner 2009). Shape can have important influences on the ecology of individual fish through its influence on many abilities including; swimming efficiency (Webb 1984), predator avoidance (Brönmark and Miner 1992, Domenici et al. 2008), and foraging success (Svanbäck and Eklöv 2004). Differences in morphology potentially reflect differences in environmental conditions (e.g., diet, structural habitat, predation risk) encountered by individuals within a population (Snowberg et al. 2015, Marklund et al. 2017) and may contribute to improved individual performance among these different environmental conditions.

Changes in morphology have been associated with different habitats, such as fusiform shapes in pelagic spaces and deeper body shapes in littoral habitats (Svanbäck and Eklöv 2002, Faulks et al. 2015). The changes in shape associated with these habitats are likely in response to environmental conditions, such as the amount of structure (Olsson and Eklöv 2005), the predation risk (Brönmark and Miner 1992), the type of prey resources available (Svanbäck and Eklöv 2004) or a combination of environmental conditions. In some cases, it appears as though different environmental conditions produce similar morphologies, such as the deep bodies formed as a response to either predator threats (Brönmark and Miner 1992) or increased structural complexity (Olsson and Eklöv 2005).

Chapter three presents experimental studies investigating how variation in morphology of yellow perch is produced through exposure to different environmental conditions. Yellow perch have been observed to have a large degree of morphological variation between habitats (Parker et al. 2009) and among populations (Kocovsky and Knight 2012). My first experiment examined morphological variation between a simulated littoral habitat and a simulated pelagic habitat. I found that morphologies differed significantly between treatments, but only when examining shape change using a holistic landmark analysis. In addition to this experiment, I exposed yellow perch to four different environmental conditions; an open water environment, increased predation threat, increased structural complexity, and an alternative prey resource (chironomids). Over a short 60 day period, yellow perch developed significant morphological differences among the treatments, with the open environment and structure environments having the greatest magnitude of difference. Shapes varied with treatment, but generally involved changes in body trunks, caudal width & length, and head orientation. This experiment had low

survivorship, therefore I replicated the experiment the following year examining only structure and open treatments. In this experiment, I again found significant differences in morphology between treatments, however the type of shape change was different from the previous year. In this experiment morphology reflected body orientation toward the ventral or dorsal direction. This may suggest that morphological responses may not be consistent annually, such as due to changing environmental factors between years (i.e. temperature, prey availability, turbidity etc.).

Individuals within populations may reside in different habitats, affecting their foraging and morphology (Parker et al. 2009, Marklund et al. 2017). Habitat differences and variable foraging could also impact the bioaccumulation of contaminants within individuals (Eagles-smith et al. 2016). Bioaccumulation describes the increasing concentration of a contaminant, such as mercury or polychlorinated biphenyl compounds (PCB) within the tissue of a fish, which typically biomagnifies with trophic level. High levels of contaminants pose health risks to both wildlife (Depew et al. 2012) and humans. Therefore a great deal of effort is taken to provide an estimate of risk (i.e. fish advisories) for individuals who consume fish (USEPA 2011). In natural systems, these compounds vary in concentration among lakes, between different habitats (Ullrich et al. 2001) and among individuals. Contaminants found within fish are absorbed through the diet and might therefore correlate with different trophic pathways present in the fish population. A growing body of research has shown that individual fish contaminant levels may also significantly vary from one another due to other individual traits. For example, both PCB's and mercury will differ between sexes of fish (Madenjian et al. 2014, 2016). However, relationships between mercury and sex do not appear to be consistent across species (Bastos et al. 2016), confounding our ability to make general predictions about contaminant concentrations. Variable trophic structures within a population of fish could also influence the pathways in which contaminants such as mercury biomagnify and should be examined as a potential source of contaminant variation.

My fourth and final chapter examined the potential for variable contaminant accumulation with variable trophic structuring by focusing on the bioaccumulation of mercury within several populations of black crappie (*Pomoxis nigromaculatus*) and yellow perch in northern Indiana lakes. A sample of black crappie and yellow perch were collected

from each lake to describe the distribution of total mercury contaminants within these lakes. Stable isotope ratios and morphology were also examined as indicators of individual diets and habitat history. Model inferencing was used to compare the ability of stable isotopes and morphology, along with fish total length and sex, to predict mercury concentration within black crappie and yellow perch. I found that both black crappie and yellow perch populations had mercury concentrations that were non-normal in distribution, often with a handful of outlier individuals with very high concentrations. Further, I found that stable isotope ratios suggested that black crappie, in general, had narrower trophic niches than yellow perch in all lakes examined. Across predictive models, I found that the top models for both yellow perch and black crappie contained morphology axes, in addition to total length. This could suggest that morphological differences were closely linked to the processes influencing the variation in bioaccumulation within fish populations.

In summary, when comparing populations of fish, it is important to consider both inter- and intra-population variation. It is clear that intra-population variation can be quite large and may even surpass inter-population differences. Intra-population variation in habitat residence and trophic structuring may influence other attributes such as morphological diversity in the population as well relate to the concentration of contaminants such as mercury. Therefore, it is prudent that we consider the ways in which variation among individuals within our study population may influence population level research in topics such as fisheries management or contaminant risk assessment.

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CHAPTER 2.   ISOTOPES AND STOMACH CONTENTS REVEAL SPATIALLY STRUCTURED TROPHIC PATHWAYS WITHIN A FRESHWATER BAY

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2.1   Abstract

The trophic structure and dominant production pathways in aquatic ecosystems can be influenced by both physicochemical and biotic factors. Within a system, spatial and temporal variation in environmental conditions can lead to variation in trophic structuring. While such patterns may be especially evident across broad spatial and temporal scales and distinct habitat types, recent evidence suggests that even across relatively fine spatial and temporal scales, production pathways supporting individual species can differ. Further, little is known how such spatiotemporal trophic variation may differ or be consistent among species and age-classes. This study examined the influence of spatial location, season, and year in structuring production pathways supporting two fish species, round goby (*Neogobius melanstomus*) and young (age 0-1) yellow perch (*Perca flavescens*) in Saginaw Bay, Lake Huron. Production patterns were indexed via short-term (stomach contents) and long-term (stable isotopes; $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$, $\delta^{18}O$) trophic indices. Across both species and between age-classes of yellow perch, spatial location had a consistent influence on trophic indices, even though locations were relatively proximate. In all cases, location had a stronger influence on diet indicators than temporal variables. Resulting spatial variation in pathways supporting these species may have ramifications for population and community resiliency, as at the population-level these consumers are less reliant on single prey types and production pathways.

Key Words: Stable Isotopes, Diet analysis, Stomach Content, Yellow Perch, Round Goby

2.2   Introduction

The energy pathways within aquatic ecosystems, from producers through consumers, may vary spatially and temporally. These pathways may differ in response to abiotic and biotic factors such as: seasonal succession of primary producers (Tallberg et al. 1999), differential nutrient inputs (Interlandi et al. 1999, Carvalho et al. 2015), upwellings (Cai et al. 2012), benthic substrates (Pan et al. 1999) and various other physico-chemical conditions. While variable primary production pathways may affect the density and composition of potential prey, the specific prey eaten by consumers, such as fish, may also reflect prey vulnerability, the realized availability of prey (Crowder and Cooper 1982, van Baalen et al. 2001), as well as individual foraging behavior of consumers (e.g. home range, diet specialization; Bolnick et al. 2003, Glover et al. 2008). Consumers of the same species may potentially rely on vastly different primary, secondary and tertiary production pathways across seasons and in different areas of an aquatic system (Happel, Creque, et al., 2015; Roswell, Pothoven, & Höök, 2013). Further, these differences may be present at both fine and coarse spatial scales (e.g. 10's of kilometers, Vizzini and Mazzola 2006; or 100's of kilometers, Jennings et al. 1997). It is informative to document this potential spatiotemporal variation to elucidate ecosystem processes and their adaptability to ecosystem perturbations (McMeans et al. 2016).

Processes acting to structure spatial variation of trophic pathways may be inconsistent across temporal scales. For instance, annual and seasonal processes structuring lower trophic levels could differ spatially creating spatial trophic patterns (e.g., upwellings in nearshore regions, riverine nutrient loading at river mouths; Piña-Ochoa et al. 2006, Rao and Schwab 2007). Similarly, temporal shifts in consumer behavior or resource availability can alter temporal trophic structuring such as through the seasonal emergence of specific invertebrate prey in some but not all habitats. These short-term changes in spatial trophic structuring could have lingering effects on long-term production pathways supporting local consumers (van Baalen et al. 2001, Rindorf et al. 2006, Bocaniov and Smith 2009). Further, the foraging range of individual organisms may be fairly local over a short time scale but may be broader over longer time frames (e.g., due to seasonal migrations), leading to shifts in the scale of seasonal trophic structuring (McCann et al. 2005).

Spatiotemporal patterns of trophic structures may not be consistent across trophically-similar species. While variable dominant primary production pathways and prey availability would be expected to affect multiple consumers, foraging behavior and range may vary among species and lead to inconsistent spatiotemporal trophic patterns. Studies examining trophic patterns among species in space and time are relatively rare but some studies have observed similar spatial (Rumolo et al. 2016) and temporal (Como et al. 2018) influences on trophic structures among species. Although not always directly compared within a single study, it appears as though several freshwater fish species from the Laurentian Great Lakes may also have trophic pathways that vary spatially. In nearshore Lake Michigan, both round goby (*Neogobius melanstomus*; Foley et al. 2017b) and age-0 yellow perch (*Perca flavescens*; Happel et al. 2015) diets varied spatially with minimal influence from seasonal variation. More direct comparisons of trophic variation in space and time of these two important freshwater species could better elucidate trophic pathway structuring, with implications for niche overlap and competition among these species.

Many species exhibit dramatic changes in diet across ontogeny, which may impact seasonal and annual variation in trophic structures. These changes often occur as size increases with age leading to the ability to exploit new food items (Werner and Gilliam 1984). Yellow perch are one example, switching from pelagic prey items such as *Daphnia* to more benthic prey such as chironomids in their first year of life (Wu and Culver 1992). Thus, ontogenetic diet shifts could impact the spatial and temporal variability of trophic pathways.

The consistency of trophic patterns can be investigated using multiple diet indicators such as stable isotopes, reflecting prey and environmental changes over a temporal period of weeks to months, and stomach contents, which indexes consumption patterns over a shorter hourly to daily temporal period. Stable isotope analysis examines the ratio of elemental isotopes (such as $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$, $\delta^{18}O$) that are incorporated into an organism's tissue (Hobson 1999, Post 2002, Fry 2006) from the environment or diet. Combining several isotope ratios such as $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$, $\delta^{18}O$ (Layman et al. 2012) can help elucidate relatively long-term differences in diet and spatial residence. Furthermore, sites may vary in their baseline isotope values (Layman et al. 2012), even among spatially

proximate locations, and fish with narrow foraging ranges may reflect site-specific differences in isotope values, whereas fish with large foraging ranges will reflect broader isotope patterns from the incorporation of isotopes from multiple sites. Specific isotopes may discern different patterns in fish foraging and residence, for instance carbon isotope ratios are commonly used to distinguish primary production origins of carbon (Fry 2006), with pelagic sources generally more depleted of ^{13}C than terrestrial and benthic sources (Bartels et al. 2018). Due to trophic fractionation, δ^{15}N increases predictably with trophic position (Fry 2006), differentiating relationships within trophic webs. Nitrogen isotopes have also been useful for identifying different trophic pathways, such as pathways reliant on benthic or pelagic feeding (Thomas and Cahoon 1993, Pinnegar and Polunin 2000). Hydrogen isotopes in consumer tissues have been consistently found to change in relation to δ^2H in both diet and ambient water, with a greater contribution from diet (Soto et al. 2013). Similarly, δ^{18}O also reflects both ambient water and diet contributions, but with a greater relative contribution by ambient water (Wang et al. 2009, Soto et al. 2013, Coulter et al. 2017). Thereby, δ^2H and δ^{18}O ratios in consumer tissues may index both trophic connections and habitat occupancy.

Isotope ratios are limited in their ability to discriminate among specific prey consumed, especially when considering diverse prey bases. Therefore, stomach content analysis is a useful supplement to isotopic studies. Prey items within stomachs can be identified to relatively low taxonomic levels, but are time sensitive, only reflecting recently consumed prey (Hyslop E. J. 1980). Stomach content may be biased by prey-specific digestion rates (Macdonald and Waiwood 1982) and may reflect prey consumed but not assimilated as diet. Studies that combine multiple diet indicators may overcome the caveats of a single indicator and are powerful explorations of trophic pathway structuring.

Through the integration of stomach contents and stable isotope ratios, we examined the importance of local spatiotemporal structures influencing trophic pathways for generalist freshwater fishes in Saginaw Bay, Lake Huron. Saginaw Bay (Figure 1) is a dynamic system, separated into an inner and outer bay with complex hydrological processes (Beeton et al. 1967, Nguyen et al. 2014) in which the inner bay is highly influenced by riverine inputs, and the outer bay is more influenced by water flow from the main body of Lake Huron. In addition to seasonal and yearly fluctuations, the bay has gone

through numerous changes in primary production and fish communities due to phosphorus reduction (Selzer et al. 2014), invasive species introductions (Nalepa et al. 2003, Foley et al. 2017a), as well as the loss of important pelagic fish prey (i.e alewife, *Alosa pseudoharengus,* Nalepa et al. 2013). These changes led to significant impacts on the Saginaw Bay fish and zooplankton community (Nalepa et al. 2013, Ivan et al. 2014) and suggest a need to understand the resulting temporal and spatial patterns in trophic pathways among important recreational and fish prey species such as yellow perch and round goby.

Previous stomach content analysis of Saginaw Bay fishes (Roswell et al. 2013, Foley et al. 2017a) suggested that short-term diets of age-0 yellow perch and round goby varied spatially. Further, stomach contents of these species appeared to be dependent on both available prey and spatially variable prey selectivity. However, it is not clear if these spatial differences in stomach contents represent long-term, distinct trophic compartments, or if spatial variation is consistent across species or age groups. Spatial trophic structuring and the flexibility of communities to consume multiple diet items could be an important component of stability within a dynamic system such as Saginaw Bay by making the broader fish community less susceptible to the fluctuations of a single diet item (MacArther 1955, Redfearn and Pimm 1988).

This study combines isotope ratios ($\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$, $\delta^{18}O$) and stomach contents, to examine the consistency of spatial and temporal patterns between two generalist fish species, yellow perch and round goby, and specifically examined two age groups (age-0 and age-1) within yellow perch. We hypothesized that both trophic indices would be characterized by strong spatial influence relative to temporal effects. Further, we predicted that each of the three fish groups would show similar spatial influences on diet indicators.

2.3   Methods

Yellow perch and round goby were collected from two offshore sites (SB5 and SB10) and two nearshore sites (N1 and V2) in Saginaw Bay, Lake Huron (Figure 1). These sites varied in substrate and vegetation; the offshore site SB5 had mainly small cobble/gravel/sand substrate, SB10 contained a silt/mud substrate (Roswell et al. 2013), nearshore sites N1 and V2 were respectively non-vegetated and vegetated. Fish were collected offshore with a 7.6 m semi-balloon trawl with a 13 mm mesh (See Roswell et al.

2013 for more details), and nearshore using a beach seine (12.2m long x 1.5m high; 2x3mm mesh). After collection, fish were immediately placed on ice and later transferred to -20°C for storage until processing. While fish were collected approximately monthly, herein we focus on fish collected in May and August of 2009 and 2010 (Table 1).

Stomach contents of yellow perch (n_{age-0}=110 n_{age-1}=60) and round gobies (n_{goby}=96) were identified using a dissecting scope with camera and individual diet items were measured using imaging analysis software ImageJ (Schneider et al. 2012). Diet items were enumerated as whole body organisms or heads of partial bodies. Lengths were measured for specimens with whole bodies, and biomass was estimated using published length-mass relationships (for more details see Roswell et al. 2013).

Isotope samples were collected from a subset of individual fish (n_{age-0}=24; n_{age-1}=34; n_{goby}=36) from each of the four sites. Following removal of stomach contents, digestive tract tissue was returned to the fish, whole fish were dried at 70°C for 3 days, and then homogenized. Lipids were removed from samples before isotopic analysis to prevent differences in isotopic values that could mask differences between groups of individuals (Post et al. 2007). Isotopes were washed in a 2:1 chloroform:methanol solution, centrifuged, and the lipid solution was pipetted off the sample. This process was repeated 3 times, after which the samples were allowed to dry at room temperature for 24 hours.

Dry samples were weighed into capsules: (0.65 mg for combined analysis of $\delta^{13}C$ and $\delta^{15}N$, and 0.3mg for combined $\delta^{2}H$ and $\delta^{18}O$ analysis) and analyzed at the Purdue Stable Isotope Facility using similar methods as described by Foley et al. (2014). In short, stable isotopes of C and N were analyzed with a Carlo Erba elemental analyzer and a SerCon 20-22 mass spectrometer (SerCon, Ltd., Cheshire, UK). Isotopes of H and O were measured with a Thermo Fisher Elemental Analyzer and a Delta V isotope ratio mass spectrometer (Termo Scientific, Inc., Massachusetts, USA). Values for each isotope are reported as δ values: $\delta X = ((R_{sample}/R_{reference})-1) \cdot 1000$‰, where X is the isotope of interest (e.g., ^{18}O), and R is a ratio of the abundance of isotope of interest to the "common" isotope (e.g., ^{16}O). Reference ratios for reporting purposes are the internationally-accepted ratios for Vienna Pee Dee Belemnite (^{13}C), air (^{15}N), and Vienna Standard Mean Ocean Water (^{2}H and ^{18}O).

2.4   Analysis

We conducted separate analyses for each fish group: age-0 and age-1 yellow perch and round goby. Stomach content data were transformed into percent biomass of individual prey categories per fish. After stomach content biomass was calculated, the diets of each fish were reclassified into one of 11 prey categories: Chironomidae, Amphipoda, Ephemeroptera, Trichoptera, Other Macroinvertebrates (see Supplemental Material Table 1 for full list.), *Daphnia* spp., Chydoridae, Predatory Zooplankton, Copepoda, Other Zooplankton (see Appendix A Supplemental Tables, Table 11 for full list.), and Fish items. Groups were based on the top contributors to individual percent biomass. We used nonmetric multidimensional scaling (NMDS) to visualize stomach content patterns, reduced to two NMDS axes, using Bray-Curtis distances for each species and age-group (see Appendix B Supplemental Figures, Figure 12). Subsequently, stomach content patterns were analyzed using permutational multivariate analysis of variance (perMANOVA) for each species and age-group, with Bray-Curtis distances and 999 iterations (Oksanen et al. 2016). Explanatory variables for each perMANOVA included fish size (total length), site, month and year (with the exception of the age-0 perch not present in May samples) along with interactions terms. For each potential explanatory variable, permutations were used to calculate partial R^2 values and p-values. The effects of site on stomach contents were evaluated using a SIMPER analysis (Clarke 1993); simper function in R package vegan (Oksanen et al. 2016). This allowed us to examine the relative contributions to dissimilarity of prey groups in pairwise comparisons of sites in the analysis using permutation techniques.

We examined isotopic variation for each fish group (age-0 perch, age-1 perch and round goby) using a perMANOVA. For age-1 perch and gobies, explanatory variables used included the site of collection, year and month, including interaction terms between the variables. Age-0 perch perMANOVA included site and year, excluding month since no age-0 perch were collected in May. The perMANOVA calculations were conducted using Euclidean distances, which compares the linear distances in isotopic space between values. If site was a significant effect, a series of six separate pairwise perMANOVAs were conducted to contrast sites. In each pairwise comparison only site was included as the independent variable to maximize the degrees of freedom and α values were Bonferroni

corrected (α=0.008) to minimize type I errors. A second series of ANOVAs were performed that examined the variation of specific isotope ratios (e.g. $\delta^{13}C$, $\delta^{15}N$, δ^2H, $\delta^{18}O$) across; sites, year, and month. If the site variable was significant a Tukey post-hoc analysis was used to examine which sites were different from one another. Analyses were conducted using the program R (R Core Team 2014) and statistical package vegan (Oksanen et al. 2016).

2.5 Results

Stomach Contents

For round goby, our perMANOVA identified length of fish, site, the interaction between sites and month; and the interaction between sites, months and year to have significant contributions in explaining stomach content composition (df $_{Total}$ =57; R^2_{Length}=0.085, p_{Length}=0.002; R^2_{Site} =0.119, p_{Site} =0.004; $R^2_{Site:Month}$=0.093, $p_{Site:Month}$=0.006; $R^2_{Site:Year:Month}$= 0.076, $p_{Site:Year:Month}$=0.034; for full results see Appendix A Supplemental Tables Table 12). We found (SIMPER analysis) that that dissimilarity among most sites was typically due to variation in chironomid consumption. However, a large degree of dissimilarity was also due to non-dominant pelagic prey items within the other zooplankton category (Figure 2), particularly within site SB10. In addition, differences in prey types, such as pelagic prey items or chironomids, were particularly evident between nearshore (N1, V2) and offshore (SB5, SB10) sites (Figure 2; See Appendix A Supplemental Tables, Table 13 for full SIMPER breakdown).

For age-0 perch, we found that all variables were influential in explaining stomach content variation (perMANOVA; df $_{Total}$ =109; R^2_{Length}=0.086, p_{Length}=0.001; R^2_{Site}=0.242, p_{Site}=0.001; R^2_{Year}=0.022, p_{Year}=0.002; $R^2_{Site:Year}$=0.120, $p_{Site:Year}$=0.001, for full results see Appendix A Supplemental Tables, Table 12). In the SIMPER analysis, we found that differences varied by site and were driven primarily by consumption of copepods, *Daphnia*, other zooplankton, and amphipods (Appendix A Supplementary Tables, Table 14).

For age-1 yellow perch, each variable appeared to contribute to variation in stomach content composition (perMANOVA; df $_{Total}$ =58; R^2_{Length}=0.142, p_{Length}<0.001; R^2_{Site}=0.187, p_{Site}<0.001; R^2_{Year}=0.045, p_{Year}=0.003; R^2_{Month}=0.027, p_{Month}=0.033; $R^2_{Site:Year}$=0.085, $p_{Site:Year}$=0.003; $R^2_{Site:Month}$=0.045, $p_{Site:Month}$=0.032; for full results see

Appendix A Supplemental Tables, Table 12). Site contributed the greatest to the partial R^2 values followed closely by fish length. Differences between sites, according to our SIMPER analysis (Appendix A Supplemental Tables, Table 15), were primarily related to variation in the consumption of chironomids. Dissimilarity among sites was also influenced by the consumption of pelagic prey such as copepods and predatory zooplankton, as well as benthic prey such as amphipods and other macroinvertebrates.

