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An Analysis of Morphometric Differentiation in Lake and River Populations of the Emerald Shiner, Notropis Atherinoides

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An Analysis of Morphometric Differentiation in Lake and River Populations of the Emerald Shiner, *Notropis atherinoides*

by

John Lang

An Abstract of a Thesis
in
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Submitted in Partial Fulfillment
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Department of Biology

ABSTRACT OF THESIS

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Understanding mechanisms that account for phenotypic variation has been of interest to biologists since the advent of Darwin's theory of evolution by natural selection. It is now understood that adaptive divergence is a key driving force of intraspecific differentiation. Further, differences in habitat (e.g., flow regime, prey regime) have been shown to drive adaptive divergence in fish. For instance, fish inhabiting faster flowing water generally exhibit more fusiform bodies than their lake counterparts. Similarly, the partitioning of benthic and pelagic morphs generally results in smaller heads with the latter. This study used geometric shape analysis to assess morphological differences between emerald shiner (*Notropis atherinoides*) populations inhabiting the Niagara River, Lake Erie, and Lake Ontario. It was expected that emerald shiners inhabiting the two lakes would have more robust bodies and smaller heads. Conversely, river emerald shiners were expected to display more fusiform bodies with larger heads. The results of this study demonstrated that emerald shiners from Lake Erie and the Niagara River had a more robust average form than individuals from Lake Ontario. This suggests that factors other than flow regime may have been responsible for this divergence. Future studies should investigate the influence that predator communities may have on the morphological divergence between these *Notropis atherinoides* populations.

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TABLE OF CONTENTS

PAGE

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Understanding the mechanisms that account for phenotypic variation has been of interest to biologists since the advent of Darwin's theory of evolution by natural selection (Pfennig and Pfennig 2010). It is now widely agreed upon that phenotypic variation is driven both by genetic divergence and by environmental differences (Langerhans 2008). Indeed, the role of environmental factors is so influential that conspecific populations generally undergo phenotypic differentiation when habitats differ (Skúlason and Smith 1995). Phenotypic differentiation is the divergence of two or more populations for a given observable trait. This differentiation typically results from one or more of three processes: genetic drift, natural selection, or phenotypic plasticity.

Genetic drift is the change in a population's allele frequencies due to stochastic events (Wright 1931). When populations are isolated, genetic drift may result in random genetic differentiation (Vrijenhoek 1998), which may in turn cause phenotypic differentiation. Therefore, this process of differentiation is unique in that environmental conditions between two populations may be similar, but mean phenotypes differ. Conversely, the other two processes that influence differentiation, natural selection and phenotypic plasticity, occur when environments differ.

Natural selection favors individuals within a population that are best suited for a given environment. This process, as it relates to the phenotypic differentiation of populations, is referred to as adaptive divergence. For conspecific populations inhabiting different environments, adaptive divergence may result in the differentiation of heritable traits such as life history, behavior, or morphology due to contrasting selective pressures (Skúlason and Smith 1995). In this way, adaptive divergence is similar to genetic drift in that both forms of

phenotypic differentiation result from differences in genetic composition. However, the process of gene flow typically opposes genetic differentiation (Slatkin 1987). Gene flow is the exchange of genetic material between populations by way of immigration and emigration (Slatkin 1987). The homogenizing effect of gene flow between conspecific populations hinders local adaptation caused by natural selection (Hendry et al. 2002). Assessing the amount of gene flow between populations can therefore aid in delineating the process that may be influencing phenotypic differentiation.

A third process that may result in differentiation is phenotypic plasticity. Phenotypic plasticity describes the nature of a single genotype's potential to express several different phenotypes due to environmental factors (Price et al. 2003). In this way, phenotypic plasticity can result in morphological differentiation between populations without differences in genetic composition. Therefore, the mean phenotypes for a given trait may differ even in panmictic populations (Young 2001). That is to say, gene flow may not necessarily hinder phenotypic differentiation by plasticity. It is clear that information about both genetic and phenotypic differentiation between populations is important for understanding their evolutionary state.