Isotopes

Variation in round goby isotope ratios (Figure 3) was influenced by site (perMANOVA; $df_{Total} = 35$; $R^2_{Site} = 0.391$, $p_{Site} = 0.001$; see full results in Appendix A Supplementary Tables, Table 16). Post-hoc analysis of pairwise comparisons of sites suggested that site specific differences were mainly driven by site N1. All other site comparisons did not meet the criteria for significance after Bonferroni correction (see Appendix A Supplementary Tables, Table 16). Analysis of individual isotopes indicated that $\delta^{13}C$ influenced differences among sites (ANOVA F=32.00, df=35, p<0.001), and $\delta^{15}N$ (ANOVA F=79.00, df=35, p<0.001). Further, δ^2H (ANOVA F=4.66, df=35, p=0.009) also contributed to differences among sites but mainly between nearshore sites. Temporal differences were also observed, mainly due to year, but only for $\delta^{13}C$ (ANOVA F=4.74, df=35, p=0.038), and $\delta^{15}N$ (ANOVA F=5.03, df=35, p=0.033; Appendix A Supplementary Tables, Table 17).

Isotope values of age-0 perch (Figure 3) were strongly influenced by the site where they were collected, and to a lesser degree by the year (perMANOVA; $df_{Total} = 23$; $R^2_{Site} = 0.726$, $p_{Site} = 0.001$; $R^2_{Year} = 0.036$, $p_{Year} = 0.019$; $R^2_{Site:Year} = 0.138$, $p_{Site:Year} = .001$; $R^2_{Residuals} = 0.101$; full results in Appendix A Supplementary Tables, Table 18). Our pairwise comparison of sites found that site V2 differed from both SB5 and SB10, and site N1 differed from site SB5 (Full breakdown see Appendix A Supplementary Tables, Table 18). Among isotope ratios: $\delta^{13}C$ (ANOVA F=38.1, df=23, p<0.001), $\delta^{15}N$ (ANOVA F=38.6, df=23 p<0.001), and δ^2H (ANOVA F=18.9, df=23 p<0.001) contributed to differences among sites. Similar to round gobies, $\delta^{13}C$ (ANOVA F=71.2, df=23 p<0.001) and $\delta^{15}N$ (ANOVA F=8.47, df=23 p<0.001; Appendix A Supplementary Tables, Table 19) isotopes were also found to have contributed to yearly differences in isotope ratios.

Isotopic composition of age-1 perch (Figure 3) were related to site and the interaction of site and year or month (perMANOVA; df_{Total}=33; R^2_{Site}=0.162, p_{Site}=0.014; $R^2_{Site:Year}$=0.126, $p_{Site:Year}$= 0.031; $R^2_{Site:Month}$=0.376, $p_{Site:Month}$= 0.001; full results in Appendix A Supplementary Tables, Table 20). However, our post hoc comparisons did not suggest any differences among isotope ratios of paired sites after Bonferroni correction (see Appendix A Supplementary Tables, Table 20). Examination of individual isotopes by ANOVA found only $\delta^{15}N$ (ANOVA F=12.2, df=33, p<0.001) contributed to differences among sites, with differences occurring between site N1 and all other sites. No differences were evident among years or months (see Appendix A Supplementary Tables, Table 21).

2.6   Discussion

The diet indicators, stomach contents and stable isotopes, expressed similar results; highlighting significant spatial differences in diet and to a lesser extent temporal differences. Our observations of spatial influences on stomach contents are analogous to previous Saginaw Bay studies on yellow perch (Roswell et al. 2013) and round goby (Foley et al. 2017). Stable isotope analyses support our hypothesis and suggest that the spatial trophic differences represent extended local foraging and residence by these three fish groups. In addition, it is worth noting that spatial influences on diet variation were generally stronger than other well studied impacts on diet, such as differential prey selection by size and age (i.e. ontogenetic diet shifts; Werner and Gilliam 1984, Wu and Culver 1992). Even though size can influence the types of food an individual may consume, they are still reliant on the prey available at a particular location.

Direct or interactive temporal effects were generally important only when examining stomach contents, which may be more sensitive to short-term differences in prey availability and foraging behavior. Spatiotemporal patterns in stomach contents could be influenced by multiple factors such as altered foraging habits (e.g. ontogenetic diet shifts; Werner and Gilliam 1984, Wu and Culver 1992) or successional changes in prey communities. Seasonal succession of prey, and annual variation in prey, could vary between locations within the inner bay (SB5, SB10 or V2) due to thermal differences or seasonal changes in river effluents, compared to locations within the outer bay (N1). River plumes and hydrodynamics within the bay could contribute to variation in temperature,

sedimentation and primary production (Beeton et al. 1967, Rao and Schwab 2007, Kraft et al. 2010, Makarewicz et al. 2012). Similarly, these factors could contribute to horizontal variation in invertebrate communities (Pinel-Alloul et al. 1999, George and Winfield 2000), although changes in horizontal distribution of prey may not always be observable within stomach contents (Marin Jarrin et al. 2015).

Our stomach content analysis yielded similar results to previous studies, for instance round goby stomach contents in Saginaw Bay were found to have varying prey selectivity with location and month (Foley et al. 2017a) and round goby in Lake Michigan displayed significant spatial variation in stomach contents, fatty acid composition and stable isotope composition (Foley et al. 2016). The stomach contents of all 3 fish groups in our study were more strongly related to spatial variables than fish length. This is particularly interesting for age-0 perch, which might have been limited in their ability to forage on a diverse range of prey, due to their smaller size. Previous work has noted that this may not necessarily be due to a lack of diverse prey availability, since age-0 yellow perch appeared to select particular diet items at each site and did not appear to consume prey in proportion to prey abundance in the environment (Roswell et al. 2013).

Consistent spatial differences in isotopic composition of round gobies and young perch suggested that fish fed regularly near their location of capture (i.e., limited movement throughout the bay) and potentially fed consistently on certain types of prey. We also observed that the majority of spatial differences were driven by $\delta^{13}C$ and $\delta^{15}N$, isotopes which are closely connected to diet. Specifically, for age-0 yellow perch, δ^2H also drove differences among sites and is associated with both diet and ambient water. Many of the spatial differences observed in our isotope analysis were driven by contrasts between the inner bay sites (V2, SB5, SB10) and the outer bay site (N1), however differences between nearshore (V2, N1) and offshore sites (SB5, SB10) were also evident.

Isotope ratios in fish tissue may vary from site to site due to baseline differences at each location (Post 2002, Layman et al. 2012). These differences could have occurred due to different river effluents near each site, or mixing with water from the greater body of Lake Huron (i.e. site N1; Nguyen et al. 2014). For example, patterns observed in $\delta^{15}N$ among our fish groups, appeared to discriminate nearshore or offshore locations. Nearshore locations can have greater terrestrial influences, such as due to agricultural runoff, which

can enrich aquatic habitats with heavier nitrogen isotopes (Larson et al. 2012). However, fish in our analysis had opposite results, in which nearshore sites actually had lower $\delta^{15}N$ relative to their counterparts in offshore sites.

We observed nearshore and offshore differences in $\delta^{15}N$ among our fish groups, but not in production pathways measured by $\delta^{13}C$. Carbon from Lake Huron, influencing site N1, might have been expected to be influenced by primary production using depleted atmospheric carbon (Fry 2006). However, the measured $\delta^{13}C$ was more enriched in this site compared to the inner bay sites. This suggests that site N1 might have been relying more heavily on benthic sources of carbon, or this site was more heavily influenced by terrestrial effluent from nearby river sources.

Most of the isotopes we examined can be affected by differences in consumption (i.e $\delta^{13}C$, $\delta^{15}N$ and δ^2H). Consumption could produce a site-specific isotope signal if prey selected by fish differed among sites and fish consistently foraged within the same location. We would have expected our fourth isotope ratio, $\delta^{18}O$, to also vary among sites, even though the ratio of oxygen isotopes is more strongly influenced by surrounding water than diets (Wang et al. 2009, Soto et al. 2013). Saginaw Bay has multiple streams and rivers that empty into the region which could drive differences in $\delta^{18}O$ among sites. However, consistent currents throughout the inner and outer bay (Nguyen et al. 2014) could homogenize the waters, and therefore the $\delta^{18}O$, and explain why our fish samples did not differ in $\delta^{18}O$, even between the furthest apart sites.

We observed significant spatial influences on isotope ratios among all three fish groups, but the extent of differences varied among each group. For instance, age-0 perch exhibited offshore-nearshore differences in $\delta^{15}N$, as well as both $\delta^{13}C$ and δ^2H; whereas age-1 yellow perch did not display similar variation. This difference in age-0 fish may reflect the faster isotope turnover, due to rapid growth of younger fish (Vander Zanden et al. 1998, Weidel et al. 2011). Therefore age-0 yellow perch may not need to reside within a site for very long to accumulate a site-specific isotope signal. In addition, age-0 yellow perch may not have been able to forage as broadly among sites due to their smaller size and limited swimming ability, compared to older fish. Similarly, round gobies are also not known for broad foraging ranges (<10km; Bergstrom et al. 2008, Marentette et al. 2011). This may have contributed to the significant differences in pairwise comparisons of isotope

values among sites for round gobies. In contrast, age-1 yellow perch only displayed significant differences among sites when all isotopes were examined in the global analysis, but the only significant pairwise test was between site N1 and site SB5. The similarity among age-1 yellow perch isotopes may have been due to a broader foraging range for age-1 perch, with some juvenile perch known to travel between 10-100km (Glover et al. 2008).

Age-0 yellow perch differed among sites in multiple isotope ratios, whereas only $\delta^{15}N$ of age-1 perch differed among sites. Both age-0 and age-1 yellow perch consumed predominately chironomids at most of the sample sites, which has also been observed in other yellow perch populations (Wu and Culver 1992, Hrycik et al. 2018) as well as the Saginaw Bay yellow perch population (Roswell et al. 2013). In addition, age-1 perch were able to feed on a larger niche of prey including fish prey, such as round gobies. Interestingly, the $\delta^{15}N$ values between age-0 and age-1 yellow perch were similar, despite increased fractionation (Post 2002) of $\delta^{15}N$ with increasing trophic level. This may suggest that the older fish were not feeding at a pronounced higher trophic level compared to young-of-year.

It is plausible that differences in trophic pathways among sites could produce additional stability in the broader community within Saginaw Bay. Several studies have suggested that compartmentalized trophic pathways, such as spatially structured pathways, could help stabilize whole communities (Krause et al. 2003, Stouffer and Bascompte 2011, McMeans et al. 2016). Fluctuations could occur in any one compartment and be less likely to have catastrophic impacts on the greater community. The differential use of resources at each site within Saginaw Bay may be an example of this stability. Such spatial compartmentalization may have improved the resilience of the Saginaw Bay fish community to the major past (Nalepa et al. 2003, 2013, Ivan et al. 2014, Selzer et al. 2014) and potential future ecosystem changes.

2.7   References

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Table 1 Distribution of individual fish by month and site for isotope & diet analysis.

Sample Sites for Isotope Analysis									
Fish	**Total**	**Year**		**Month**		**Site**			
		2009	*2010*	*May*	*August*	*N1*	*V2*	*SB10*	*SB5*
Goby	36	18	18	13	23	11	10	6	9
Perch Age-0	24	12	12	0	24	6	6	6	6
Perch Age-1	34	17	17	16	18	7	10	7	10
Sample Sites for Stomach Content Analysis									
Fish	**Total**	**Year**		**Month**		**Site**			
		2009	*2010*	*May*	*August*	*N1*	*V2*	*SB10*	*SB5*
Goby	96	37	21	33	25	10	11	10	27
Perch Age-0	110	58	52	0	110	12	19	41	38
Perch Age-1	63	25	38	34	29	7	20	16	20
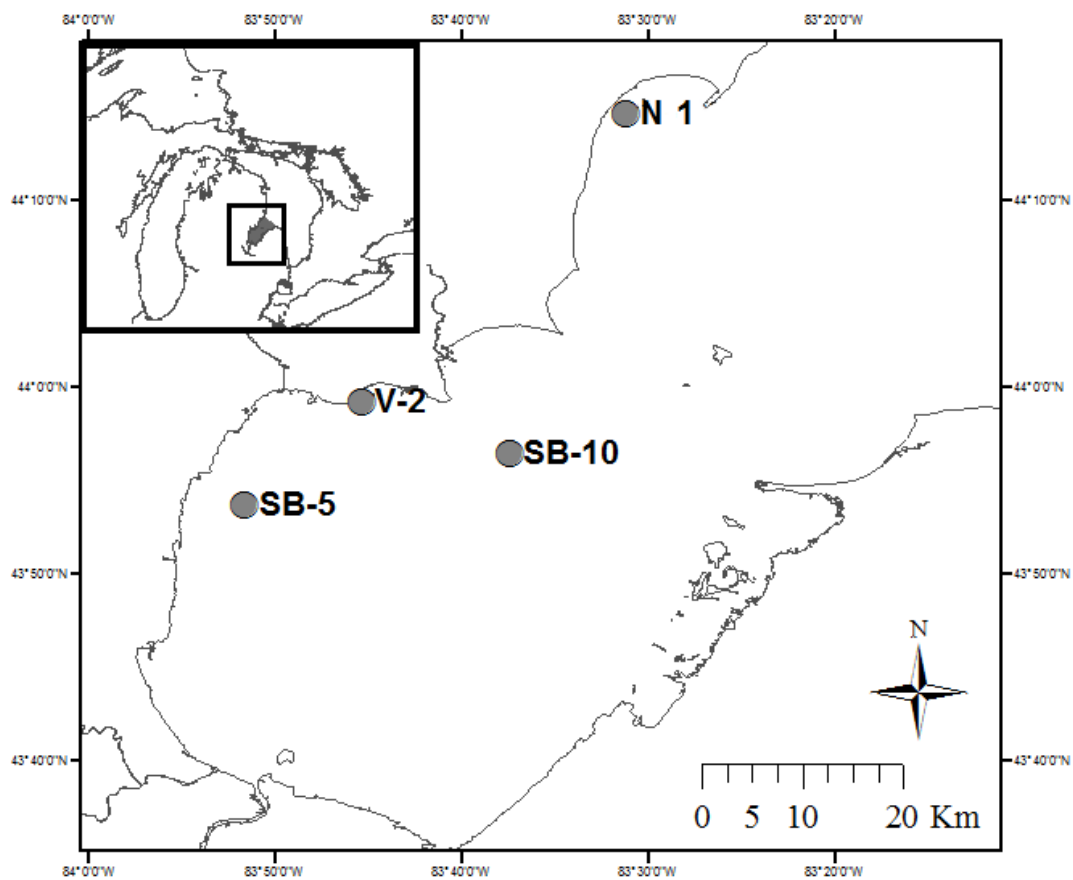
Figure 1 Location of sampling sites within Saginaw Bay, Lake Huron for yellow perch and round goby collections used in isotope and stomach contents.
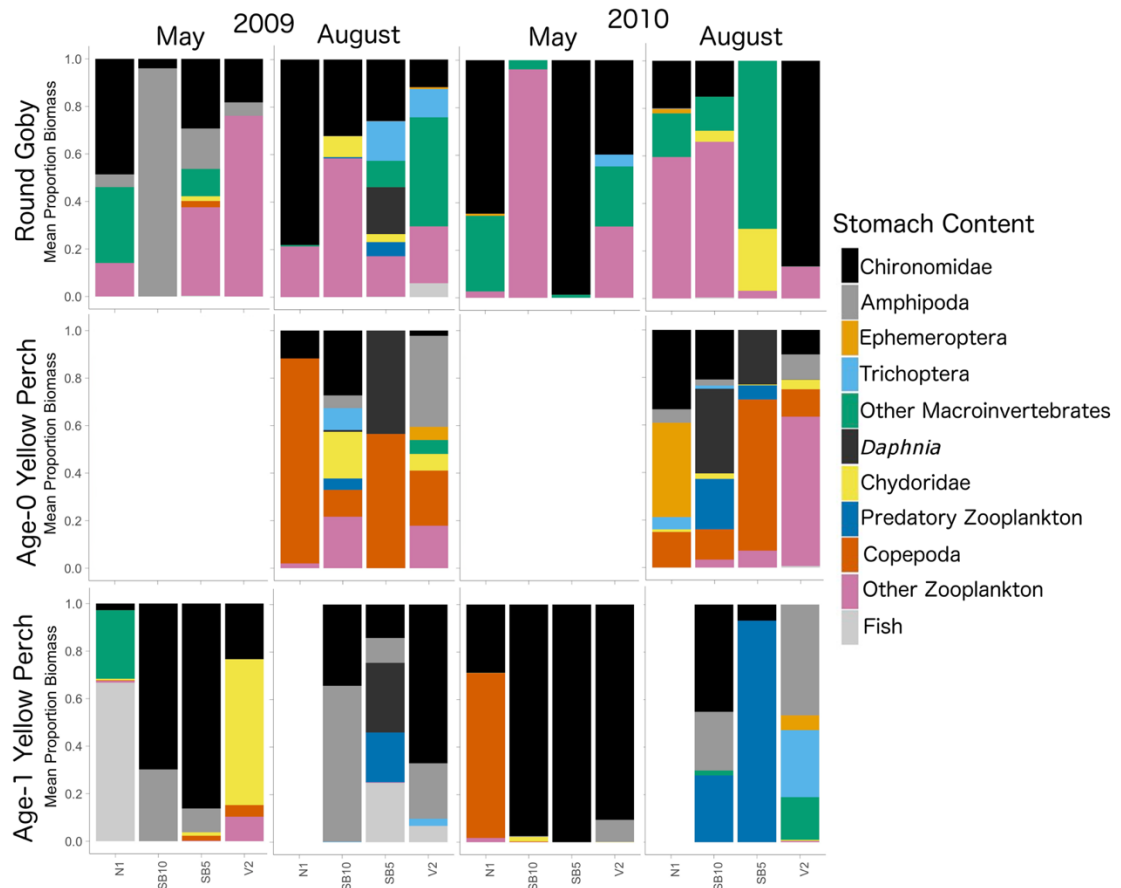
Figure 2 Mean proportional stomach content composition for round goby (top), age-0 yellow perch (middle) and age-1 yellow perch (bottom) within each of 4 sites across years and months. Note: No age-0 yellow perch were collected in May and no age-1 yellow perch were collected from site N1 during August of 2009 or 2010.
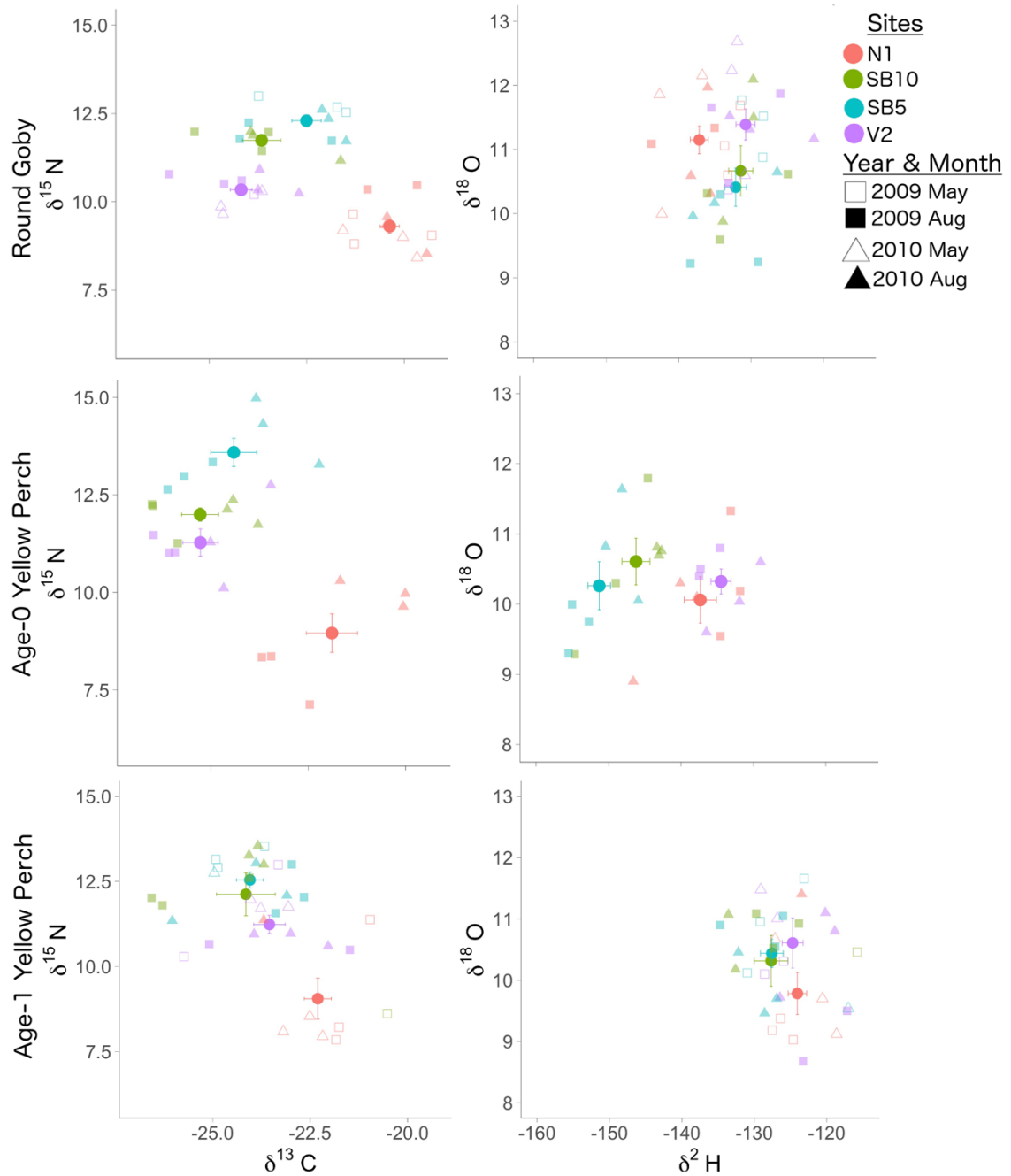
Figure 3 Isotope ratios of individual (small points) round goby (top), age-0 perch (center) and age-1 perch (bottom) presented by site, season and year. Mean (larger round points) and standard error bars for each site are also depicted in each bi-plot.

CHAPTER 3.   SIMULATED ENVIRONMENTAL INFLUENCES ON YELLOW PERCH MORPHOLOGY

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3.1   Abstract

Morphological variation among individuals of the same species is influenced by various processes, including selection for both canalized morphology and phenotypic plasticity. Among fishes, morphology often varies consistently with habitats, such as the deep bodied shapes associated with littoral, structured habitats or fusiform shapes within pelagic zones. However, other environmental conditions such as the presence of predators, can also induce similar morphological differences within populations. We conducted a series of experiments with yellow perch, *Perca flavescens*, to examine the influences of specific environmental conditions on perch morphology. We first conducted a mesocosm experiment with age-1 yellow perch using simulated littoral and pelagic habitats and examined morphology using a traditional aspect ratio approach, as well as a landmark analysis. We found significant differences in morphology among simulated habitats, with observed shape changes similar to yellow perch morphologies observed in natural systems. We then tested yellow perch within four different environmental conditions in 2015; open, chironomid diet, predation risk, and structured environments. Again, we found significant differences in morphology among treatments. Concerned about low survivorship within our previous experiment, we replicated the structure and open environmental conditions in 2016 and found that the types of shape change between treatments were different than what was observed previously. The differences in shapes we observed are likely the combination of differential mortality among our treatments, as well as potential plastic responses to environmental treatments.