Phenotypic differentiation in fish has been widely studied, with differentiation in form being of special interest. Fish form (i.e., body shape) directly influences swimming performance, which in turn directly affects an individual's fitness (Domenici 2003). The steady-unsteady swimming model was developed by Langerhans and Reznick (2010) to explain how various ecological factors influence the evolution of different swimming modes in fishes. This model proposes that morphological features that enhance performance in one swimming mode (i.e., steady vs. unsteady), will necessarily decrease performance in the other swimming mode. This tight link between fish morphology and swimming performance results in a tradeoff that is

strongly influenced by a number of environmental drivers. In particular, the steady-unsteady swimming model can be used to make predictions about how factors such as habitat complexity, flow regime, and predator density influence the body shape of fish.

The influence of flow regime on fish body shape has been of particular interest to evolutionary biologists. Langerhans (2008) laid the foundation for using the steady-unsteady swimming model to make predictions regarding fish form and flow velocity. This model predicts that fish inhabiting lentic waters (i.e., low flow) will typically display a deeper body. This more robust form allows for increased maneuverability and burst-speed, which are swimming abilities associated with the unsteady swimming mode. On the other hand, fish living in lotic systems (i.e., fast-flowing waters) generally exhibit a more fusiform shape, which reduces drag and enhances steady swimming in flowing waters. This pattern is widely observed in the cyprinids, a family of freshwater fish that primarily includes the carps and true minnows. Following river impoundment, some cyprinid species undergo a morphological shift to deeper bodies and smaller heads (Haas et al. 2010; Franssen 2011; Cureton and Broughton 2014). It is thought that when these streams were dammed, the flow regime was altered drastically, resulting in a shift toward more robust individuals.

The steady-unsteady swimming model also offers predictions about the influence of predator regimes on fish body shape. The model predicts that a predator-dense environment will select for deeper bodies in the prey fish (Langerhans and Reznick 2010). This more robust form enhances thrust and burst speed in fast-start performance, improving predator evasion (Langerhans 2004). On the other hand, an environment with fewer predators should select for a more streamlined form, which enhances competitive behaviors, such as food and mate acquisition (Domenici 2003). This pattern of divergence is seen in a number of fish species. For

instance, western mosquitofish (*Gambusia affinis*) in predator-dense environments display deeper bodies, as well as significantly faster burst speeds, than conspecific populations that do not coexist with piscivorous fish (Langerhans 2004). Similarly, populations of threespine stickleback (*Gasterosteus aculeatus*) that do not encounter their native predatory fish typically display a more streamlined shape than their counterparts that do encounter native predators (Walker and Bell 2000). Additionally, when the red shiner (*Cyprinella lutrensis*), a small-bodied cyprinid, is reared in an environment containing predators, individuals typically develop deeper bodies than those raised in predator-free environments (Franssen 2011). Clearly the steady-unsteady model provides a robust framework for making predictions about the influence of a number of environmental factors on fish body shape.

To better understand the processes that influence phenotypic differentiation, I investigated morphological divergence between populations of the emerald shiner, *Notropis atherinoides*. The emerald shiner is a species well-suited for morphological studies for a number of reasons. First, emerald shiners only exhibit sexual dimorphism during spawning periods (Flittner 1964). Therefore, outside of the spawning season, both sexes can be pooled for morphometric analyses. Second, emerald shiners display high levels of morphological variability. Based on morphological differences, Hubbs and Lagler (1958) identified two subspecies of *Notropis atherinoides*: river emerald shiners (*N. a. atherinoides*) and lake emerald shiners (*N. a. acutus*). According to this distinction, lake emerald shiners possess deeper bodies and shorter heads. However, this taxonomic distinction has since been refuted and *N. atherinoides* is now considered a single, highly variable species (Flittner 1964). A more recent study suggested that morphological variation between river and reservoir emerald shiner populations may be attributed primarily to phenotypic plasticity (Young 2001). However,

phenotypic differentiation of emerald shiner populations has not yet been investigated in the Great Lakes.