3.2   Introduction

Fish morphology represents an expression of individual genotype and may be reflective of environmental conditions through phenotypic plasticity (i.e. different expressions of a genotype). Populations of fish may have a large degree of morphological variation (Olsson and Eklöv 2005, Torres-Dowdal et al. 2012, Yavno and Fox 2013) while others have highly canalized, genetically fixed, morphologies (Waddington 1942). Populations may express high morphological variation through individual differences in development and environmental interactions; including variable predation risk, prey availability and structural environment (Brönmark and Miner 1992, West-Eberhard 2003, Langerhans 2008, Svanbäck and Schluter 2012). Morphological variation, particularly among populations, has been related to genetic variation (McCairns and Bernatchez 2010, Yavno and Fox 2013) and may reflect adaptations to particular environmental conditions, such as the adapted ecotypes of rainbow trout, *Oncorhynchus mykiss* (Keeley et al. 2007). Morphological variation within populations may also reflect phenotypic plasticity (but see Faulks et al. 2015) and favor improved individual performance within specific habitats, such as the foraging efficiency of different morphologies for Eurasian perch, *Perca fluviatilis* (Svanbäck and Eklöv 2003).

There is no shortage of morphological studies that have examined intra-specific morphological variation of fish. Consistent patterns have emerged as to the types of habitats which may drive divergence in morphology, as well as the types of shape differences among habitats. Various studies have identified morphological contrasts between benthic-littoral and pelagic habitats (Svanbäck and Eklöv 2003, Olsson and Eklöv 2005, Svanbäck and Schluter 2012, Gaston and Lauer 2015, Marklund et al. 2017), limnetic and riverine environments (Keeley et al. 2007), as well as morphological changes in response to predation risk (Brönmark and Miner 1992, Ghalambor et al. 2007). In many cases, the morphologies associated with different habitats and environments are related to changes in body depth (i.e. deep bodied contrasted to fusiform shapes) and body orientation (bodies arched towards the ventral or dorsal direction). These differences in shape have been associated with foraging (Svanbäck and Eklöv 2003), the risk of predation (Brönmark and Miner 1992), and swimming behaviors (Webb 1984). While there is consistency in the

types of habitats and shape change influencing intra-specific fish morphology, less is known on how more specific environmental conditions influence body shape.

Studies have previously examined intra-specific fish morphology using coarse differences between natural environments (i.e. pelagic vs benthic/littoral; Svanbäck and Eklöv 2004, Svanbäck and Schluter 2012). However, habitats are characterized by a variety of environmental conditions, many of which have been separately observed to influence morphology; such as temperature (Martsikalis et al. 2018), structure (Olsson and Eklöv 2005), predation risk (Brönmark and Miner 1992), and diet complexity (Heermann et al. 2007). Differences in such individual factors could affect the morphological differentiation between habitats; for example differences in water clarity among lakes had a significant influence on the degree of divergence in habitat-specific morphologies for Eurasian perch (Scharnweber et al. 2016a).

Few controlled experiments have contrasted the influences of individual environmental conditions on fish morphological expression. Thus, it may be difficult to predict how morphological expression will respond to individual or interacting environmental factors. For example, pelagic environments may induce fusiform morphologies in fishes (Faulks et al. 2015), and habitats with greater predation risk may induce fishes with deeper body morphologies (Brönmark and Miner 1992). Therefore, fish residing in pelagic habitats with high predation risk could have contrasting influences on their body shape. This could result in morphologies less-fit for either environment. It is therefore valuable to examine influences from different environment types to identify how morphologies respond as well as the magnitude of response.

Yellow perch, *Perca flavescens*, are a popular fish for both commercial and recreational fisheries, in North America. Morphological studies examining the variation among yellow perch have been associated with genetic differences among populations (Sepulveda-Villet and Stepien 2012), as well as occupation of different habitats (i.e. nearshore and offshore; Parker et al. 2009). Parker et al. (2009) identified longer and deeper bodied perch in nearshore lake habitats compared to the more structurally complex wetland regions. Another study contrasted morphologies in lakes with and without predators and found that yellow perch in lakes with predators had deeper bodies (Lippert et al. 2007). Yellow perch are congeners of Eurasian perch, which have been the focus of a series of

morphological plasticity studies (e.g. Svanbäck and Eklöv 2006, Mustamäki et al. 2014, Faulks et al. 2015) linking Eurasian perch morphology to habitat occupation and foraging behavior.

Prior to examining the influences of specific environmental conditions, we began our study by first identifying if simulated habitats (pelagic and littoral) could produce divergent morphologies similar to those observed in the field. We then examined the influences of four environmental conditions (open, structure, predation, chironomid diet) over two years. Following this experiment we then replicated the structure and open conditions the following year, where we aimed to improve survivorship within our pools as well as examine if the environmental treatments would produce morphological changes similar to the previous year. Throughout our experiments we aimed to examine morphological change using simple, traditional morphometrics, and holistic morphological analyses (i.e. landmark morphometrics) ascertaining if changes to morphology were observed, as well the types of shape change. We hypothesized that simulated littoral and pelagic habitats would produce divergent morphologies in perch, with shape differences similar to morphologies found in natural systems. Following this experiment, we hypothesized that open and structure conditions, would have the greatest divergence in yellow perch morphology, similar to what has been seen in past morphological studies (Olsson and Eklöv 2005). Finally, we expected that the environmental conditions, specifically structure and open conditions, would produce similar changes in yellow perch morphology across different years.

3.3   Simulated Habitat Mesocosms

Our first experiment examined the influences of two simulated habitats, in 2015, on morphological expression of age-1 yellow perch. Yellow perch in this experiment originated from a population that was collected between 1992-1993 in the Perquimans River in North Carolina, USA (Personal Communication with Dr. Paul Brown, Purdue University). Since its initial collection, the population has been maintained at Purdue's Baker Aquaculture Research facility in West Lafayette, Indiana. A small pilot study conducted in 2014 utilized the same cohort of yellow perch, at age-0, within a similar mesocosm design (Appendix C. Supplemental Analysis) and suggested that these yellow

perch had a capacity for morphological change in response to simulated habitats. Our 2015 mesocosm study, began on June 2 and utilized twenty fish per mesocosm (total length mean±sd; 92.29±10.8mm, Table 2), with eight mesocosms simulating four pelagic and four littoral habitats for 135 experimental days.

Mesocosm cages were constructed using 6mm meshed plastic and PVC piping (dimensions 100x100x30cm). Approximately 50 pieces (25cm/piece) of polypropylene rope were added to each littoral cage to simulate structural features of littoral zones, and the cages themselves were anchored to the pond benthos approximately 1 meter from the shoreline. Pelagic cages were suspended just below the surface near the center of a quarter acre aquaculture pond. Mesocosms were designed to provide fish in each simulated habitat with natural prey found in the pelagic and littoral regions. Littoral cages were moved approximately 1m each week to provide fish with fresh benthic foraging material.

At the conclusion of the 135 day period, fish were removed and lightly anesthetized using MS-222 to capture images for morphometrics. Digital images of fish were taken on a concave board with a scalebar in each image and photographed using a Panasonic CMC-TS5 camera. These images were used for morphometric analysis (described further below.). Fish total lengths were measured to the nearest mm and weights to the nearest 0.01g. The growth of fish (mm/day) in each habitat were compared using a nested ANOVA, nesting among cages. Average length was calculated for fish pre-experiment and used to calculate growth between the start of the experiment and its conclusion after 135 days.

3.4   Morphometric Analysis: Simulated Habitat Mesocosms

Our examination of morphology occurred in two separate analyses. First, we examined individual yellow perch using an aspect ratio (AR) approach, similar to traditional morphometric studies, in which an individual's AR was indexed as the ratio of body length (measured as standard length from the jaw to the end of the caudal peduncle) and body depth (measured from the anterior base of the first dorsal fin to anterior base of the pectoral fin; Figure 4). This ratio has been shown by previous studies to be representative of morphological differences between littoral and pelagic fishes (Gaston and Lauer 2015). A second AR index quantified the ratio of body length to caudal depth (measured as the narrowest point of the caudal peduncle; Figure 4), which has been

correlated to fish swimming speeds and behaviors (Webb 1984). These were used to compare the habitat treatments using separate nested ANOVAs for each AR, nesting individuals within their respective cages.

In addition to the AR analysis, we compared morphology using landmark analysis, which provides a more holistic examination of morphological variation for the entire fish body. This approach, unlike traditional morphometrics, quantifies whole body shapes by comparing the relative positions of assigned landmarks to identify differences in shape among treatments (Zelditch et al. 2004). Landmark analysis was prepared by selecting 18 landmark points (Figure 4), similar to other Percidae studies (Olsson and Eklöv 2005, Scharnweber et al. 2016), using the program TpsDig (Rohlf 2005). A procrustes procedure was carried out on all images which scaled, translated and rotated images to reduce variation related to image effects (e.g. size and orientation), focusing on the shapes of individual fish (Zelditch et al. 2004). In short, this normalized images for analysis and placed them into a coordinate system centered around 0.

Fish within experimental mesocosms had different sizes at the conclusion of the study and some shape change can be attributable to differences in size and development (i.e. allometry; Klingenberg 2016). To account for potential allometric-based variation in shape, a permutational multivariate analysis of variance (perMANOVA) was conducted using the procrustes coordinates as dependent variables and the individual centroid sizes as the independent variable (See Faulks et al. 2015 for similar methods). Centroid size is correlated with fish size and represents the average distance between each landmark and the center of individual shapes (Klingenberg 2016). Residuals for individual fish were extracted from this perMANOVA, removing variation in shape expected from allometry. These allometry-corrected residuals were then used to compare morphology among habitats through a nested perMANOVA, nesting individuals among cages.

In order to visualize the differences in morphology between our two treatments, we conducted a canonical variance analysis (CVA). This analysis collapsed the morphology of our perch into a single canonical variate axis, which represented changes in shape that discriminated the pelagic and littoral treatments. A CV axis may be interpreted similarly to the AR values calculated earlier in our study, except an AR value represents changes in 2 variables (e.g. length and width) while the CV axis represents changes across 18

landmarks. This method can therefore assist us in identifying when complex changes in shape occur. We tested the validity of this axis by cross-validating the CV axis via Jackknife cross validation which provided a percent classification accuracy for which individuals could be reassigned to their correct treatment. All statistical and morphological analyses were conducted in R (R Core Team 2014) using the packages vegan (Oksanen et al. 2016), lme4 (Bates et al. 2015), geomorph (Adams et al. 2018), and Morpho (Schlager 2017).

3.5   Environmental Conditions

Our examination of how specific environmental conditions influenced yellow perch morphology was conducted using two similar experiments. The first experiment was conducted in 2015 and examined four environmental conditions (open, structure, predation and chironomid forage). Survivorship in this study was lower than expected and therefore a second experiment was conducted in 2016 and focused only on open and structure environments. Study systems were constructed within 24, two-meter wide, circular cattle pools. Pools received a constant flow of water from a common well source and maintained mean temperatures of 21.5° C (range 19.3° C to 24.0° C) and 22.3° C (range 19.3° C to 23.7° C) during 2015 and 2016, respectively. Flow rate into each pool during either experiment was minimal and maintained at approximately 14 cm^3 per minute. Yellow perch in both experiments descended from wild populations within Lake Erie but were maintained in an aquaculture setting for several generations (Personal Communication with Ohio Department of Natural Resources). These fish were hatched at St. Mary's Fish Hatchery in St. Mary's, Ohio before being brought to Purdue's Baker Aquaculture Research Center.

In our first experiment, each pool was stocked with 50 perch (total length mean±sd: 54.7±8.2 mm) and the experiment was conducted for 60 days. Any fish that died during the experiment were recorded and removed from the pool, however mortality was generally only observed if fish were floating due to bloat and therefore were unsuitable for morphometric analysis. Following the experimental period, fish were euthanized using tricaine methanesulfonate (MS-222) and frozen at -20° C for morphometric analysis. All fish were frozen at the same time following euthanasia, however storage lengths may alter

the morphologies of fish over time (Kočovský 2016). Fish in the 2015 pool experiment were imaged over a period ranging from October 27th to December 30th, 2015. To account for impacts due to variable storage lengths, the imaging date was included as a variable in the final morphology analysis (see below).

The four treatments: open, structure, predation and chironomid forage were simulated as follows. Open treatments received no special augmentations to the pools. Structured treatments were built using 50cm length pieces of polypropylene rope, glued to the pool base, to mimic vegetation. Rope was randomly distributed, and density was approximately 200 lines per square meter. Predation pools contained a perceived, olfactory only, predator threat from 4 large, adult yellow perch. These predators were fed young-of-year yellow perch several times each week and water from the predator tank was distributed continuously into each predator treatment, maintaining the average flow rate for each pool (14 cm^3 min^{-1}). The consumption of young-of-year yellow perch likely induced the predators to produce kairomones in their tank, which have been previously observed to illicit anti-predator responses, including increased growth rates, from young-of-year perch (Pangle et al. 2012, Barry et al. 2017). The final treatment provided an alternative diet item, chironomids, to alter the forage behavior of the fish. Each experimental day, 5g of frozen chironomids were provided to the alternative diet treatment pools. The amount of chironomids provided was decreased when mortality was observed within the pool, to maintain equal proportions of food per individual within the chironomid diet pools. Perch were fed by placing frozen chironomids into 2 weighted sections (10 cm^2 each) of artificial turf, which was intended to stimulate benthic-littoral foraging in yellow perch by forcing them to feed from the bottom of the pool.

The open, structure and predator treatments received zooplankton as prey for feeding. Zooplankton were collected daily from a nearby aquaculture pond, counted and distributed to each of these treatment except the chironomid pools. Zooplankton densities varied during the experimental period reflecting natural changes in pond zooplankton populations, including a large increase in cladocerans during the second half of the 2015 experiment. When fish mortality was observed within a pool, the amount of supplemental zooplankton to be added was adjusted to keep the proportion of food per individual equivalent across pools. Zooplankton within pools were sampled on a weekly basis by

siphoning out 15L of water and filtering through a 50μm mesh. Zooplankton density did not appear to differ among pools receiving zooplankton over the 60 day period.

A second environmental conditions experiment was conducted in 2016, following lower than expected survivorship in the 2015 experiment (see Results Environmental Conditions). This experiment duplicated only the open and structure treatments from the previous year. Several additional changes were also made to our experimental setup. First, the structured pools were altered slightly by attaching artificial vegetation to chicken wire and anchoring it to the bottom. Further the structured treatments used an increased density of vegetation compared to the previous experiment (250 pieces per square meter). Wire was also anchored to the base of open pools, but with no artificial vegetation. Finally, following a shorter 50 day experimental period, each fish was lightly anesthetized using MS-222, during which fish total length (mm) and mass (g) was recorded, and each fish was photographed live with a scalebar on a concave surface using a Panasonic CMC-TS5 camera.

A new cohort of age-0 Lake Erie yellow perch (total length; mean±sd; 65.59 ±9.5 mm) were distributed into either an open or structured pool. Into these treatments we stocked 40 fish per pool, a lower fish density compared to the previous experiment to reduce intraspecific competition for food. Fish were stocked within 20 pools, for a total of 400 fish per environmental treatment at the start of the experiment. The experimental period lasted for 50 days during which all pools were provided supplements of zooplankton from a nearby aquaculture pond. While the physical conditions of the pools were generally similar between 2015 and 2016, zooplankton counts (Appendix A Supplemental Tables, Table 22) were lower in 2016. Since zooplankton were less abundant, all pools received the same amount of fresh zooplankton each day and were not reduced when fish mortality was observed in order to maintain an abundance of food items for fish.

3.6   Morphometric Analysis: Environmental Conditions

The preparation of morphometric information for both environmental condition experiments was analogous to the landmark analysis procedure used within the mesocosm study. This included the same 18 landmark points and adjustments for allometry for all individuals before morphometric analysis. In addition, we compared the growth of yellow

perch individuals in each treatment. Average length from a subset of perch measured at the start of each experiment was subtracted from the final length of each individual perch, from each pool, and divided by the number of experiment days (60 for 2015 and 50 for 2016). Mass was not measured for individuals at the start of the 2015 experiment but was measured in 2016. Differences in growth (mm d^{-1}) among treatments were examined in each experiment using a nested ANOVA, nesting individuals to their pools. Survivorship was also examined among treatments, separately for each experiment, using ANOVA and followed up by a post-hoc Tukey test when significant differences were found.

A nested perMANOVA tested the influence of environment treatments on individual morphologies for both environmental condition experiments. The 2015 experiment used a two-way perMANOVA design, which included the treatment along with the date of imaging, to account for variable freezing storage lengths on body shape, and nested individuals by their pool. A one-way perMANOVA tested only the treatment variable for the 2016 experiment since fish were imaged before freezing. This analysis, in both experiments, was followed by a canonical variance analysis (CVA), which examined the magnitude and types of shape change occurring among treatments. The 2015 experiment CVA collapsed the shapes of fish into 3 axes based on known groups (the environmental treatments), while the 2016 experiment utilized only a single axis. In both experiments, we calculated a Mahalanobis distance between treatment group means. Greater Mahalanobis distances between treatment group means along the CV axes indicated greater shape differences between treatments. In addition, we cross-validated CVA axes using a jackknife cross validation and evaluated the percent classification accuracy.

3.7   Results: Simulated Habitat Mesocosms

At the conclusion of the mesocosm experiment, we removed 89 fish from our eight mesocosms (Table 2). This corresponded to 69% of the fish initially placed in the littoral mesocosms and 43% within the pelagic mesocosms; perch were lost through mortality, or potentially, escapement. Based on a nested ANOVA, there were no significant differences in growth between pelagic and littoral treatments in log growth of all fish (mean±sd=0.21±0.1 mm d^{-1}; $F_{1,87}$=1.42, p=0.28); however, the nesting factor, cage, had a

significant effect on growth (p<0.001). Mean length-depth and length-caudal aspect ratios of littoral perch were not significantly different than pelagic perch (nested ANOVA simulated habitat; length-depth; $F_{1,81}$=3.27, p=0.14; length-caudal; $F_{1,81}$=1.94, p=0.21); however cage had a significant effect for length-caudal measurements, but not for length-depth measures (nested ANOVA cage; length-depth; p=0.11; length-caudal; p=0.015).

In contrast to AR analyses, the more holistic landmark morphology analysis revealed significant differences in morphology between habitat treatments (nested perMANOVA; pseudo-$F_{1,81}$=2.14; R^2=0.034, p=0.04), as well as among the cages (pseudo-$F_{6,81}$=1.5, R^2=0.10, p=0.01). The CVA had an 88.7% classification accuracy and the Mahalanobis distance between our pelagic and littoral treatment was 2.3 (Figire 5A). Examination of the shape differences, using CVA, helped to explain why the landmark analysis was in contrast to the aspect ratios. Shape differences in body depth and caudal peduncle depth were not evident; whereas differences in fin placements were evident, resulting in longer body trunks, but not deeper trunks, for fish in littoral treatments. We also observed that individuals from pelagic treatments displayed longer heads and longer peduncles, but with slightly shorter body trunks, particularly along the ventral side. This change in shape would not have been picked up in the AR test which only examined the entirety of fish standard length, not specific segments of fish length. Finally, the CV axis appears to suggest that head orientation may also differ between our treatments, with landmarks shifting in angles that extend the jaw and orient the head dorsally in pelagic fish, and vice versa in littoral fish.

3.8 Results: Environmental Conditions

During the 2015 experiment, yellow perch displayed limited growth. The open (mean±sd 0.02±0.17 mm d^{-1}) and chironomid (mean±sd 0.02±0.13 mm d^{-1}) treatments grew positively, whereas predation (mean±sd -0.06±0.12 mm d^{-1}) and open (mean±sd 0.00020±0.19 mm d^{-1}) treatments had decreasing or no change in length. These differences in growth were only borderline insignificant ($F_{3,625}$=3.24, p=0.051; see final lengths and mass Table 3.2). Survivorship was significantly different among treatments (ANOVA; $F_{3,20}$=10.38, p<0.001). Pairwise comparisons of treatments using a Tukey post-hoc test found that significant differences were driven only by chironomid treatments (mean±sd

survivorship, 84±0.8%) with all other treatments having statistically similar survivorships (mean±sd survivorship; predation= 50±2.4%, open 36±1.6%, and structure 39±1.3%; Table 3). The nested two-way perMANOVA conducted to test morphologies among the environment treatments indicated that the environmental treatment had a significant effect (R^2=0.085, $F_{3,628}$=20.5, P=0.001). In addition, the nesting factor, pool, was significant (R^2=0.048, $F_{14,628}$=2.5, P=0.001), but duration of freezing storage was not (R^2=0.046, $F_{16,628}$=0.84, P=0.882).

The CVA also supported the separation among treatment groups (Figure 5B) using 3 axes with a classification accuracy of 82%. The first CV axis appears to closely reflect the differences between fusiform bodies and deeper bodies (higher values). Unexpectedly, chironomid-fed yellow perch were more likely to have these fusiform shapes in comparison to open and predation risk treatments. Neither of the remaining axes appeared to show separation of chironomid-fed perch from the other treatments. The second axis highlighted changes in landmark positions which produced a larger, almost 'rounder' trunk in perch shapes (lower values). Along this axis, both structure and predation treatments were distributed toward this rounder morphology, opposite of open treatments. Finally, the third CV axis reflected both differences in head orientation and caudal peduncle length, wherein individuals with lower values had heads directed dorsally with shorter peduncle lengths. Along this third axis, predation treatments were distributed lower on the axis, opposite of structured and open treatments. For more detailed changes in shape, see Figure 5B. Mahalanobis distances (For full D_m results see Table 4) calculated between pairwise comparisons of treatments, suggested that the predation and structure treatments were the most similar (D_m=2.71) and the open and structure treatments were the most divergent in morphology (D_m=3.59).

We replicated the structure and open environments in 2016 with the intention to improve fish survivorship but continued to have low survivorship (mean±sd survivorship; open=25±13.0 %, structure=18±14.8 %). In 2016, this was likely due to lower zooplankton counts (See Table 3 and Appendix A Supplemental Tables, Table 22). However, this did not result in statistical differences in survivorship (ANOVA; $F_{1,18}$=1.256, p>0.05). In addition, no differences in growth (0.03±0.18 mm/day) were observed between treatments (nested ANOVA; $F_{1,170}$=1.58, p=0.211), nor among the nested variable pool (p=1.0). These

conditions likely influenced the different morphologies observed between the two treatments (Figure 5C). While the R^2 value was low, we found a significant difference in morphology between environmental treatments (R^2=0.014, $F_{1,135}$=3.05, P=0.020), as well as significant differences among the nesting variable, pool (R^2=0.17, $F_{18,135}$=1.5, P=0.001). Finally, the CVA produced a single axis to separate the treatments and calculated a Mahalanobis distance between open and structured fish to be 2.24, with a classification accuracy of 86.6%. Shape changes in this axis reflected body orientation, with structure treatment fish oriented more ventrally, lower CV values, compared to open treatment fish.