Emerald shiners are widespread throughout the Great Lakes and its tributaries. In this study I assessed potential divergence of emerald shiners between populations in Lake Erie, Lake Ontario, and the Niagara River. The Niagara River is the only natural connecting waterway between Lake Erie and Lake Ontario. This river consists of two main sections: the upper Niagara and the lower Niagara River. Eastern Lake Erie drains into the upper Niagara River, flows over Niagara Falls into the lower Niagara River, and then drains into the western basin of Lake Ontario. Therefore, this unique aquatic system allows for the comparative assessment of river and lake emerald shiners and provides an opportunity to better understand the environmental factors potentially driving morphological divergence.

As previously mentioned, understanding levels of gene flow between populations helps infer the potential mechanism of morphological divergence. Due to the geography of the study area, gene flow is expected to be mostly unidirectional. The Niagara Falls act as a barrier to upstream migration, allowing gene flow to occur only over the falls (although canals and human transfer may provide vectors for migration). There are no physical barriers separating Lake Erie and the upper Niagara River, suggesting that migration may facilitate bidirectional gene flow between these two water bodies. However, the shorelines of the Niagara River headwaters have been modified by vertical seawalls, which has dramatically increased the water velocity in this corridor. Hydrodynamic models have identified these areas as likely migration barriers, potentially hindering migration from the upper Niagara River to Lake Erie (Allen 2015; Sood 2015); however, emerald shiners have been observed upstream of these locations, swimming toward Lake Erie (personal observation). Potential hydrodynamic barriers between the lower

Niagara River and Lake Ontario have not yet been investigated, therefore migration of emerald shiners between these two water bodies may be bidirectional. Clearly, there are a number of questions surrounding the connectivity of emerald shiners in this system.

Population genetic analysis is a useful tool for understanding levels of migration. Recently, collaborators used next-generation sequencing to assess the genetic structure of emerald shiners collected from Lake Erie, Lake Ontario, and the lower and upper Niagara River. The results suggest that there was no statistically discernible genetic structuring of these emerald shiner groups (P. Michalak, personal communication). In other words, emerald shiners collected from these four water bodies were genetically similar at the markers analyzed. Identifying patterns of morphological divergence in light of these population genetic data should provide insight into the level of connectivity of the emerald shiners inhabiting these areas.

Understanding the connectivity between these emerald shiner populations may have potential management implications. The emerald shiner is a common cyprinid in the Lake Erie basin where it plays a key role as a forage fish in the food web (Flittner 1964; Werner 1980). These fish are an important food source for a number of sport fish (Knight et al. 1984; J. Cochran, personal communication) and piscivorous birds (DeBruyne et al. 2013), including the threatened common tern. However, very little is known of the emerald shiner populations in Lake Erie, Lake Ontario and the Niagara River. A better understanding of the population dynamics of these emerald shiners is critical for sustaining higher trophic levels within this region's aquatic food web.

Lake Erie and Lake Ontario are both lentic systems, whereas the Niagara River is a lotic system. It follows that emerald shiners from these lakes will experience flow velocities that are different from what the river populations experience. The primary objective of this study was to

determine if there are morphological differences between lake and river emerald shiner populations due to the difference in flow regime. As mentioned earlier, previous morphological studies on emerald shiners have shown that they are highly variable. These studies found that lentic (i.e., lake or reservoir) populations display a more robust body, while lotic (i.e., river or stream) populations exhibit a more fusiform body (Flittner 1964; Young 2001). These differences are consistent with the steady-unsteady swimming model as it applies to differences in flow regime. Therefore, I hypothesized that Lake Erie and Lake Ontario emerald shiners would generally exhibit a deeper, more robust body. Conversely, the Niagara River emerald shiners, inhabiting areas with stronger currents, would possess more fusiform bodies.