3.9   Discussion

Our simulated habitat and environmental condition treatments influenced the morphology of yellow perch, supporting our hypotheses that habitats and specific environmental conditions would impact perch morphology. We also predicted that structure and open environmental conditions would have the greatest influence on morphology, and the results from our environmental conditions experiments supports this. However, our prediction that shape differences would be similar between treatments across years was not supported due to differences, between years, in our environmental condition experiments.

The changes in body shape in response to our treatments potentially reflect a combination of selection for particular body shapes (through differential mortalities), as well as plastic responses to the different environments. Our mesocosm experiment, which utilized older fish, only found significant morphological differences when examining fish using a multivariate, landmark approach which was then repeated in the environmental pool experiments. The differences in morphology, explained by treatment, were greater within our environmental condition experiments than our mesocosm experiment. This may be due to our use of a younger yellow perch population from Lake Erie, compared to the age-1 Perquimans River perch in the mesocosm experiment. This influence should be considered when utilizing yellow perch morphology as a discriminating factor for populations. It may also suggest that morphology could potentially be used as an indicator of habitat or foraging mode of yellow perch in the future.

In the simulated habitat mesocosms, we observed no differences in mean AR between simulated habitat types but did observe differences based upon landmark analyses. We expected that AR differences between treatments would roughly match shape differences based upon landmark analyses (i.e., contrast between deep bodied and fusiform shaped fish), but there was no strong agreement between AR and landmark analyses. Nonetheless, some of the shape differences appeared to match morphologies observed in past perch studies. For example, our CVA found increased caudal peduncle lengths, and decreased head depth and head length in pelagic fish, relative to littoral fish. These measures were also found to be important differences in yellow perch residing within wetland and nearshore habitats in Lake Michigan (Parker et al. 2009).

Morphological changes measured within our simulated habitats could be due to plastic responses to different habitats or selection against different morphologies. The low growth measured within our habitats could have indicated a low capacity for body shape change, suggesting that selection may have played a greater role in morphological differences. Within lakes, populations may have genetic differences between morphologically distinct individuals due to selective pressures between habitats, but this may not be consistent among different species or among different populations (Faulks et al. 2015).

In addition to selective impacts, our simulated habitats may not have been severe enough to elicit a plastic morphological response in our age-1 fish compared to younger fish. Developmental windows (West-Eberhard 2003) within the first year of perch life could play an important role in their juvenile morphology. If yellow perch have a limited development window during early-life, it is possible that the older perch might have had a limited capacity to undergo morphological change. Many other morphologically plastic species, including Eurasian perch, continue to alter shape at older ages, sometimes reversing morphological responses to previously experienced environments (Olsson and Eklöv 2005, Sánchez-hernández and Amundsen 2015). For yellow perch, development windows for plastic body shape changes could be limited by 1) ontogenetic diet changes occurring during the first year of life or 2) sexual maturation during later life in which larger fish devote more resources toward growth that favors reproduction.

Future studies may consider examining how morphology relates to ontogenetic diet shifts and specialized foraging in yellow perch. Foraging efficiency has been related to Eurasian perch morphology (Svanbäck and Eklöv 2003) and could have similar impacts on yellow perch groups which exhibit ontogenetic diet shifts (Wu and Culver 1992). Differences in morphology could have similar influences on yellow perch foraging efficiency, driving perch to forage more on prey items for which their morphology is best suited. This could occur even when alternative prey may be present in greater abundance, or the environment is less conducive for foraging, such as when yellow perch are observed diving into hypoxic waters (Roberts et al. 2009) for their preferred chironomid prey. Of note, within our environmental pools in 2015, the second greatest difference in morphology, measured by Mahalanobis distances, was between the open environment and chironomid diet treatments.

Habitats could have a strong influence on morphology through selective pressures toward canalization or selective pressures promoting morphological plasticity (Keeley et al. 2007, Faulks et al. 2015). The environmental differences among systems may not be greater than the environmental differences between habitats within systems, such as pelagic or littoral habitats. Populations that span multiple habitat types may be likely to express greater plasticity than populations in more homogenous systems (Olsson and Eklöv 2005, Sánchez-González and Nicieza 2017). Further, habitats such as pelagic or littoral environments could vary in multiple environmental conditions, such as predation risk, prey availability and structural complexity. The morphological responses of our study perch among four different environmental conditions resulted in statistically distinguishable shapes, as well as differences in the magnitude of shape change, measured by Mahalanobis distances. It will be valuable for future studies to consider the effects of differences in temporal and environmental factors on morphology, even among similar habitats such as structured and non-structured littoral regions.

Each of our tested environmental conditions appeared to produce distinguishable morphologies in perch, with the strongest differences measured between structured and open environments. The major shape differences between these two treatments, in 2015, appeared consistent with other polymorphic species known for littoral (i.e. deep bodies in structured conditions) and pelagic (i.e. fusiform bodies in open conditions) morphologies

(Svanbäck and Eklöv 2002, Faulks et al. 2015). However, shape differences in our 2016 pool experiment were more closely related to body orientation toward the dorsal or ventral position. Unfortunately, the morphologies of fish within the two pool experiments could not be directly compared due to the differences in when fish images were taken (i.e. live vs frozen fish) and differences in treatment setup. However, given the differences in the CVA shapes, we would suggest that treatments may not induce shapes consistently, perhaps due to annual environmental differences such as temperature or food availability. Little is known on the consistency of morphological differences between morphologies across time, but others have shown that morphological divergence is impacted by other environmental variables such as water clarity (Scharnweber et al. 2016a).

3.10 Summary

To our knowledge, our study is the first to utilize simulated pelagic and littoral habitats to induce morphological changes in yellow perch. Previous studies have shown that different environmental conditions may affect body shape (Brönmark and Miner 1992, Olsson et al. 2007), and we demonstrate that observed morphologies of yellow perch appear to respond to environmental factors operating individually. These environmental conditions could exist in many different combinations and at different intensities (i.e. high predation risk or low predation risk within structured or unstructured environments). We suggest that morphologies of fish in natural systems may be sensitive enough to reflect their environments, potentially making fish morphology a valuable indicator of habitat residence. However, morphologies may also vary due to differences in age among fish, as well as annual differences in environmental factors from one year to the next.

3.11 References

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Table 2 Total lengths (mm; mean±sd, with sample size in parenthesis) and aspect ratios (mean±sd) of age-1 yellow perch at the conclusion of mesocosm experiment.

		mean±sd [a]
Littoral	Length	117.47± 10.38(55)
	Length-Depth	3.88±0.11
	Length-Caudal	11.39±0.69
Pelagic	Length	124.47± 17.10(34)
	Length-Depth	3.81±0.12
	Length-Caudal	11.08±0.60

[a]All mesocosms were stocked with 20 fish at the start of the study and numbers in the parenthesis represent the remaining number of individuals in all 4 replicates for each treatment.

Table 3 Yellow perch individual sizes at the conclusion of the environment conditions experiments.

Experiment	Treatment (replicates[a])	Total Length (mm; mean±sd)	Mass (g; mean±sd)	n[b]
2015	Open (6)	56.03±10.04	1.47±1.3	109
	Structure (6)	54.71±11.21	1.47±1.9	118
	Predation (6)	51.31±7.29	0.95±0.63	150
	Chironomid (6)	55.93±7.71	1.54±0.99	252
2016	Open (10)	64.68±9.46	2.48±1.60	104
	Structure (10)	66.97±9.53	2.85±1.65	68

[a]Number of replicates for each treatment
[b]Total number of individuals remaining across all replicate pools.

Table 4 Pairwise CVA Mahalanobis distances between the 2015 environmental condition treatments.

	Chironomid	Open	Predation
Open	3.52	-	-
Predation	2.86	3.00	-
Structure	2.91	3.59	2.71
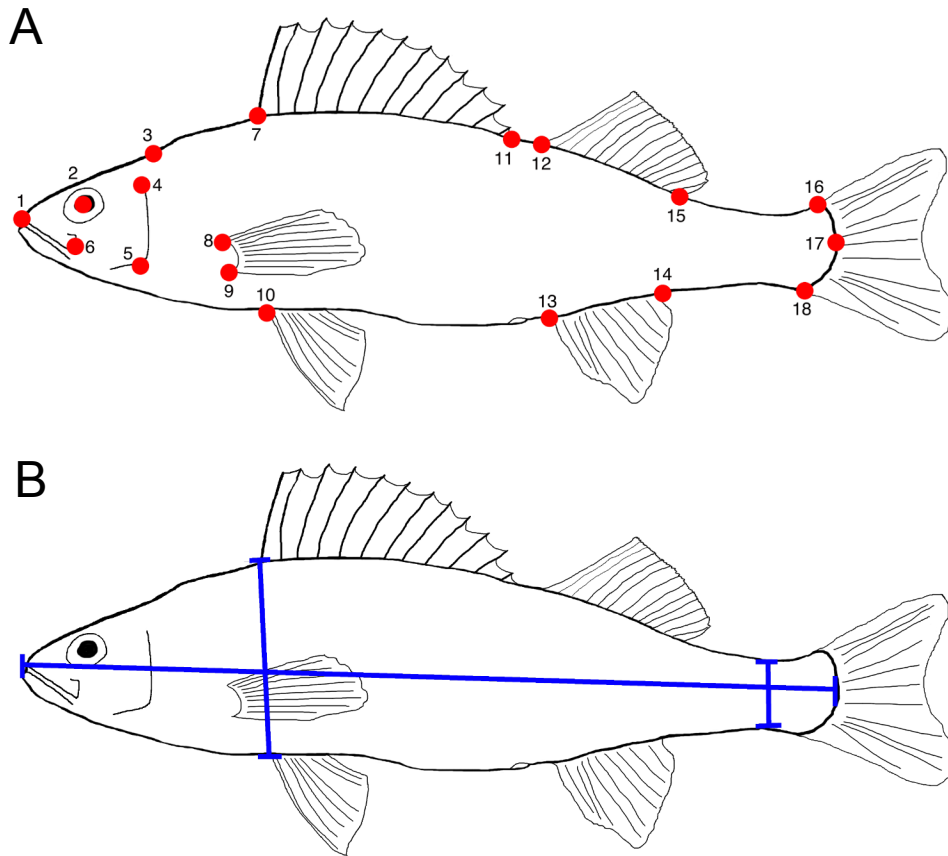
Figure 4 Diagrams of landmark locations (A) and length measures for aspect ratio calculation (B).
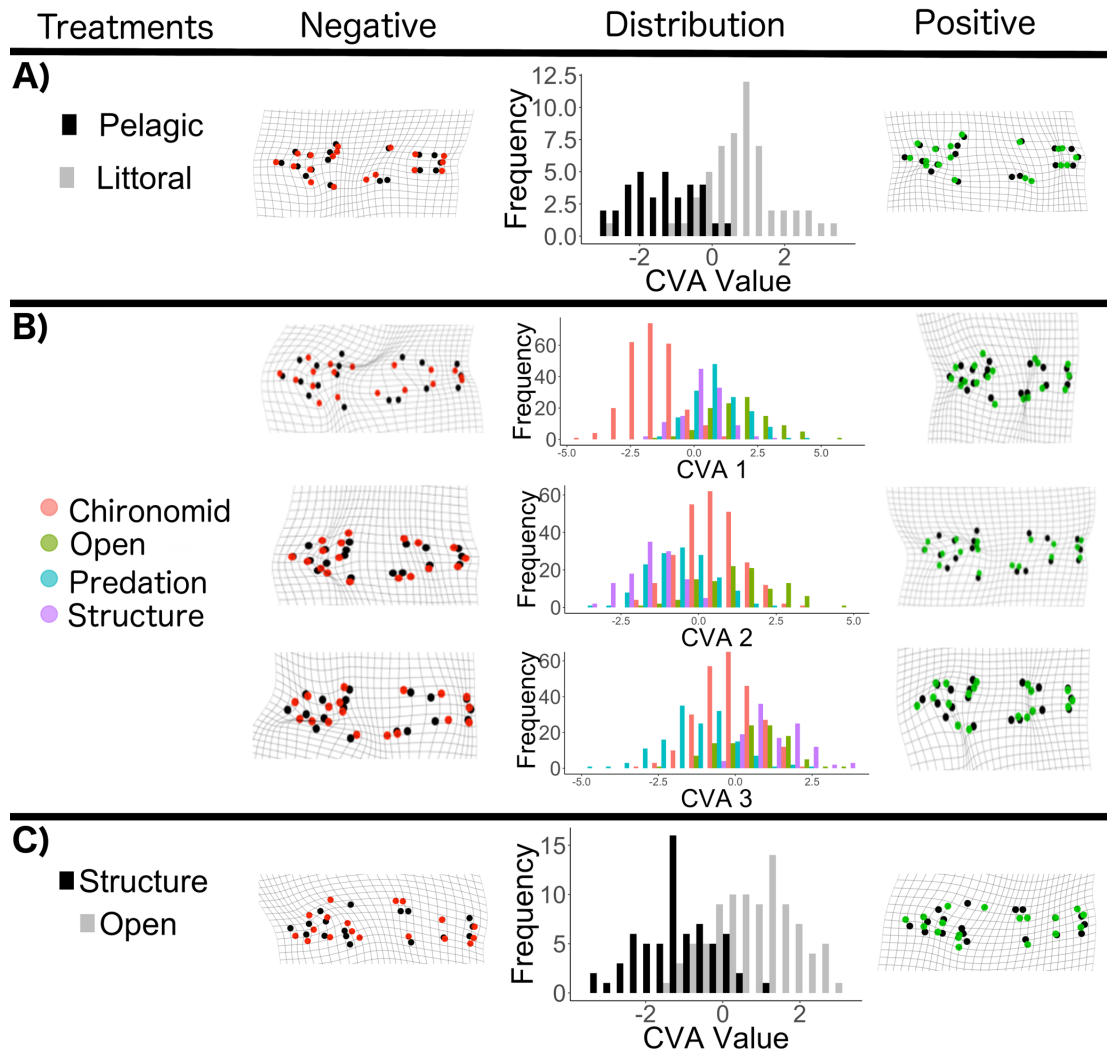
Figure 5. Distributions of the canonical variate results as well as warp grids depicting the change in shape from a CV value of 0 (black) toward the negative direction (red) or positive direction (green). A) Canonical variate axis for 2015 simulated habitat experiment. Mahalanobis distances between the two treatment groups was 2.3 and the percent classification accuracy was 88.7%. B) The three canonical variate axes for the 2015 environmental pool experiment. Mahalanobis distances are reported in Table 4 and the percent classification accuracy was 82.0%. C) The canonical variate axis for the 2016 environmental pool experiment. The Mahalanobis distances between the treatment groups was 2.24 and the percent classification accuracy was 86.6%.

CHAPTER 4.   TROPHIC AND MORPHOLOGY METRICS AS PREDICTORS OF INTRASPECIFI VARIATION IN MERCURY CONCENTRATIONS OF FISH

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4.1   Abstract

Contaminants such as mercury accumulate in organisms through their diet. Because of this connection between consumption and contaminant accumulation, mercury concentration among individual organisms, such as fish, may vary due to differential production pathways, variable trophic level feeding, and varying habitat use within aquatic systems. We examined the variation of mercury concentrations within two fish species, yellow perch (*Perca flavescens*) and black crappie (*Pomoxis nigromaculatus*) among five glacial lakes in northern Indiana. Further, we indexed the trophic positions of individual fish for each species, using two approaches: stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) and morphological variation. Both stable isotope ratios and morphology have been shown to reflect differential trophic roles within species. We related mercury concentration of individual fish to their morphology, stable isotopes, as well as sex, length and lake using multiple linear regression with model averaging. We found that isotopic niches were different among and within lakes for each of our species, but $\delta^{13}C$ and $\delta^{15}N$ were not good predictors of mercury concentration within individual fish. Instead, morphology, summarized by principle components analysis, was among the best predictors of mercury concentration for both black crappie and yellow perch. We suggest that mechanisms influencing the morphology of yellow perch and black crappie within our study lakes may be similar to mechanisms influencing the concentration of mercury in fish tissue.

Keywords: Stable Isotopes, Landmark morphology, Model Inference, Trophic Niches

4.2   Introduction

Intra-specific variation in prey consumption and variable spatial-temporal production pathways complicate trophic niches within aquatic systems. Variation in trophic niches may also affect the accumulation of contaminants, such as mercury (Hg), which are acquired and incorporate into organismal tissue as a result of prey consumption (i.e. diet). It follows therefore that variation in mercury contamination within populations would reflect differences in trophic niches among individuals. In the United States, Hg is the top contaminant of concern in waterways and contributes to over 80% of fish advisories (US EPA, 2011). Mercury, particularly its organic form methylmercury (MeHg), is a persistent toxin which bioaccumulates through diet consumption in popular sport fish and may reflect variable trophic patterns within the population. MeHg can make up to 85-90% of total mercury within fish (Ullrich et al. 2001) and can have negative health effects on human consumers, particularly at-risk populations such as pregnant women and children. The U.S. Environmental Protection Agency (EPA) has set maximum limits of Hg at 300 µg/kg of fish tissue wet weight (US EPA, 2001) to protect human health. In addition to being a danger to human health, Hg may also have lethal and sub-lethal impacts on fish and wildlife (Depew et al. 2012). Presently, there is a need to understand the variation in mercury loads within fish populations, to better inform management decisions regarding health risks to human consumers as well as fish, and other wildlife.

Variation in Hg among individual fish may begin with spatio-temporal differences of Hg within the environment, such as through the distribution of anthropogenic sources of Hg (i.e. mining waste or fossil fuel burning; Eagles-Smith et al., 2016), and physical and chemical processes among and within lakes (Greenfield et al. 2001). In addition, methylmercury, produced by methylation via microbial communities, varies with local processes influencing microbe activity (i.e. temperature, pH, and the presence of oxygen; Ullrich et al., 2001). Temporal factors may also influence the variation in MeHg, for instance in lakes and reservoirs, higher MeHg is generally recorded in water samples and fish tissue (Sorensen et al. 2005) during warmer summer periods (Ullrich et al. 2001) and during periods of lower water levels (Eagles-Smith et al. 2016).

In addition to variation in available Hg among and within lakes, bioaccumulation of Hg may vary across species and within fish populations in a lake. High variation in Hg concentration could confound attempts to calculate appropriate fish consumption advisories distributed around mean concentration levels. Intra-specific variation of Hg can be related to individual differences in the size, sex and trophic specialization of fish. In particular, increasing trophic level consumption as fish increase in size is commonly observed to relate to increased Hg concentration through bioaccumulation (Lavoie et al. 2013). However, relationships between some of these individual characteristics are not fully defined or are inconsistent among species. For example studies examining the relationships between sex and Hg concentration have found several species of fish with sex bias (Madenjian et al. 2014, 2015), but within at least one survey of 41 species the majority of fish exhibited no significant sex bias in total Hg (Bastos et al. 2016).

Variable trophic pathways and foraging specializations within fish populations could potentially have a large degree of influence on the variation in Hg concentration among fish. If groups of individuals consume prey items that are high in Hg, compared to other prey items, this could lead to a non-normal distribution of Hg loads within the population. For example, Karimi et al. (2016) observed higher Hg concentrations in zooplankton relative to benthic invertebrates, and their models predicted that benthivorous fish would have lower Hg concentrations as a result. Spatial differences in the concentration of MeHg could occur near river effluents from large watersheds with high Hg contamination (Hurley et al. 1995), producing variation in the baseline Hg levels within a lake. Additionally, MeHg could differ spatially within hypoxic regions of lakes or wetlands (Greenfield et al. 2001, Ullrich et al. 2001, Sorensen et al. 2005, Hanna et al. 2016) and could alter fish exposure. While most fish avoid hypoxic regions and therefore may avoid food with higher Hg levels some fish such as yellow perch, *Perca flavescens*, are known to dive into hypoxic zones to consume benthic invertebrates (Roberts et al. 2009), potentially putting them at greater risk for MeHg contamination. Temporal differences in trophic pathways, and the pathways for Hg bioaccumulation, could reflect changing prey availability such as in seasonal succession of invertebrates (Winemiller 1990, Flory and Milner 2000), or changing rates of primary production (Tallberg et al. 1999) between years or seasons. Changing temperatures, oxygen levels (Roberts et al.

2009), predation risk, and changes in prey availability could drive fish out of some habitats (Wu and Culver 1992), creating spatial differences in consumption and altering Hg exposure.

Spatial-temporal variation in trophic pathways can be measured through trophic indices such as stable isotope ratios (Post 2002). In addition, fish may become morphologically specialized for feeding within different habitats or upon different prey items (Svanbäck and Eklöv 2004), and this morphology could reflect differential foraging patterns. Stable isotope ratios such as $\delta^{13}C$ and $\delta^{15}N$ are particularly well studied and useful diet indicators (Layman et al. 2012) for fish communities. Carbon isotope ratios (Post 2002, Ives et al. 2013) may discriminate among pelagic (i.e. atmospheric in origin and therefore less enriched), benthic and terrestrial production pathways (more enriched). Nitrogen isotope ratios become more enriched with increasing trophic levels making this index useful for comparing trophic variation among consumers within the same system (Post 2002).

We hypothesized that variation in mercury content could be observed within populations and be related to individual differences such as sex and size, but also trophic indices (i.e. $\delta^{13}C$, $\delta^{15}N$ and morphological landmarks). Our study focused on two freshwater fish species, yellow perch and black crappie, *Pomoxis nigromaculatus,* among five northern Indiana lakes. Both species are popular targets of anglers and could act as conduits for mercury contamination to the human populace. Yellow perch are a generalist fish species known to have variable trophic relationships (Roswell et al. 2014, Happel et al. 2015) as well as individual specialization for particular prey (Wu and Culver 1992). Diets of black crappie may also vary, particularly with respect to size, changing from a benthic invertebrate diet to a predominantly piscivore diet (Seaburg and Moyle 1964).

4.3   Methods

Fish and Lakes

Black crappie and yellow perch were collected in five northern Indiana glacial lakes (Backwater, Jimmerson, Skinner, Sylvan, and Wawasee: see Figure 6) by the Indiana Department of Natural Resources as a part of annual surveys in March-June 2016 (See

Sullivan et al. 2015 for methods). The lakes varied from one another in terms of morphometrics and water quality metrics (Table 5 and Appendix A Supplemental Tables Table 23), and also varied in past (1987-2005) measured Hg concentration in fish tissue examined by the Indiana Department of Environmental Management (IDEM; Table 6). Fish were collected by trap net and preserved in a -20˚C freezer for approximately 1 week prior to sample processing. In the laboratory, fish were digitally imaged on a concave board using a Panasonic DMC-TS5 camera with a ruler for scale. In addition, each fish was sexed by internal examination of gonads, measured for total length (to 1 mm), mass (to 0.1 g), and had 1-3 g of dorsal muscle tissue removed from just below the anterior end of the dorsal fin to quantify stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) and mercury concentration (total mercury). Size has a significant influence (Bastos et al. 2016) on mercury concentration therefore a subset of fish with total lengths between 150 and 300 mm were used for analysis (see Table 7 for size information by lake and fish type). In total, 96 yellow perch (mean total length 212.7±29.6 mm) and 140 black crappie (mean total length 220±41.0 mm) were selected.

In August of 2016, invertebrates from four lakes (Jimmerson, Shipshewana, Skinner, Sylvan, and Wawasee) were collected from two replicate samples of benthic invertebrates and zooplankton at four sites around the lake, two in the nearshore (~1.5 m of water depth within 5m of the shoreline) and two in offshore regions (near the center of the lake). Unfortunately, the isotope ratios from these samples displayed a large degree of variation from fish samples, which may have been related to temporal differences in collection (i.e. invertebrates were collected in the fall and fish were collected in the spring). We therefore elected not use invertebrate isotope ratios to make baseline corrections among lakes (see analysis: isotopes below).

Stable Isotopes

Fish samples for $\delta^{13}C$ and $\delta^{15}N$ isotope ratio analysis were stored at -20°C prior to preparation. All samples were dried (60 °C) for 3 days, ground, and sent to the Cornell Isotope Laboratory for analysis, where they were analyzed using a ThermoFinnigan Delta Plus mass spectrometer and NC2500 elemental analyzer. Values for each isotope are reported as δ values: $\delta X = ((R_{sample}/R_{reference})-1) \cdot 1000‰$, where X is the isotope of interest

(e.g., ^{15}N), and R is a ratio of the abundance of isotope of interest to the "common" isotope (e.g., ^{14}N). Reference ratios are the internationally-accepted ratios for Vienna Pee Dee Belemnite (^{13}C), and atmospheric air (^{15}N). Carbon isotope ratios were mathematically adjusted to account for lipids using the following equation for aquatic organisms from Post et al. (2007). Where C:N represents the ratio by mass between carbon and nitrogen.

$$\delta^{13}C_{adjusted} = \delta^{13}C_{unadjusted} - 3.32 + 0.99 \times C{:}N$$

Morphometrics

Similar methodologies were used to prepare morphometric data for black crappie and yellow perch. Landmark points were used to measure morphology, using 18 points for yellow perch and 13 points for black crappie (Figure 7). Landmarks were assigned using the program TpsDig (Rohlf 2005). Three perch and one black crappie were excluded due to poor images, reducing morphology samples sizes to 93 perch and 139 black crappie. Fish shapes were normalized via the procrustes procedure in the program R (R Core Team 2014) using the function GeoMorph (Adams et al. 2018). This method adjusts images for differences due to size, scale, and position through rescaling, translation and rotation of images. Some variation in shape is attributable to differences in size (i.e. allometry) and this was accounted for through a residual analysis. Procrustes points were analyzed by perMANOVA (999 permutations using procrustes distances) with the centroid sizes of individuals, which measures the average distance of each landmark to the image center and is closely related to fish size (Klingenberg 2016). The residuals from the perMANOVA were extracted and used for subsequent analyses (see Parsons et al. 2016 for similar methods).

Mercury

Total mercury (Hg) content was measured using a thermal decomposition (gold) amalgamation atomic absorption spectrophotometer direct mercury analyzer (DMA-80, Milestone Inc.; for similar methods see Cladis et al. 2014). Individual fish samples were run in duplicate, along with a standard (Tort-3). Mercury concentration was calculated as a wet weight (μg/kg). Following initial quantification of mercury, the coefficient of

variation (CV) was calculated for each individual. Individual samples with CV over 10% were analyzed a second time in duplicate. After this rerun of samples, the mean individual mercury values were recalculated using the addition of the re-analyzed samples, which then met our CV requirements. These means were used for subsequent analyses. Mercury concentrations represent the total mercury content within the muscle tissue and may include both inorganic and organic (i.e. methylmercury). However, previous studies have shown that the vast majority of mercury within muscle tissue consists of methylmercury due to its ability to accumulate in tissue (Boening 2000). We expected that concentrations of Hg would differ among lakes due to different biotic and abiotic factors.

4.4   Analysis

Isotopes

Analyses that included stable isotopes all included lake as a random effect, which accounted for isotope baseline differences among lakes for $\delta^{15}N$ and $\delta^{13}C$, rather than utilizing baseline corrected isotope ratios. We compared the isotope niches of individuals among lakes, using $\delta^{15}N$, along with lipid corrected $\delta^{13}C$, for both yellow perch and crappie. Comparisons of fish isotopes were made using a permutational multivariate analysis of variance (perMANOVA; R function ADONIS within package vegan; Oksanen et al., 2016) a nonparametric version of MANOVA which can partition variance among factors based upon Euclidian distances and we fitted species-specific models using permutation tests (999 permutations) with psuedo-F ratios. These models included lake as a nested factor, total length of fish, as well as an interaction term between lake and total length. Following this we conducted separate univariate ANOVA and Tukey post-hoc tests of $\delta^{15}N$ and lipid corrected $\delta^{13}C$ to determine which lakes differed from one another in terms of specific stable isotope ratios.

Morphology

Intra-specific morphological differences among lakes were first compared using a perMANOVA (999 permutations using allometry-corrected procrustes distances). Following this, species-specific principal components analyses (PCA) were used to summarize multivariate morphology. These analyses collapsed the landmark data into a

series of principal components (PC). To identify an appropriate number of PC axes for subsequent analyses, we first plotted the variance for each PC (Appendix B Supplemental Figures, Figure 13) to visualize the change in variance among PCs. We then further conducted a randomization-eigenvalue procedure, to provide a more analytical approach to selecting the number of principal components to examine. This method, recommended by Peres-Neto et al. (2005), randomized the morphology matrix and conducted a PCA 999 times and extracted the calculated eigenvalues. For each PC axis, a p-value was estimated based on the proportion of eigenvalues that were greater than or equal to the original morphology dataset (package PCDimension; Coombes and Wang 2018). Based on the 'elbow' within the scree plot and the randomized procedure, we proceeded with our analysis using the first 4 PC axes for yellow perch and first 5 PC axes for black crappie. For each selected PC, we examined how shape differed across the axis using warp grids of the minimum and maximum PC scores. Axes were tested for their relevance as potential trophic discriminators using a multiple linear regression, nesting individual fish within their respective lakes and examined how well shape was explained by: sex (except for yellow perch due to a bias in perch sex ratios) and both isotope ratios. Length was not included since it was previously extracted from morphology.

Mercury

Mercury distributions within fish populations were first examined using the Shapiro-Wilk test for normality. In situations where distributions were non-normal, Hg values were natural log transformed for subsequent analyses. For each species, we conducted an ANCOVA to examine among lake differences, including total length and sex as covariates.

Different individual characteristics (e.g. sex, length, morphology, and isotope ratios) were examined as potential predictors of Hg through a series of models. In each model, log total Hg was the dependent variable and the following independent variables were included: total length, sex, lipid corrected $\delta^{13}C$, $\delta^{15}N$, and the principal components of interest (yellow perch: PC1-4; black crappie: PC1-5). The variable lake was used as a random effect, allowing us to nest individual fish within each lake. Every combination of independent variables was considered (256 total YEP models & 512 total BLC models).

Models were compared using corrected Akaike Information Criterion (AICc). The Δ AICc (The difference calculated between each model AICc value and the lowest model AICc value) and AICc weights (w_i; the relative likelihood of each model divided by the sum of all relative likelihood values) were used to represent the differences among models.

Model averaging was used to calculate new averaged coefficients for each variable within a species-specific model. This process reduced potential model selection bias through the inclusion of many models (Burnham and Anderson 2002), and may more accurately indicate how a model variable influenced total Hg within fish. We selected only models with a w_i greater than 0.01 for model averaging, removing models with little to no influence. This left us with a subset of 15 top yellow perch models and 22 top black crappie models, within which new Δ AICc and w_i were calculated for the model averaging process. Coefficients for each variable were averaged across each model in which they appeared and are weighted by their w_i so that models with lower w_i have less influence on the final average model coefficients. Further, the output of this analysis calculated an importance value for each variable from the sum of w_i over all of the models in which the variable was present. Variables with higher importance values may represent more influential predictors, but we supplemented this inference by also considering other model inference techniques, such as coefficients of determination (Galipaud et al. 2014, Cade 2015) in our model comparison. This analysis was conducted in program R using the package MuMln (Barton 2018).

4.5   Results

Isotopes

Fish isotope ratios differed among lakes (perMANOVA: YEP n=96, R^2=0.60, p=0.0001; n=140 BLC R^2=0.63 p=0.0001; Figure 8), after accounting for the differences in fish total length (YEP n=96, R^2=0.12, p=0.0001; BLC n=140, R^2=0.27, p=0.0001). Interactions between lake and fish total length were significant for perch and crappie (YEP n=96, R^2=0.035, p=0.0001; BLC n=140, R^2=0.025, p=0.0001). For yellow perch, post-hoc ANOVA and Tukey tests found differences were attributed to $\delta^{13}C$ ($F_{2,94}$=263.6, p<0.0001) and $\delta^{15}N$ ($F_{2,94}$=6.602, p=0.002) in lakes Wawasee and Sylvan (p<0.05). In black crappie,

ANOVA found differences among lakes were also driven by $\delta^{15}N$ ($F_{4,136}$=239.6, p<0.0001) and by $\delta^{13}C$ ($F_{4,136}$=316.6, p<0.0001), and Tukey tests identified that most differences were between Lake Jimmerson and all other lakes (p<0.05).

Morphology

        Morphology of yellow perch and black crappie varied among lakes and provided some support for specialization of fish within lakes. Yellow perch displayed morphological differences related to both lake and sex (perMANOVA: n= 93; Lake R^2=0.06, p=0.002; Sex R^2= 0.03, p=0.026). Black crappie also demonstrated differences in morphologies among lakes and between sexes (n=139; Lake R^2=0.13, p=0.001; Sex R^2=0.02, p=0.001), however lake effects explained more shape variation for crappie than for perch. The ratio of male to female perch was skewed toward one sex or another in all perch lakes except Lake Wawasee, which likely influenced the explanatory contribution of sex within the perMANOVA.

        Morphological variation was summarized by PCA and our randomization method selected the first 5 PCs for black crappie (Figure 9) and 4 PCs for yellow perch (Figure 10). This represented 67% and 69% of the morphological variation for black crappie and yellow perch respectively. In both species, some axes appeared to reflect lake specific differences (i.e. black crappie PC2, yellow perch PC2 & PC4). There were some similar morphological axes between the two species for instance, PC1 reflected a ventral-dorsal orientation in the body shape. Further, several PCs appeared to reflect changes to the body trunk such as through changes in depth, particularly PC 2, 3, and 5 for black crappie and PC2 and 4 for yellow perch.

        In most cases, we found no relationship (P>0.05) with either stable isotope or sex within the multiple linear regression analysis of black crappie morphology, with a few exceptions. Sex did significantly explain morphology along PC3 ($F_{1,138}$ =4.54, P_{sex}=0.035) and PC4 ($F_{1,138}$ =22.89, P_{sex}<0.001), where males had higher PC3 and PC4 values. In shape, this meant that males were generally more fusiform (PC3, Figure 9) and their pelvic & anal fins were closer together (PC4, Figure 9). In addition, $\delta^{15}N$ had a negative and significant relationship with PC4 ($F_{1,138}$=11.26, $P_{\delta15N}$ =0.032). Similar to black crappie, the morphology principal components for yellow perch also did not have significant

relationships with the stable isotopes measured, except in two cases. Yellow perch δ^{15}N was negatively related to PC3 ($F_{1,92}$=4.34, $P_{\delta15N}$=0.04) and δ^{13}C was negatively related to PC 4 ($F_{1,92}$=7.99 P $_{\delta13C}$ =0.006). In this case, perch with higher δ^{15}N had larger trunks in both length and width, particularly along the base of their dorsal fins (Figure 10). Perch with lower δ^{13}C also had larger trunks and caudal peduncles, while perch with higher values were more fusiform (Figure 10).

Mercury

Mercury contamination, tested by Shapiro-Wilk tests, were not always normally distributed within populations. In yellow perch, lakes Sylvan and Backwater both exhibited non-normal distributions. Black crappie populations also exhibited non-normal distributions within lakes Backwater, Jimmerson, Skinner, and Sylvan. Most of the lake specific mean total Hg values (Table 8) generally agreed with previous samples collected by IDEM (Table 6 and Figure 8). The Lake Wawasee samples were higher than previously reported IDEM samples, and this could be due to temporal changes in mercury loading, since the most recently reported IDEM samples from Lake Wawasee were from 1996. Of note, each lake had individuals with higher mercury loads than the average individual fish from that lake, for example in Backwater Lake the mean total mercury was 59.79 µg/kg and the maximum total mercury measured in an individual fish was 114.76 µg/kg (Table 8).

Mercury content varied among lakes for both species of fish but not between different sexes (Figure 11, ANCOVA: perch $F_{4,\,89}$=61.63, p<0.0001; crappie $F_{4,129}$=341.9, p<0.0001). For both species, total length was an important predictor of mercury content (perch, $F_{1,\,89}$=13.00, p=0.007; crappie, $F_{1,129}$=245.2, p<0.0001). No significant differences were evident between sexes in either black crappie ($F_{1,129}$=2.325, p=0.69) or yellow perch ($F_{1,89}$=3.713, p=0.057). In perch, this analysis may have been hindered by the bias in sex ratios in all but Lake Wawasee.

Our model inference analysis suggested several associations between the variables tested and mercury content of yellow perch. However, even the top model from this analysis had a low coefficient of determination (R^2=0.56). The top two models had high AIC weights (0.27 & 0.22 respectively) compared to the remaining 15 perch models

(see Table 9 for the full list of models). The top model contained all of the morphology PCs and total length, while the second top model contained the same variables except for morphology PC1. The relative importance values (IV) in this analysis were: PC4 (IV=0.98, 14 models), PC2 (IV=0.90, 11 models), total length (IV=0.84, 11 models), PC3 (IV=0.83, 13 models), PC1 (IV=0.55, 8 models), sex (IV=0.06, 2 models), and δ^{15}N (IV=0.04, 2 models). The isotope ratio, δ^{13}C was not included in the average model since none of the models containing this variable met the 0.01 w_i criteria. Three of the morphology PCs were negatively associated with mercury content (see full average model results in Table 10 and morphology in Figure 10), suggesting that perch with benthic orientation (PC1), longer trunks (PC2), and fusiform shapes (PC4) had higher mercury. As expected, length was positively associated with mercury in the average model. Between the sexes, male perch had generally higher mercury content than female perch. Finally, δ^{15}N was negatively associated with total mercury.

In black crappie, the top models were nearly identical in model weight and coefficients of determination (Table 9). In addition, the coefficients of determination values were higher compared to yellow perch models. The top model (R^2=0.90, w_i=0.12;), similar to perch, contained each morphology PC and total length. In contrast to yellow perch, δ^{13}C contributed to the selected models but δ^{15}N did not. Sex also does not appear in any of the selected models. The calculated importance values were high for total length (IV=1.00, 22 models), morphology axis PC 4 (IV=1.00, 22 models), PC 5 (IV=0.89, 16 models), PC2 (IV=0.65, 12 models), PC 3 (IV=0.60, 12 models), PC 1 (IV=0.55, 11 models), but low for δ^{13}C (IV=0.28, 8 models). Length and δ^{13}C had positive relationships with total Hg (Table 10). The morphology PCs each had a negative relationship (Table 10), suggesting that fish with ventral orientation (PC1), larger trunks (PC2 and PC3), and narrower caudal peduncles (PC 4 and PC5) had higher mercury levels.

4.6   Discussion

We explored the complex inter- and intrapopulation patterns in trophic ecology and total mercury contamination of two sport fish species, yellow perch and black crappie. Mercury measured within each species varied greatly within and among the

different lake populations, with some individuals having greater total Hg concentrations than reported in previous fish surveys. In addition to a strong relationship with total length, variation in Hg appears to be more strongly related to variation in morphology for both black crappie and yellow perch, than to sex or differences in trophic niches as quantified by δ^{15}N and δ^{13}C.

Within lakes, we found that total Hg within our fish populations generally did not follow parametric distributions and suggests that a more robust sampling of fish populations may be required to capture the true distributions of mercury. In general, the average total Hg for each lake was comparable to values measured by Indiana's Department of Environmental Management (IDEM). However, the differences in Hg ranges and distributions, observed between yellow perch and black crappie, suggests that samples of all fish species, particularly targeted sport fish, may be necessary to properly assess inter-lake variation in Hg loads. Total Hg values calculated from only a handful of representative fish may underestimate the risk of Hg consumption for recreational anglers. Many of the study lakes have complex shorelines that could create spatial differences within the fish population and individuals may reside within different habitats and feed on different food resources. This could lead to different rates of bioaccumulation. For example, differences in Hg accumulation due to diets and environment have also been observed in Artic char, *Salvelinus alpinus* (Kahilainen et al. 2016), and European whitefish, *Coregonus lavaretus* (Kahilainen et al. 2017).

We expected that the bioaccumulation of total mercury within individual black crappie or yellow perch would be correlated with δ^{15}N or δ^{13}C, however found little connection between these variables. The small differences in δ^{13}C values in perch may have reflected different intra-population pathways of production, such as terrestrial and offshore pathways. Also of note, yellow perch had a negative relationship, in the model averaged coefficients between total Hg and δ^{15}N, opposite of what others have noted for this isotope ratio (Bank et al. 2007). However, lower δ^{15}N could also indicate that perch were feeding on zooplankton which have been shown to have more Hg content than some benthic invertebrates (Karimi et al. 2016). However, the relationship in yellow perch between δ^{13}C and total mercury was opposite of what we might expect if zooplankton,

with lower $\delta^{13}C$, were being consumed. Nonetheless, larger perch and black crappie in the study did have positive associations with total Hg, similar to other fish research (Farkas et al. 2003). Follow up correlations indicated that $\delta^{15}N$ and fish total length were positively associated with one another in both species. The unexpected patterns in yellow perch may be further evidence that relationships between total Hg and trophic niches may not be clear cut and fish may be feeding in different trophic pathways with different risks to Hg accumulation.

Contrary to yellow perch, black crappie isotope ratios were narrower within their mutual lakes and suggested that within a population, crappie feed more consistently within the same trophic level and within similar production pathways. Our average model for black crappie indicated that fish with higher mercury were possibly feeding closer to the benthos or terrestrial systems (i.e. increasing $\delta^{13}C$). However, models containing isotope ratios did not have large coefficients of determination.

For both black crappie and yellow perch, morphology metrics were among the best predictors of total Hg. Fish morphology has been frequently found to reflect the environments and diets of individuals (Marklund et al. 2017), particularly in species known to exhibit morphological plasticity (Olsson et al. 2007, Faulks et al. 2015). The connection between morphology and total Hg may be the result of morphological responses to different habitats or diets with variable Hg exposure. In our average model for black crappie, fish that were more ventrally oriented, with larger trunks and narrower peduncles had higher mercury concentrations, a morphology which we might associate with benthic habitats (Olsson et al. 2007). This is opposite of what we might expect since fish feeding closer to benthos would be more likely to feed on benthic invertebrates, which may have lower mercury (Karimi et al. 2016). Fusiform shapes, fish with narrower trunks, may be more suited for foraging on zooplankton or other fish, in the case of larger individuals. Both zooplankton and fish consumption could have increased mercury accumulation within the consumer, compared to benthic diets.

Similar patterns to black crappie were found in the average perch model, where ventral orientation was also significantly associated with higher mercury. However, fusiform shaped perch were found to have greater mercury in the case of morphology PC4 and PC3, while PC2 would seem to suggest that an expansion in the dorsal portion

of the body trunk, in length but not depth, was associated with higher mercury. The similarity in ventral orientation among our study species may suggest a need to investigate the associations between morphology and mercury content in future work. The dissimilarity between our two species in body shape patterns related to mercury may have occurred due to differences in habitat or prey selection. We would not suggest using morphology as a direct predictor of Hg content in fish as the model coefficients of determination were not always high without the presence of additional model variables such as total length, particularly for yellow perch. However, individual morphology may be more sensitive to the environmental variation impacting mercury exposure when compared to other environmental indicators such as stable isotopes.

Several past morphology studies identified habitats and diets that may influence the morphology of individual fish (Faulks et al. 2015, Marklund et al. 2017). We instead aimed to use morphological shapes as trophic/habitat indicators. We were able to identify axes that appear to summarize body shapes noted in other morphology studies (Brönmark and Miner 1992, Parker et al. 2009, Faulks et al. 2015), such as fusiform-deep body shapes or ventral-dorsal orientation. We found that while some of our morphological PCs were related to stable isotopes, another trophic indicator, most of our morphological metrics were not. For example, black crappie morphology generally had no association with either isotope, except for between PC4 and nitrogen isotope ratios. It's possible that the diets of crappie did not differ greatly enough for $\delta^{13}C$ to vary with morphology.

However, the differences in shapes may still suggest some variation in habitat residence. Yellow perch had two morphology PCs associated with isotopes (PC3 and $\delta^{15}N$, PC4 and $\delta^{13}C$), which may have been more likely since they had greater variation in isotope signatures. Both the negative relationship with $\delta^{15}N$ and positive relationship with $\delta^{13}C$ could be possible if fusiform shaped perch were feeding on pelagic prey such as zooplankton. In research on a yellow perch congener, Eurasian perch (*Perca fluviatilis*), foraging on zooplankton was closely linked with fusiform body shapes (Svanbäck and Eklöv 2004). If the yellow perch in our lakes were feeding on different food items or within different habitats, the isotope and morphology metrics may parallel each other. Similar results between isotopes and morphology have been observed in North American

minnow species (Burress et al. 2016) and Eurasian perch (Mustamäki et al., 2014) and build support for using morphological diversity as an indicator for trophic-habitat diversity within populations.

There is considerable evidence relating total length to total Hg within fish tissue due to bioaccumulation (Ullrich et al. 2001). In this study, the relationship between length and total Hg, while significant, often had a poor model fit without additional variables. This has been noted for yellow perch in other studies focused on factors influencing the Hg concentration in this species (Greenfield et al. 2001, Sorensen et al. 2005). In addition, different sexes in some fish (Madenjian et al. 2015, Bastos et al. 2016) may vary in total Hg due to consumption rates and the rate at which Hg can be eliminated from the body, which may change with season and across years. Biased sex ratios may have hindered our analyses that examined differences in total Hg between sexes, but model averaging did not suggest that there was any significant influence of sex on Hg in either species. Overall, the inference techniques used suggested that ideal predictive models of total Hg would include length along with one or two additional individual characteristics that measure trophic niches such as morphology, or diet indicators (i.e. stable isotopes).