MATERIALS AND METHODS

Fish Collection and Preservation

Emerald shiners were collected by electrofishing in the early summer of 2015 (see Appendix A for seasonal differences in body shape). Figure 1 shows the sampling locations for the four sites used in this study. Lake Ontario (LO) shiners were sampled from within Wilson Boatyard Marina of Tuscarora Bay (the mouth of the east branch of Twelve Mile Creek) on 17 June 2015. Lake Erie (LE) individuals were taken from the mouth of Cattaraugus Creek on 19 June 2015. Upper Niagara River (UN) emerald shiners were collected from the vacant Gratwick Park Marina on 24 June 2015. Lower Niagara River (LN) emerald shiners were collected from Lewiston Landing Waterfront on 2 July 2015. A minimum of 30 individuals were collected from each site. From this point forward, fish from each site will be referred to by the name of the body of water they were collected from (e.g., individuals from the mouth of Cattaraugus Creek will be referred to as Lake Erie or LE emerald shiners, etc.). Additionally, each sampling group will at

times be referred to as a distinct population, referring to the qualitative definition of a population offered by Krebs (2008): a group of individuals occupying the same space at the same time.

Upon returning to the lab, fish total lengths were measured and individuals were sorted into the following age groups, based on size (R. Snyder, personal communication): age 0 (< 60 mm), age 1 (60-84 mm), and age 2+ (> 85 mm). Age-1 individuals were the target size range for the geometric morphometric analysis (see Appendix B for an explanation and justification). For each site, 30 age-1 emerald shiners were placed in 95% ethanol. Although this method of preservation distorts the true shape of emerald shiners to some extent (see Appendix C), it is the best known option for short-term preservation (Berbel-Filho et al. 2013). For each sampling event, fish were preserved for 14 days. Individuals were then photographed on their lateral left side using an Olympus Camedia C-5060 digital camera, mounted on a macro stand. The camera was mounted at a standard height; although a reference scale was photographed, it was not used.

Geometric Morphometric Analysis

To investigate differences in shape between these populations of emerald shiners, I used a technique known as geometric morphometrics. Morphometrics is the study of variation in biological form. Historically, morphometrics employed linear measurements, masses, and ratios. What is now referred to as 'traditional' morphometrics involves the application of multivariate statistical analyses to linear distance measurements (Bookstein 1991). The traditional method known as the box-truss analysis examines linear distance measurements between homologous landmarks (Strauss and Bookstein 1982). However, exclusively analyzing linear measurements has several limitations. For instance, traditional methods lack size standardization, and offer relatively poor statistical power in identifying shape variation (Parsons et al. 2003). Additionally, these methods offer only limited visual representations of differences in shape between groups

(Parsons et al. 2003). Specifically, traditional methods rely on data tables for reporting shape variation. However, newer techniques offer visual representations, such as deformation grids. The more recent method of geometric morphometrics has overcome the limitations listed above. This technique quantifies the geometry of landmarks relative to one another, archiving these data throughout the analysis (Bookstein 1991). The geometric morphometric technique utilizes computer software to remove non-shape variation, including translation (i.e., every landmark moves the same distance, in the same direction), rotation, and scale. Additionally, such software is used to statistically compare samples, and to create graphical representations of shape (Adams et al. 2004). This powerful technique is often used to compare the body shapes of related populations, providing information on morphological differentiation.

In this analysis, I used 12 homologous landmarks adapted from previous work on emerald shiners (Figure 2; Young 2001). Geometric morphometric data were collected using the thin-plate spline (tps) software packages (http://life.bio.sunysb.edu/morph/). Landmarks (LM) were set digitally on the photographs using the program tpsDig2 v2.17 (Rohlf 2013a). To account for the bending of specimens due to preservation, I used the 'unbend specimens' function in tpsUtil v1.58 (Rohlf 2013b). This function aligns a designated subset of landmarks and uses their spatial displacement to correct the position of all other landmarks. In this procedure, I used the tip of the premaxilla (LM 1) and four temporary landmarks along the dorsal side of the lateral stripe. Once landmark positions were corrected, temporary landmarks were removed. The output of this procedure is a set of 2D coordinates for each landmark on each individual in the analysis. The program tpsRelw v1.54 (Rohlf 2014) was used to perform a general Procrustes analysis (GPA). This analysis uses a partial least squares method to remove non-shape components of shape variation, including translation, rotation and scale. The tpsRelw

program was also used to calculate centroid size, which is a standardized measure of size. Lastly, I used tpsRegr v1.42 (Rohlf 2015) to produce mean shapes of individuals from the two habitat types (lake vs. river) and from each of the four sites.