Accurate predictions of mercury content are necessary when determining risk factors to recreational anglers. We found that including trophic measures and individual characteristics such as morphology can help improve predictive models. Further, robust sampling within a lake, as well as representative samples of each species, may be ideal for examining true distributions of mercury. These recommendations may be necessary as lakes within temperate zones may be experiencing changes in their mercury content. For instance Weiss-Penzias et al., (2016) and Zhou et al., (2017) observed increasing Hg deposition across numerous sites within the United States and Laurentian Great Lakes region and increasing Hg content within Great Lakes fishes. Although the factors underlying these changes are not fully understood, they may be related to environmental changes on biotic influences to trophic pathways (Blukacz-Richards et al., 2017) or abiotic influences such as increased precipitation events (Holmes et al. 2016). The influence of these changes on fish tissue Hg could be more accurately assessed using

multiple metrics of intra-population variation including those examined in this study; length, morphology, sex, and stable isotopes.

4.7　References

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Table 5 The average physical and chemical characteristics from the 5 study lakes. Data acquired from Indiana's Clean Lake Program (IDEM).

	Backwater	Jimmerson	Skinner	Sylvan	Wawasee
Max Lake Depth (m)	1.5	15.32	9.0	8.0	20.9
Area (ha)	56.66	114.53	50.59	254.96	1059.5
Perimeter (km)	9.05	22.73	4.36	21.69	46.05
Total Catch (km)	-	105.40	36.14	-	67.91
Temperature (C) (at 1m/ 5m/10m)	24.43/-/-	26.21/21.34/11.11	27.12/15.52/-	26.72/19.79/-	27.13/25.51/17.18
Dissolved Oxygen (mg/L) (at 1m/5m/10m)	6.63/-/-	8.05/7.61/1.87	10.37/0.10/-	8.28/0.38/-	7.58/7.38/0.06
SRP (mg/L) (Epilimnion/Hypolimnion)	0.04/0.01	0.01/0.01	0.01/0.27	0.01/0.13	0.01/0.02
NH_3 (mg/L) (Epilimnion/Hypolimnion)	0.04/0.02	0.03/0.71	0.03/2.10	0.04/1.11	0.02/0.43
pH (Epilimnion/Hypolimnion)	7.9/8.0	8.2/7.6	8.6/7.4	8.4/7.6	8.6/7.6

Table 6 Indiana Department of Environmental Management (IDEM) records (prior to 2016) on total mercury values within the 5 study lakes. No values were available for Backwater Lake.

Lake	Year(s) collected	Total Mercury (µg/kg wet weight; Mean±SD[1])	Fish
Jimmerson	1988	123 (16)	Centrarchidae (Mixed)
	1988	318 (6)	*Micropterus salmoides*
	1988, 1996	249.7±183.54 (15)	*Ameiurus natalis*
Skinner	2005	56 (6)	*Pomoxis nigromaculatus*
	2005	46 (12)	*Lepomis macochirus*
	2005	94.2 (1)	*Cyprinus carpio*
	2005	142±50.9 (4)	*Micropterus salmoides*
	2005	69.8 (2)	*Ameiurus natalis*
Sylvan	2005	0 (3)	*Ameiurus melas*
	2005	34 (6)	*Pomoxis nigromaculatus*
	2005	26.4 (8)	*Lepomis macochirus*
	1987	44 (1)	*Ameiurus nebulosus*
	2005	53.6 (1)	*Cyprinus carpio*
	1987, 1996, 2005	91.2±44.7 (25)	*Micropterus salmoides*
	2005	105 (1)	*Esox lucius*
	2005	65.9 (12)	*Sander vitreus*
	2005	15.7 (3)	*Catostomus commersonii*
	1996	48.2 (1)	*Ameiurus natalis*
Wawasee	1987	25.8±1.5 (12)	*Ameiurus melas*
	1987, 1996	21.9±4.4 (4)	*Cyprinus carpio*
	1987, 1996	201.5±37.4 (4)	*Micropterus salmoides*
	1987	70.5±14.8 (8)	*Lepomis gulosus*
	1996	25.4 (2)	*Ameiurus natalis*
	1987	51±18.4 (14)	*Perca flavescens*
	1996	126 (3)	*Amia calva*
	1996	12.2 (2)	*Ameiurus nebulosus*

[1]Mean total mercury is provided for all fish. For fish that were collected over multiple years, the standard deviation is also provided. Standard deviation was not available for fish that were collected from a single year. Sample size is included in parentheses.

Table 7 Yellow perch and black crappie, sex specific total length (mm; mean ± standard deviation) and sample size (in parentheses) by study lake.

	Yellow Perch		Black Crappie	
Lake	Male	Female	Male	Female
Backwater	195±0 (1)	194.9±13.0 (25)	206.2±27.2(9)	226.4±16.9(7)
Jimmerson	(0)	(0)	247.9± 14.6(11)	257.3±17.7 (21)
Skinner	(0)	(0)	156.4±18.1 (19)	155.5±16.1 (11)
Sylvan	238.4±19.9 (37)	250±0 (1)	250.5±24.3 (13)	240.1±15.1 (13)
Wawasee	199.2±28.4 (12)	194.1±26.6 (20)	225.5±28.6 (17)	235.7±7.6 (19)

Table 8 Yellow perch and black crappie, total mercury concentration (µg/kg of wet weight; mean ± standard deviation), sample size (in parentheses), and the minimum; maximum total mercury concentration for each Indiana Lake examined.

Lake	Yellow Perch Mean±SD	Min;Max	Black Crappie Mean±SD	Min;Max
Backwater	59.79±20.22(26)	32.55;114.76	106.80±36.13(16)	25.63;166.27
Jimmerson	(0)	-	246.72±75.26(32)	133.00;442.90
Skinner	(0)	-	65.07±10.90(30)	52.40;102.61
Sylvan	37.92±12.94(38)	19.06;77.69	34.03±10.65(26)	17.21;56.37
Wawasee	38.67±6.84(32)	24.90;189.37	106.54±19.27(36)	78.08;172.46

Table 9 A subset of the top models (original $w_i > 0.01$) for yellow perch (Top) and black crappie (bottom). Models were used for calculating the final average model (Table 10)[1]. Each model was run nesting fish within lake.

Yellow Perch	Intercept	PC1	PC2	PC3	PC4	$\delta^{13}C$	$\delta^{15}N$	Length	Sex	R^2	df	logLik	AICc	$\Delta AICc^2$	w_i
	2.40	-0.88	-4.56	1.88	-7.50	-	-	0.01	-	0.56	8	-31.50	80.73	0	0.27
	2.44	-	-4.41	2.01	-7.77	-	-	0.01	-	0.56	7	-32.90	81.13	0.4	0.22
	2.35	-0.94	-4.87	-	-6.70	-	-	0.01	-	0.56	7	-33.69	82.71	1.98	0.1
	2.40	-	-4.74	-	-6.92	-	-	0.01	-	0.56	6	-35.12	83.23	2.5	0.08
	4.02	0.08	-1.67	4.16	-12.55	-	-	-	-	0.48	7	-34.21	83.76	3.03	0.06
	4.02	-	-1.68	4.15	-12.54	-	-	-	-	0.48	6	-35.47	83.93	3.2	0.05
	2.38	-0.85	-3.79	2.56	-6.79	-	-	0.01	+	0.56	9	-32.37	84.93	4.2	0.03
	2.58	-0.68	-	2.85	-9.21	-	-	0.01	-	0.54	7	-34.91	85.15	4.43	0.03
	2.42	-	-3.63	2.71	-7.02	-	-	0.01	+	0.56	8	-33.76	85.25	4.52	0.03
	2.61	-	-	2.93	-9.37	-	-	0.01	-	0.54	6	-36.23	85.45	4.72	0.03
	4.02	0.12	-	4.44	-13.00	-	-	-	-	0.47	6	-36.32	85.64	4.91	0.02
	4.02	-	-	4.43	-12.98	-	-	-	-	0.47	5	-37.58	85.85	5.12	0.02
	3.05	-0.87	-4.65	1.26	-6.81	-	-0.05	0.01	-	0.57	9	-32.89	85.98	5.25	0.02
	3.09	-	-4.51	1.39	-7.07	-	-0.05	0.01	-	0.57	8	-34.29	86.31	5.59	0.02
	2.19	-1.11	-5.72	0.17	-	-	-	0.01	-	0.54	7	-35.56	86.45	5.72	0.02

Table 9 continued

Black Crappie	Intercept	PC1	PC2	PC3	PC4	PC5	δ13C	δ15N	Length	Sex	R²	df	logLik	AICc	Δ AICc	wi
	2.61	-0.78	-0.74	-0.48	-4.67	-2.93	-	-	0.01	-	0.90	9	8.98	1.44	0.00	0.12
	2.60	-	-0.29	-0.52	-4.53	-2.95	-	-	0.01	-	0.90	8	7.71	1.69	0.24	0.11
	2.59	-0.79	-0.67	-	-4.68	-2.95	-	-	0.01	-	0.90	8	7.46	2.18	0.74	0.08
	2.58	-	-0.21	-	-4.53	-2.96	-	-	0.01	-	0.90	7	6.19	2.47	1.02	0.07
	2.58	-0.66	-	-0.41	-4.56	-2.98	-	-	0.01	-	0.90	8	7.24	2.63	1.19	0.07
	5.00	-1.25	-1.25	-0.46	-4.45	-3.30	0.08	-	0.01	-	0.90	10	9.52	2.67	1.23	0.07
	2.59	-	-	-0.49	-4.49	-2.97	-	-	0.01	-	0.90	7	6.07	2.72	1.27	0.06
	4.98	-1.26	-1.18	-	-4.46	-3.32	0.08	-	0.01	-	0.90	9	8.04	3.32	1.88	0.05
	2.56	-0.68	-	-	-4.57	-2.99	-	-	0.01	-	0.90	7	5.75	3.36	1.92	0.05
	2.57	-	-	-	-4.50	-2.98	-	-	0.01	-	0.90	6	4.57	3.49	2.05	0.04
	4.81	-	-0.50	-0.52	-4.24	-3.29	0.07	-	0.01	-	0.90	9	7.87	3.66	2.22	0.04
	4.90	-1.05	-	-0.34	-4.26	-3.38	0.07	-	0.01	-	0.90	9	7.69	4.02	2.58	0.03
	4.79	-	-0.42	-	-4.25	-3.31	0.07	-	0.01	-	0.90	8	6.37	4.37	2.93	0.03
	2.65	-0.79	-0.95	-0.54	-4.98	-	-	-	0.01	-	0.90	8	6.33	4.45	3.01	0.03
	4.89	-1.06	-	-	-4.28	-3.39	0.07	-	0.01	-	0.90	8	6.23	4.66	3.21	0.02
	4.78	-	-	-0.47	-4.18	-3.33	0.07	-	0.01	-	0.90	8	6.22	4.66	3.22	0.02
	2.64	-	-0.50	-0.58	-4.83	-	-	-	0.01	-	0.90	7	5.05	4.75	3.30	0.02
	2.62	-0.80	-0.87	-	-4.99	-	-	-	0.01	-	0.90	7	4.80	5.25	3.80	0.02
	4.76	-	-	-	-4.19	-3.34	0.07	-	0.01	-	0.90	7	4.75	5.36	3.92	0.02
	2.61	-	-0.40	-	-4.84	-	-	-	0.01	-	0.90	6	3.52	5.59	4.15	0.02
	2.60	-0.64	-	-0.45	-4.84	-	-	-	0.01	-	0.90	7	4.55	5.76	4.31	0.01
	2.62	-	-	-0.52	-4.77	-	-	-	0.01	-	0.90	6	3.39	5.85	4.41	0.01

[1] The - indicates variable was not present within the model and a + indicates a variable was present.

[2] The Δ AICc and w_i represent only the subset of models used to calculate the average model.

Table 10 ANOVA tables for the final averaged models for yellow perch (top 15 models) and black crappie (top 22 models). Averaged coefficient values were calculated only from models (Table 9) which contained the variable.

		Estimate	Std. Error	z value	P-value
Yellow Perch	Intercept	2.69	0.76	3.50	<0.001
	Length	0.01	0.00	4.21	<0.001
	PC1	-0.74	1.35	0.54	0.59
	PC2	-4.17	2.77	1.49	0.14
	PC3	2.44	3.11	0.77	0.44
	PC4	-8.31	4.43	1.86	0.06
	$\delta^{15}N$	-0.05	0.04	1.41	0.16
	Sex (Male)	0.09	0.13	0.72	0.47
Black Crappie	Intercept	3.24	1.21	2.66	0.01
	Length	0.01	0.00	7.93	<0.001
	PC1	-0.88	1.10	0.79	0.43
	PC2	-0.65	2.11	0.31	0.76
	PC3	-0.48	1.73	0.27	0.78
	PC4	-4.54	1.91	2.36	0.02
	PC5	-3.07	2.06	1.48	0.14
	$\delta^{13}C$	0.0687	0.0308	2.21	0.0270
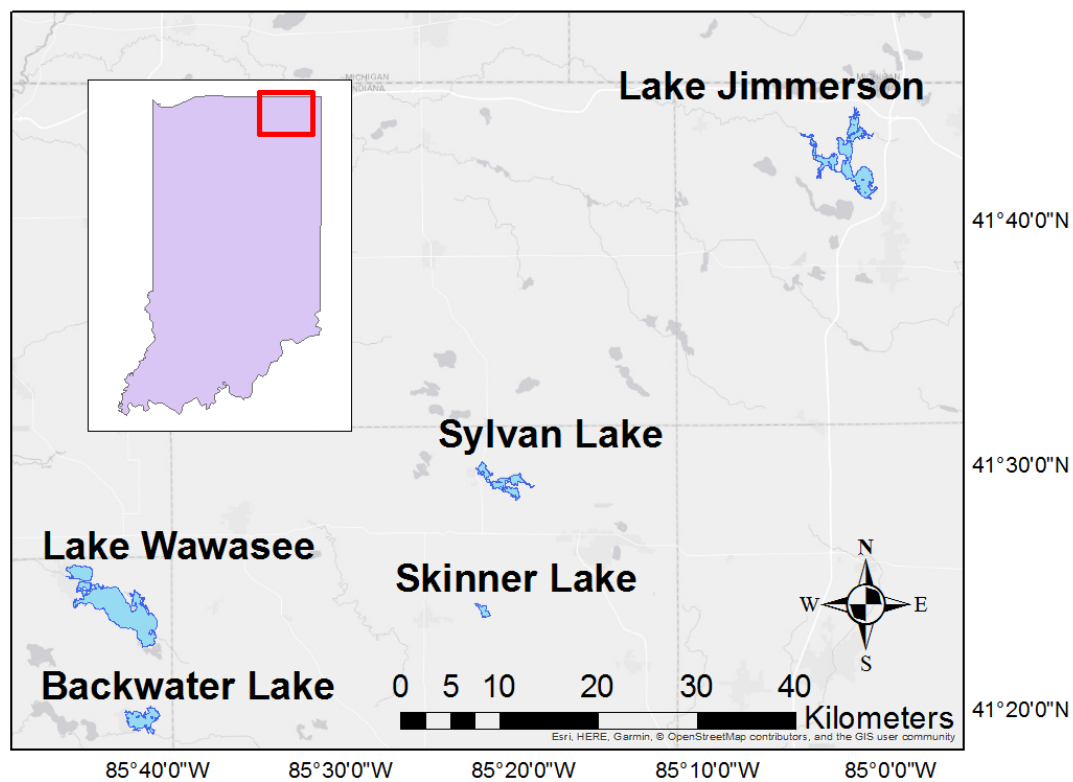
Figure 6 Map of Indiana lakes (in UTM) in which yellow perch and black crappie were collected for isotopes (δ^{13}C and δ^{15}N), morphology, and total mercury analysis.
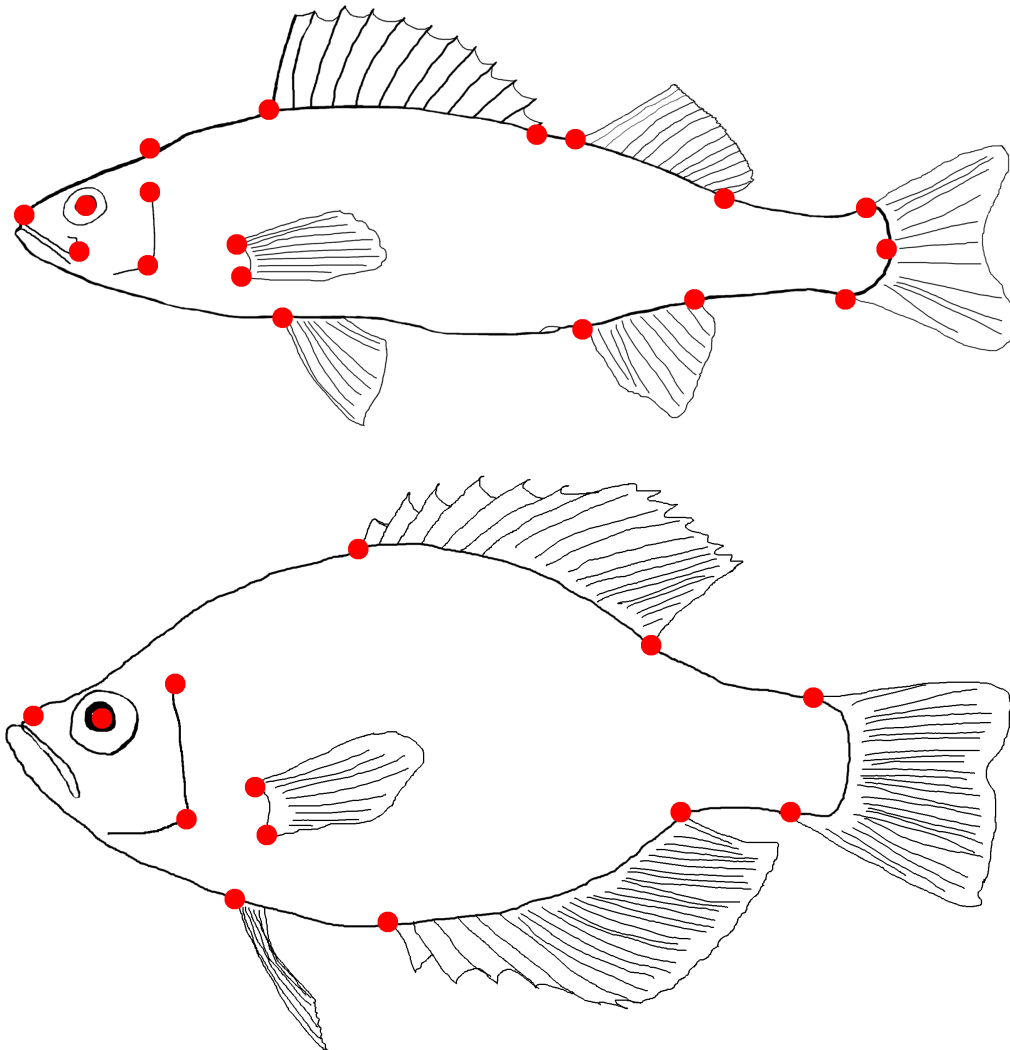
Figure 7 Landmark points on yellow perch (top) and black crappie (bottom) used for morphometric analysis.
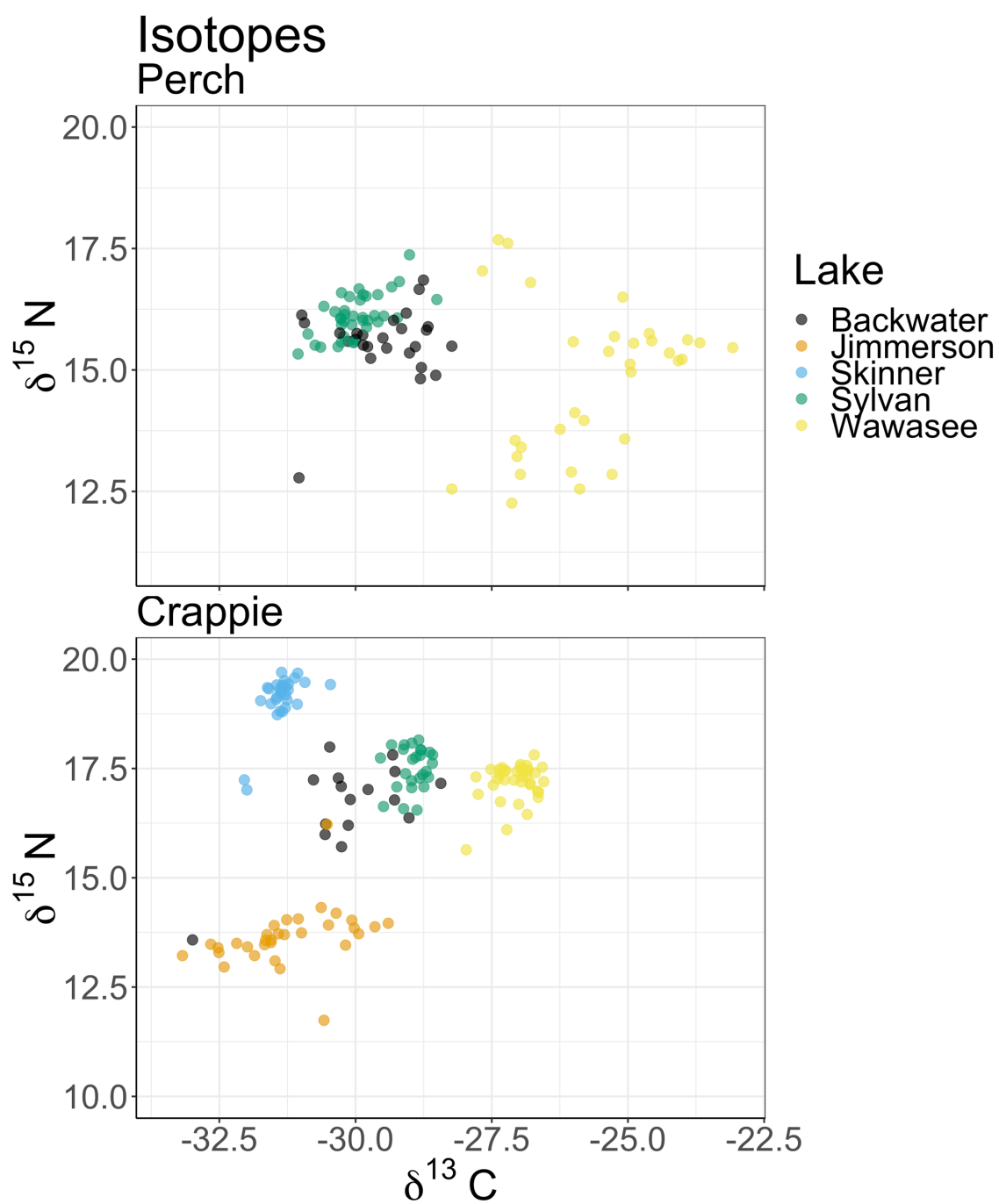
Figure 8 Isotope ratio biplots for yellow perch (top) and black crappie (bottom). Carbon ratios are lipid corrected.
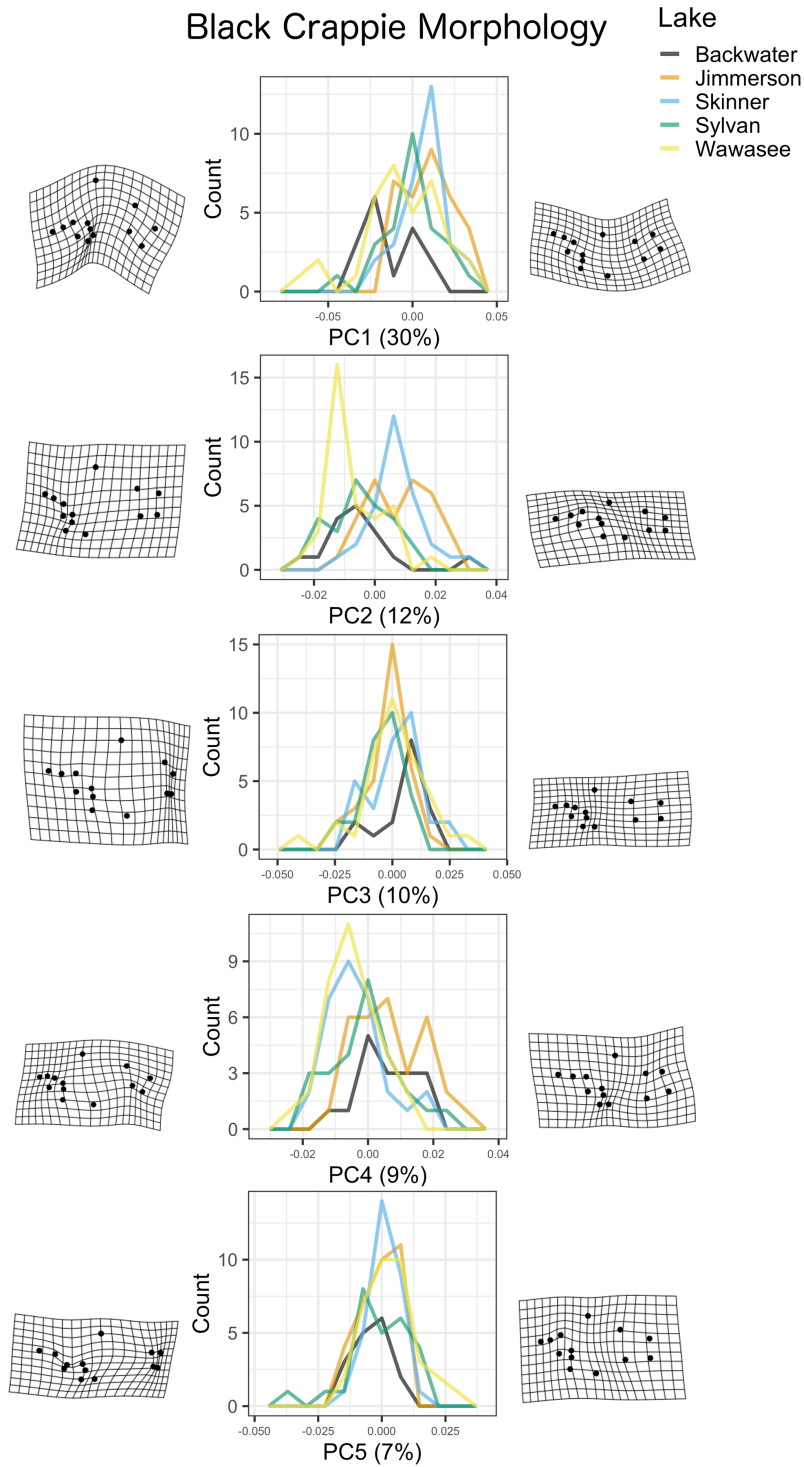
Figure 9 Landmark morphology of black crappie collapsed into five principle component axes (68% total morphological variation explained). Warp grids (grids magnified: PC1 5x, PC2 6x, PC3 x4, PC4 x5, PC5 x4) represent the morphology of individuals at the minimum or maximum of the axis.
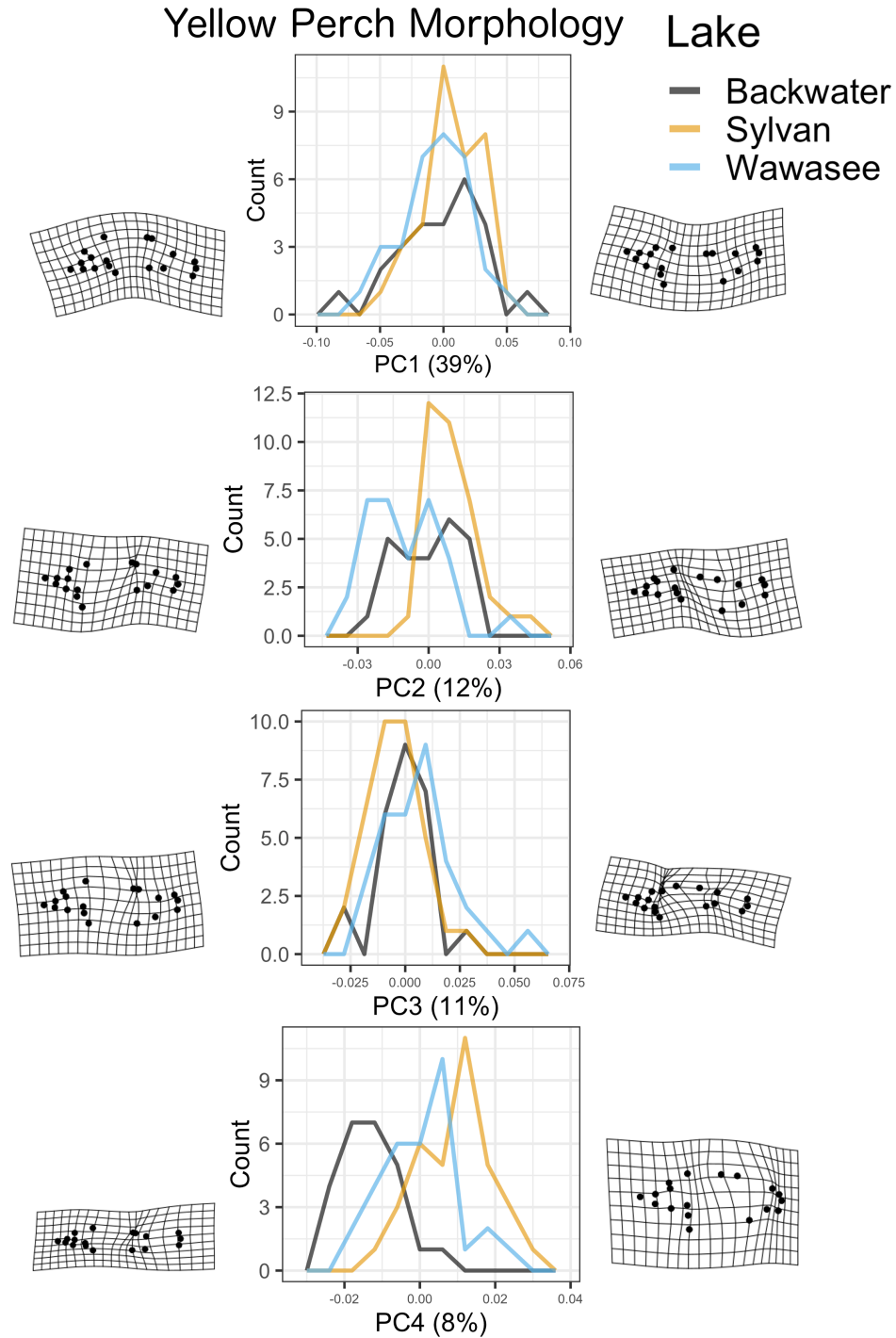
Figure 10 Landmark morphology of yellow perch collapsed into 4 principle component axes (70% total morphological variation explained). Warp grids (grids magnified: PC1 2x, PC2 3x, PC3 x3, PC4 x5) represent the morphology of individuals at the minimum or maximum of the axis.
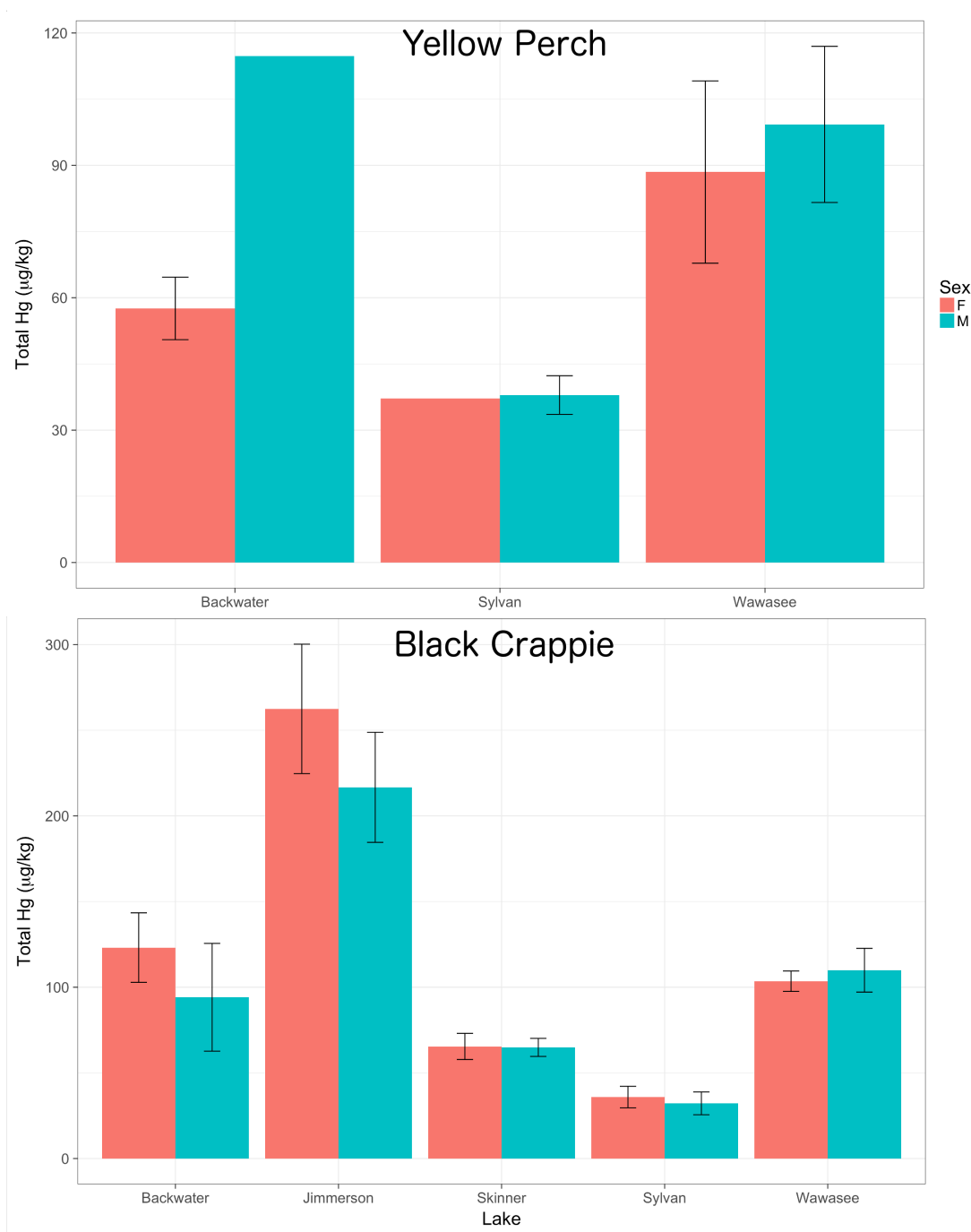
Figure 11 Mean with 95% confidence interval of total mercury for male (blue) and female (red) fish in yellow perch (top) and black crappie (bottom) populations. Note, male yellow perch in Backwater and female yellow perch in Sylvan are missing error bars since only a single representative of this sex was collected in 2016.

APPENDIX A. SUPPLEMENTAL TABLES

Table 11 Distribution of stomach contents into the 11 prey categories.

Category	Prey Item (lowest taxonomic level identified)
Chironomidae	Chironomidae
Ephemeroptera	*Hexagenia* Caenidae Unidentified Ephemeroptera
Chydoridae	Chydoridae
Amphipoda	Amphipoda
Daphnia	*Daphnia*
Predatory Zooplankton	*Bythotrephes* *Hemimysis* *Leptodora*
Copepoda	Calanoida Cyclopoida Nauplii stage copepods Harpacticoida
Trichoptera	Trichoptera Unidentified Hydroptilidae Hydrosychidae
Fish	Unidentified fish larvae Fish eggs *Neogobius melanostomus*
Other Zooplankton	*Diaphanosoma* Veliger stage molluscs *Ilyocryptus* *Eubosmina* Ostrocoda *Bosmina*

Table 11 continued

	Holopedium
Other MacroInverts	Acari
	Coleoptera
	Diptera
	Sphaeriidae
	Odonata
	Isopoda
	Hemiptera
	Oligochaete
	Dreissena bugensis
	Dreissena polymorpha
	Unidentified *Dreissena*
	Nematoda
	Lymnaeidae
	Unidentified Gastropod

Table 12 PerMANOVA comparisons of stomach contents among the four sites for round goby, age-0 yellow perch and age-1 yellow perch. Round goby and age-1 yellow perch variables included site, month, year, total length, and the interaction terms among site and the temporal variables. Analysis of age-0 yellow perch did not include month as a variable.

Round Goby		Df	F	R^2	P-value
	Total Length	1	6.74	0.085	0.002
	Site	3	3.14	0.119	0.004
	Month	1	2.59	0.033	0.059
	Year	1	0.760	0.009	0.533
	Site*Month	3	2.45	0.093	0.006
	Site*Year	3	1.42	0.054	0.195
	Month*Year	1	0.890	0.011	0.455
	Site*Month*Year	3	2.01	0.076	0.034
	Residuals	41		0.519	
Age-0 Yellow Perch		Df	F	R^2	P-value
	Total Length	1	16.4	0.086	0.001
	Site	3	15.4	0.242	0.001
	Year	1	4.18	0.022	0.002
	Site*Year	3	7.64	0.120	0.001
	Residuals	101		0.529	
Age-1 Yellow Perch		Df	F	R^2	P-value
	Total Length	1	14.2	0.142	<0.001
	Site	3	6.23	0.187	<0.001
	Month	1	2.73	0.027	0.033
	Year	1	4.53	0.045	0.003
	Site*Month	2	2.27	0.045	0.032
	Site*Year	3	2.83	0.085	0.003
	Month*Year	1	1.46	0.015	0.205
	Site*Month*Year	1	0.370	0.004	0.824
	Residuals	58		0.450	

Table 13 SIMPER results for pairwise comparisons for dissimilarity between sites and the stomach content differences in round goby. Table shows the top 5 prey categories that contribute to dissimilarity.

Round Goby	Average Value[a]		Average Dissimilarity[b]	SD[c]	Cumulative Contribution[d]
Site	V2	SB10			
Other Zooplankton	0.274	0.592	0.255	0.176	37.66
Chironomidae	0.380	0.193	0.186	0.156	65.11
Other Macroinverts	0.255	0.061	0.142	0.183	86.06
Amphipoda	0.004	0.096	0.052	0.150	93.69
Chydoridae	0.00	0.054	0.028	0.057	97.79
Site	V2	SB5			
Chironomidae	0.380	0.282	0.201	0.152	29.78
Other Macroinverts	0.255	0.176	0.171	0.189	55.20
Other Zooplankton	0.274	0.298	0.170	0.140	80.51
Amphipoda	0.004	0.126	0.067	0.151	90.45
Chydoridae	0.000	0.048	0.025	0.057	94.21
Site	V2	N1			
Chironomidae	0.380	0.505	0.214	0.146	36.87
Other Macroinverts	0.255	0.226	0.180	0.182	67.96
Other Zooplankton	0.274	0.247	0.160	0.135	95.56
Fish	0.023	0.000	0.011	0.036	97.51
Amphipoda	0.004	0.015	0.010	0.023	99.17
Site	SB10	SB5			
Other Zooplankton	0.592	0.298	0.249	0.175	36.14
Chironomidae	0.193	0.282	0.166	0.163	60.25
Other Macroinverts	0.061	0.176	0.104	0.152	75.37
Amphipoda	0.096	0.126	0.099	0.182	89.77
Chydoridae	0.054	0.048	0.043	0.065	96.04
Site	SB10	N1			
Other Zooplankton	0.592	0.247	0.253	0.175	36.59
Chironomidae	0.193	0.505	0.229	0.160	69.71
Other Macroinverts	0.061	0.226	0.122	0.156	87.41
Amphipoda	0.096	0.015	0.054	0.142	95.26
Chydoridae	0.054	0.000	0.027	0.055	99.14
Site	SB5	N1			
Chironomidae	0.282	0.505	0.223	0.165	33.59
Other Zooplankton	0.298	0.247	0.167	0.146	58.74
Other Macroinverts	0.176	0.226	0.152	0.167	81.69
Amphipoda	0.126	0.015	0.068	0.142	91.94
Chydoridae	0.048	0.000	0.024	0.055	95.62

[a] Average value of the factor examined within each site
[b] Average contribution to overall dissimilarity
[c] Standard Deviation of contribution
[d] Percent Cumulative contribution

Table 14 SIMPER results for pairwise comparisons for dissimilarity between sites and the stomach content differences in age-0 yellow perch. Table shows the top 5 prey categories that contribute to dissimilarity.

Age-0 Yellow Perch	Average Value[a]		Average Dissimilarity[b]	SD[c]	Cumulative Contribution[d]
Site	**V2**	**SB10**			
Other Zooplankton	0.444	0.127	0.210	0.175	24.90
Chironomidae	0.070	0.241	0.134	0.170	40.76
Amphipoda	0.219	0.040	0.117	0.160	54.59
Copepoda	0.164	0.120	0.109	0.134	67.57
Daphnia	0.001	0.177	0.089	0.133	78.07
Site	**V2**	**N1**			
Copepoda	0.164	0.644	0.2925	0.188	34.56
Other Zooplankton	0.444	0.014	0.219	0.185	60.45
Amphipoda	0.219	0.017	0.112	0.161	73.72
Chironomidae	0.070	0.184	0.106	0.150	86.28
Ephemeroptera	0.022	0.122	0.068	0.127	94.29
Site	**V2**	**SB5**			
Copepoda	0.164	0.601	0.246	0.130	29.54
Other Zooplankton	0.444	0.035	0.211	0.182	54.87
Daphnia	0.001	0.333	0.166	0.130	74.75
Amphipoda	0.219	0.000	0.110	0.165	87.88
Chironomidae	0.070	0.000	0.035	0.092	92.06
Site	**SB10**	**N1**			
Copepoda	0.120	0.644	0.301	0.206	35.19
Chironomidae	0.241	0.184	0.157	0.170	53.61
Daphnia	0.177	0.000	0.089	0.134	63.98
Other Zooplankton	0.127	0.014	0.066	0.118	71.68
Predatory Zooplankton	0.128	0.000	0.064	0.113	79.15
Site	**SB10**	**SB5**			
Copepoda	1.989	0.120	0.266	0.134	33.9
Daphnia	1.328	0.177	0.161	0.122	54.44
Chironomidae	0.686	0.241	0.121	0.176	69.80
Predatory Zooplankton	0.641	0.128	0.068	0.106	78.42
Other Zooplankton	0.583	0.127	0.066	0.114	86.84
Site	**N1**	**SB5**			
Copepoda	0.644	0.601	0.215	0.137	36.51
Daphnia	0.000	0.333	0.166	0.130	64.69
Chironomidae	0.184	0.000	0.092	0.149	80.27
Ephemeroptera	0.122	0.000	0.061	0.129	90.62
Other Zooplankton	0.014	0.035	0.022	0.029	94.30

[a] Average value of the factor examined within each site
[b] Average contribution to overall dissimilarity
[c] Standard Deviation of contribution
[d] Percent Cumulative contribution

Table 15 SIMPER results for pairwise comparisons for dissimilarity between sites and the stomach content differences in age-1 yellow perch. Table shows the top 5 prey categories that contribute to dissimilarity.

Age-1 Yellow Perch	Average Value[a]		Average Dissimilarity[b]	SD[c]	Cumulative Contribution[d]
Site	**V2**	**SB10**			
Chironomidae	0.543	0.512	0.236	0.168	37.18
Amphipoda	0.210	0.296	0.181	0.170	65.66
Predatory Zooplankton	0.000	0.175	0.088	0.153	79.46
Trichoptera	0.087	0.000	0.044	0.127	86.36
Other Macroinverts	0.054	0.014	0.033	0.109	91.53
Site	**V2**	**SB5**			
Chironomidae	0.543	0.573	0.243	0.216	36.49
Amphipoda	0.210	0.071	0.123	0.184	54.98
Predatory Zooplankton	0.000	0.228	0.114	0.193	72.13
Trichoptera	0.087	0.000	0.044	0.127	78.69
Chydoridae	0.063	0.007	0.034	0.093	83.83
Site	**V2**	**N1**			
Chironomidae	0.543	0.176	0.261	0.220	29.40
Copepoda	0.005	0.397	0.199	0.228	51.78
Fish	0.007	0.286	0.144	0.224	68.02
Amphipoda	0.210	0.000	0.105	0.184	79.82
Other Macroinverts	0.054	0.124	0.082	0.172	89.08
Site	**SB10**	**SB5**			
Chironomidae	0.512	0.573	0.234	0.167	37.85
Predatory Zooplankton	0.175	0.228	0.158	0.191	63.31
Amphipoda	0.296	0.071	0.155	0.161	88.33
Daphnia	0.000	0.059	0.029	0.099	93.09
Fish	0.000	0.050	0.025	0.109	97.12
Site	**SB10**	**N1**			
Chironomidae	0.512	0.176	0.241	0.172	26.94
Copepoda	0.000	0.397	0.199	0.231	49.13
Fish	0.296	0.000	0.148	0.163	65.67
Predatory Zooplankton	0.000	0.286	0.1429	0.227	81.63
Other Macroinverts	0.175	0.000	0.088	0.154	91.43
Site	**SB5**	**N1**			
Chironomidae	0.573	0.176	0.271	0.217	30.92
Copepoda	0.011	0.397	0.199	0.226	53.64
Fish	0.050	0.286	0.154	0.231	71.15
Predatory Zooplankton	0.228	0.000	0.114	0.194	84.17
Other Macroinverts	0.000	0.124	0.062	0.152	91.24

[a] Average value of the factor examined within each site
[b] Average contribution to overall dissimilarity
[c] Standard Deviation of contribution
[d] Percent Cumulative contribution

Table 16 PerMANOVA and post-hoc pairwise comparisons of sites for round goby isotope ratios. All isotopes ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{2}H$) are included as dependent variables. Independent variables include the site, year, month and all interactions. The global permutation p-value represents the comparison of all sites after 999 permutations and the corresponding R^2 value. Pairwise comparisons only include site and the significance value is corrected using the Bonferroni method (α=0.008). Significant pairwise comparisons are denoted in bold.

Round Goby		Df	F	R^2	P-value
Global					
	Site	3	7.1	0.392	0.001
	Month	1	0.36	0.007	0.617
	Year	1	0.51	0.009	0.537
	Site*Month	2	1.29	0.047	0.281
	Site*Year	3	0.84	0.046	0.496
	Month*Year	1	3.02	0.055	0.074
	Site*Month*Year	1	1.19	0.022	0.286
	Residuals	23		0.422	
V2*SB10					
	Site	1	0.630	0.043	0.462
	Residuals	14		0.957	
V2*SB5					
	Site	1	2.19	0.114	0.143
	Residuals	17		0.886	
V2*N1					
	Site	1	15.8	0.455	**0.001**
	Residuals	19		0.545	
SB10*SB5					
	Site	1	0.373	0.028	0.623
	Residuals	13		0.972	
SB10*N1					
	Site	1	10.3	0.406	**0.003**
	Residuals	15		0.594	
SB5*N1					
	Site	1	9.67	0.349	**0.004**
	Residuals	18		0.650	

Table 17 Isotope ANOVA and post-hoc results for round goby.

Round Goby	Isotope	Df	Variable	F	P
ANOVA	C	3	Site	32.0	<0.001
		1	Month	0.0340	0.85
		1	Year	4.74	0.038
Tukey			**Comparison**	**P**	
	Site		SB10 v N1	<0.001	
			SB5 v N1	<0.001	
			V2 v N1	<0.001	
			SB5 v SB10	0.119	
			V2 v SB10	0.711	
			V2 v SB5	0.003	
	Month		May v August	0.865	
	Year		2009 v 2010	0.042	
	Isotope	**Df**	**Variable**	**F**	**P**
ANOVA	N	3	Site	78.8	<0.001
		1	Month	1.93	0.18
		1	Year	5.03	0.033
Tukey			**Comparison**	**P**	
	Site		SB10 v N1	<0.001	
			SB5 v N1	<0.001	
			V2 v N1	<0.001	
			SB5 v SB10	0.134	
			V2 v SB10	<0.001	
			V2 v SB5	<0.001	
	Month		May v August	0.210	
	Year		2009 v 2010	0.037	
	Isotope	**Df**	**Variable**	**F**	**P**
ANOVA	H	3	Site	4.66	0.0090
		1	Month	0.250	0.62
		1	Year	0.190	0.67
Tukey			**Comparison**	**P**	
	Site		SB10 v N1	0.063	
			SB5 v N1	0.066	
			V2 v N1	0.010	
			SB5 v SB10	0.99	
			V2 v SB10	0.99	
			V2 v SB5	0.90	
	Month		May v August	0.65	

Table 17 continued

Isotope	Df	Year Variable	2009 v 2010 F	0.674 P
ANOVA **O**	3	Site	2.91	0.051
	1	Month	3.10	0.089
	1	Year	1.20	0.28
Tukey		**Comparison**	**P**	
Site		SB10 v N1	0.627	
		SB5 v N1	0.184	
		V2 v N1	0.900	
		SB5 v SB10	0.928	
		V2 v SB10	0.306	
		V2 v SB5	0.053	
Month		May v August	0.114	
Year		2009 v 2010	0.295	

Table 18 PerMANOVA and post-hoc pairwise comparisons of sites for age-0 yellow perch isotope ratios. All isotopes ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{2}H$) are included as dependent variables. Independent variables include the site, year, their interaction. The global permutation p-value represents the comparison of all sites after 999 permutations and the corresponding R value. Pairwise comparisons only include site and the significance value is corrected using the conservative Bonferroni method (α=0.008). Significant pairwise comparisons are denoted in bold.

Age-0 Yellow Perch Global		Df	F	R^2	P-value
	Site	3	38.442	0.726	0.001
	Year	1	5.63	0.036	0.019
	Site*Year	3	7.299	0.138	0.001
	Residuals	16		0.100	
V2*SB10					
	Site	1	21.689	0.684	**0.003**
	Residuals	10		0.316	
V2*SB5					
	Site	1	54.169	0.844	**0.001**
	Residuals	10		0.156	
V2*N1					
	Site	1	3.112	0.237	0.057
	Residuals	10		0.763	
SB10*SB5					
	Site	1	4.0723	0.289	0.064
	Residuals	10		0.711	
SB10*N1					
	Site	1	10.124	0.503	0.014
	Residuals	10		0.497	
SB5*N1					
	Site	1	25.268	0.716	**0.002**
	Residuals	10		0.283	

Table 19 Isotope ANOVA and post-hoc results for age-0 yellow perch.

Age-0 Yellow Perch	Isotope	Df	Variable	F	P
ANOVA	**C**	3	Site	38.1	<0.001
		1	Year	71.2	<0.001
Tukey			**Comparison**	**P**	
	Site		SB10 v N1	<0.001	
			SB5 v N1	<0.001	
			V2 v N1	<0.001	
			SB5 v SB10	0.119	
			V2 v SB10	0.999	
			V2 v SB5	0.125	
	Year		2009 v 2010	<0.001	
	Isotope	**Df**	**Variable**	**F**	**P**
ANOVA	**N**	3	Site	38.6	<0.001
		1	Year	8.47	<0.001
Tukey			**Comparison**	**P**	
	Site		SB10 v N1	<0.001	
			SB5 v N1	<0.001	
			V2 v N1	<0.001	
			SB5 v SB10	0.009	
			V2 v SB10	0.382	
			V2 v SB5	<0.001	
	Year		2009 v 2010	0.009	
	Isotope	**Df**	**Variable**	**F**	**P**
ANOVA	**H**	3	Site	18.9	<0.001
		1	Year	1.35	0.26
Tukey			**Comparison**	**P**	
	Site		SB10 v N1	0.012	
			SB5 v N1	<0.001	
			V2 v N1	0.677	
			SB5 v SB10	0.223	
			V2 v SB10	<0.001	
			V2 v SB5	<0.001	
	Year		2009 v 2010	0.260	
	Isotope	**Df**	**Variable**	**F**	**P**
ANOVA	**O**	3	Site	0.528	0.668
		1	Year	0.0900	0.767

Table 19 continued

Tukey		Comparison	P
	Site	SB10 v N1	0.608
		SB5 v N1	0.967
		V2 v N1	0.931
		SB5 v SB10	0.860
		V2 v SB10	0.916
		V2 v SB5	0.999
	Year	2009 v 2010	0.767

Table 20 PerMANOVA and post-hoc pairwise comparisons of sites for age-1 yellow perch isotope ratios. All isotopes ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, δ^2H) are included as dependent variables. Independent variables include the site, year, month and all interactions. The global permutation p-value represents the comparison of all sites after 999 permutations and the corresponding R value. Pairwise comparisons only include site and the significance value is corrected using the conservative Bonferroni method (α=0.008). Significant pairwise comparisons are denoted in bold.

Age-1 Yellow Perch Global		Df	F	R^2	P-value
	Site	3	3.99	0.162	0.014
	Month	1	0.240	0.003	0.759
	Year	1	0.340	0.004	0.660
	Site*Month	2	9.26	0.376	0.001
	Site*Year	3	3.11	0.126	0.031
	Month*Year	1	1.98	0.027	0.146
	Site*Month*Year	1	2.29	0.031	0.119
	Residuals	20		0.270	
V2*SB10					
	Site	1	1.235	0.076	0.259
	Residuals	15		0.924	
V2*SB5					
	Site	1	1.97	0.098	0.144
	Residuals	18		0.901	
V2*N1					
	Site	1	1.422	0.087	0.208
	Residuals	15		0.913	
SB10*SB5					
	Site	1	0.0250	0.002	0.994
	Residuals	16		0.998	
SB10*N1					
	Site	1	2.97	0.198	0.098
	Residuals	12		0.802	
SB5*N1					
	Site	1	5.15	0.256	0.023
	Residuals	16		0.744	

Table 21 Isotope ANOVA and post-hoc results for age-1 yellow perch.

Age-1 Yellow Perch	Isotope	Df	Variable	F	P
ANOVA	**C**	3	Site	2.73	0.0630
		1	Month	0.0280	0.869
		1	Year	0.381	0.661
Tukey			**Comparison**	**P**	
	Site		SB10 v N1	0.085	
			SB5 v N1	0.075	
			V2 v N1	0.289	
			SB5 v SB10	0.999	
			V2 v SB10	0.816	
			V2 v SB5	0.852	
	Month		May v August	0.884	
	Year		2009 v 2010	0.672	
	Isotope	**Df**	**Variable**	**F**	**P**
ANOVA	**N**	3	Site	12.2	<0.001
		1	Month	0.127	0.724
		1	Year	0.523	0.476
Tukey			**Comparison**	**P**	
	Site		SB10 v N1	<0.001	
			SB5 v N1	<0.001	
			V2 v N1	0.006	
			SB5 v SB10	0.900	
			V2 v SB10	0.472	
			V2 v SB5	0.106	
	Month		May v August	0.755	
	Year		2009 v 2010	0.491	
	Isotope	**Df**	**Variable**	**F**	**P**
ANOVA	**H**	3	Site	1.164	0.341
		1	Month	0.0870	0.771
		1	Year	0.121	0.731

Table 21 continued

Tukey			Comparison	P	
	Site		SB10 v N1	0.540	
			SB5 v N1	0.494	
			V2 v N1	0.993	
			SB5 v SB10	0.999	
			V2 v SB10	0.631	
			V2 v SB5	0.582	
	Month		May v August	0.797	
	Year		2009 v 2010	0.740	
	Isotope	**Df**	**Variable**	**F**	**P**
ANOVA	**O**	3	Site	0.905	0.451
		1	Month	0.721	0.403
		1	Year	0.365	0.550
Tukey			**Comparison**	**P**	
	Site		SB10 v N1	0.779	
			SB5 v N1	0.591	
			V2 v N1	0.397	
			SB5 v SB10	0.995	
			V2 v SB10	0.941	
			V2 v SB5	0.983	
	Month		May v August	0.459	
	Year		2009 v 2010	0.565	

Table 22 Physical Conditions within pools during 2015 and 2016.

	2015 (60 days)	2016 (50 days)
Temperature	20.9 C	22.3 C
Oxygen	6.4 mg/L	7.1 mg/L
pH	7.8	7.9
Zooplankton Counts[a]	975/ml	107/ml

[a] Values represent average counts collected from a nearby aquaculture pond for use in the experiments. In 2015, zooplankton additions were adjusted with changing pool densities, in 2016 all pools received the same amount of zooplankton supplements. All zooplankton within the sample including; Copepods, *Diaphanosoma*, *Eubosmina*, *Daphnia*, Rotifers, Ostrocods, and small members of Insecta.

Table 23 Source and years used for physical measurements from the study lakes. Values used in the study were averaged across years.

Lake	Indiana Clean Lakes Program[1]
Backwater	1994, 1998, 2003
Jimmerson	1989, 1992, 1997, 2002, 2006
Skinner	1990, 1993, 2000, 2003, 2006, 2013
Sylvan	1991, 2000, 2003, 2010, 2012, 2014
Wawasee	1994, 2000, 2003, 2006, 2010

[1]Includes pH, Primary Producer community make-up, Dissolved Oxygen, Temperature, Lake Depth, Lake Perimeter, Lake Surface Area, Lake SDI
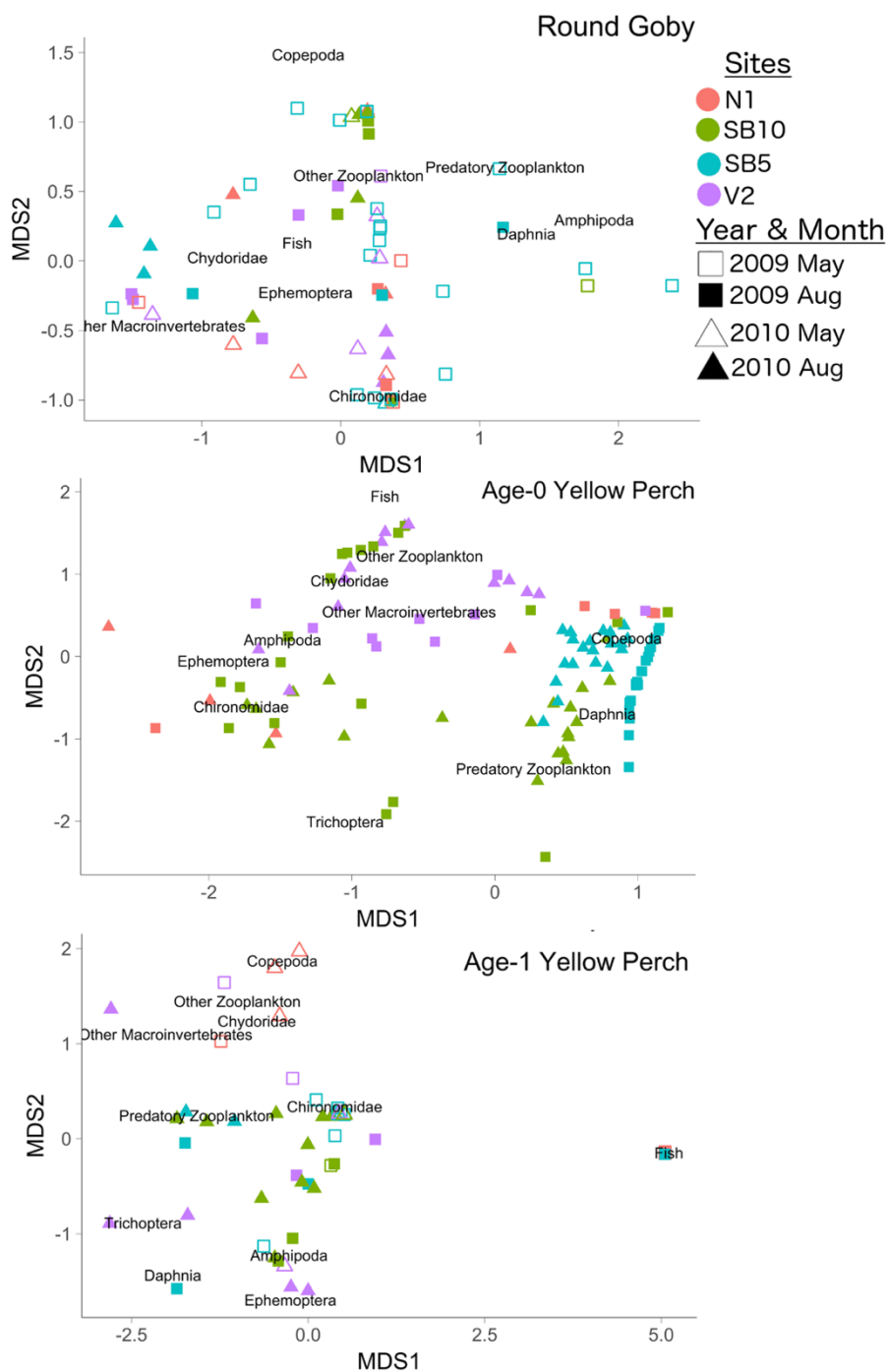
APPENDIX B. SUPPLEMENTAL FIGURES

Figure 12 Biplots of the 2 MDS axes summarizing the stomach contents from round goby (Top; stress=0.144), age-0 yellow perch (middle; stress= 0.159) and age-1 yellow perch (bottom; stress=0.84) for each site, in each year and month.
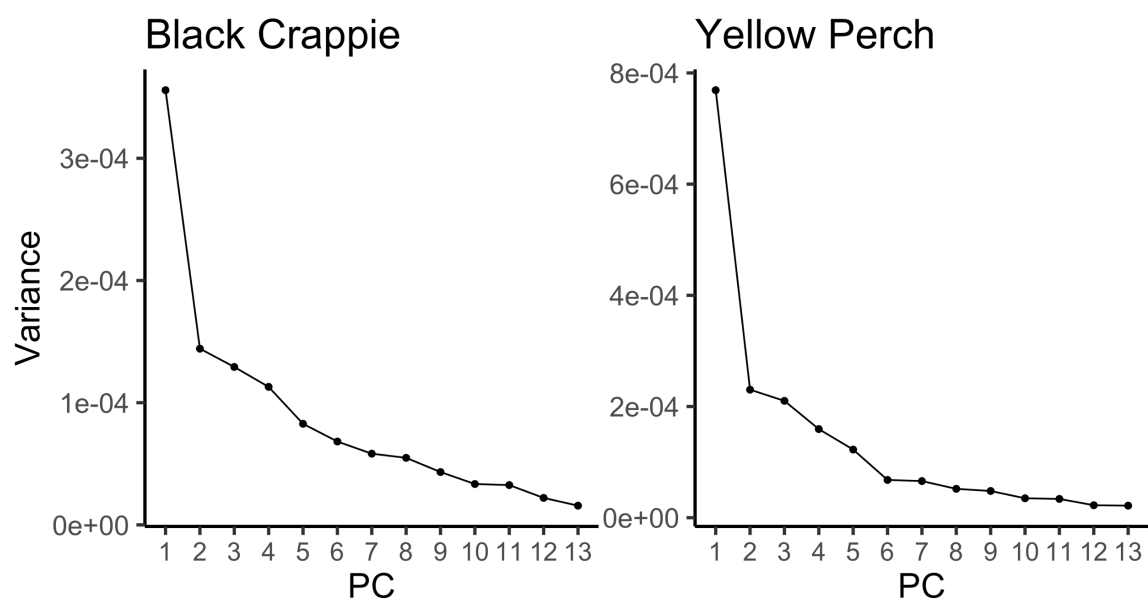
Figure 13 Scree plots containing the first 13 principle components used to summarize morphological variance for black crappie (Left) and yellow perch (Right).

APPENDIX C. SUPPLEMENTAL ANALYSIS

2014 Pilot study; Population and Simulated Habitat effects on Age-0 Yellow Perch

Introduction & Methods

Prior to our 2015 experiment to examine morphological change in response to simulated habitats, we conducted a pilot study using a smaller mesocosm setup in 2014. This study examined two populations of age-0 yellow perch. The first from North Carolina's Perquimans river, and the second was a population descended from Ohio's Lake Erie (Personal Communication with Ohio Department of Natural Resources) which resided at St. Mary's Fish Hatchery in St. Mary's, Ohio before being moved to Purdue for the study. Prior to experimentation, young-of-year perch from each population were housed indoors, for 2-3 weeks within separate, 750L circular flow tanks and were fed zooplankton collected from a nearby aquaculture pond. On July 23, 30 fish from each population (60 total) were measured for length (Table S1) and then placed into a mesocosm simulating a littoral or pelagic habitat condition (See mesocosm description in Chapter 3 Methods). In addition, 32 randomly selected individuals from each population were euthanized at the start of the experiment and frozen for morphometric analysis of pre-experiment shapes. In total, there were 4 mesocosms: Ohio-Littoral, Ohio-Pelagic, North Carolina-Littoral, North Carolina-Pelagic. The lack of replication in this study prevented us from including this work in the main manuscript but did suggest unique changes in shape in response to simulated habitats. In addition, many fish either died or escaped our mesocosms in this pilot study, leaving no mesocosm with more than 11 fish or some with less (See Table S1).

We compared both the change in length and aspect ratios (AR) of our experimental perch in the pilot study. Change in length, (i.e. growth; mm/day) was measured at the conclusion of the study, calculated using the average total lengths of fish placed into each cage at the start of the mesocosm experiment and subtracted from the final total length of the individuals removed from the cage (see Table S1 for a full list of total length values by habitat and treatment). The measured change in length was then divided by 100, the experiment time period, and used to compare the growth of fish across treatments. To compare morphologies among the 4 mesocosm treatments, we measured two AR (See the

mesocosm experiment methods and Figure 4) for each of the fish remaining at the conclusion of the experiment and compared the means of each mesocosm.

Results & Discussion

All fish, in each mesocosm cage, grew during the 100 day experiment. Ohio yellow perch began the experiment at larger sizes than NC yellow perch (Table S1). In general individuals from the NC population, from both habitats, had greater changes in mean length (0.249mm/day) than OH fish (0.174mm/day) after the 100 day experiment (Table S1). Interestingly, our simulated habitats did not appear to differ in growth despite the fact that our littoral mesocosms would have had access to additional benthic/littoral food items such as chironomids.

The two populations of perch appeared to have different AR, pre-experiment, with NC fish being smaller than OH perch (Table S1). By the end of the experiment, only pelagic NC fish appeared to still have smaller AR than pelagic OH fish, with littoral treatments having similar ratios. Across habitats, littoral fish appeared to have generally larger AR, but mostly only for the length-caudal ratios in both populations. This is in contrast to other morphology studies which often note differences related to body depth (Faulks et al. 2015, Scharnweber et al. 2016), particularly between pelagic and littoral environments.

Table S1: Mean total lengths (mm; mean±sd) and aspect ratios (mean±sd) of fish (pre & post experiment) within a 2014 pilot study on the morphological variation attributable to population-habitat effects.

		Ohio		N. Carolina	
	Habitat	Pre-Mesocosm[a]	Post-Mesocosm[a]	Pre-Mesocosm[a]	Post-Mesocosm[a]
Total Length	Littoral	68.1±12.3 (30)	85.9±9.6 (10)	57.43±6.1 (30)	84.8±7.8 (10)
	Pelagic	66.8±10.96 (30)	83.8±2.5 (5)	55.4±5.3 (30)	77.8±4.9 (11)
Length-Depth	Littoral	3.55±0.21 (32)[b]	3.76±0.17	3.54±0.14 (32)[b]	3.72±0.13
	Pelagic	-	3.74±0.15	-	3.60±0.14
Length-Caudal	Littoral	10.18±0.63 (32)[b]	10.63±051	9.72±0.50 (32)[b]	10.61±0.46
	Pelagic	-	10.2±0.2	-	10.02±0.41

[a]All mesocosms were originally stocked with 30 fish and values in parentheses represent the final sample size collected from each cage.

[b]Pre-Mesocosm fish measured for length-depth or length-caudal ratios were represented by 32 fish sampled prior to the mesocosm experiment and have no affiliation with either littoral or pelagic mesocosms at this stage.