Statistical Analyses

Statistical analyses were performed using the Integrated Morphometrics Package (IMP) software suite (http://www3.canisius.edu/~sheets/IMP%208.htm). I used the program TwoGroup v8 (Sheets 2006b) to perform a pairwise comparison between lake and river populations of *Notropis atherinoides*. The Lake Erie and Lake Ontario samples were pooled to produce the "lake" sample, and the upper and lower Niagara River samples were pooled to produce the "river" sample. The TwoGroup program quantifies the amount of differentiation between populations using Goodall's F-statistic. This value represents the amount of variation between the two groups compared to the variation within each group. Goodall's F-statistic increases with the level of divergence between two groups. To increase robustness, TwoGroup offers a resampling technique for calculating Goodall's F. Using this function, I performed a 900-bootstrap pairwise comparison between habitat types for each sampling event. Under this resampling framework, the p-value associated with Goodall's F is a nonparametric value, which provides a descriptive level of significance. Specifically, this p-value represents the fraction of resamples in which Goodall's F is greater than or equal to the value for the original data. Together, Goodall's F and its associated p-value provide a quantitative description of the degree of differentiation between two groups.

The program CVAGen v8 (Sheets 2006a) was used to perform a canonical variates analysis (CVA) and a "Jackknife Groupings" test. The CVA is a useful tool for visualizing broad patterns of variation between two or more groups. This analysis determines the axes of

differentiation that account for the greatest amount of variation between groups, and then plots the CV scores for each individual. The "Jackknife Groupings" test first calculates the distances from each specimen to the mean value of each group. Then, one known specimen is removed at a time and assigned to the closest group. Ultimately, this analysis outputs the number of individuals that are correctly placed into its *a priori* group based on shape. The percent of correct assignment increases with increasing divergence between groups.

After analyzing differences in shape between habitat types, I analyzed differences in shape between each of the four sites: Lake Erie, Lake Ontario, the upper Niagara River, and the lower Niagara River. I used TwoGroup to perform site-wise comparisons. I then used CVAGen to perform a canonical variate analysis and a Jackknife Groupings test.

RESULTS

The shapes of 120 *Notropis atherinoides* individuals were analyzed from four different sites across the lower Great Lakes. The analysis included 30 emerald shiners each from Lake Erie (LE), the upper Niagara River (UN), the lower Niagara River (LN), and Lake Ontario (LO). Emerald shiners from LE and LO were pooled to make the "lake" sample (N = 60), and emerald shiners from UN and LN were pooled to make the "river" sample (N = 60).

The pairwise comparison between lake and river populations indicates that there was a significant difference between the two groups ($p = 0.0067$) with some degree of habitat differentiation (Figure 3). This differentiation in shape occurs exclusively along the first canonical variate (CV1). Individuals from the river sites cluster relatively tightly to the left of the plot, while lake individuals are more spread out to the right. The "Jackknife Groupings" test assigned 63.33% of the individuals correctly with an expected random rate of correct assignment

of 50.89% (Table 1). This relatively low correct assignment rate suggests that the two groups did not differ greatly. Mean shapes of the individuals from the two habitat types display the differences between lake- and river-dwelling emerald shiners (Figure 4). When differences are magnified ten times, it appears that emerald shiners from the river sites were deeper-bodied, possessed larger caudal regions and smaller heads with more upturned mouths.

To better understand this pattern of divergence, I analyzed the shapes of these emerald shiners by sample location, in addition to using the pooled "river" and "lake" samples as outlined above. Pairwise comparisons across the four sites showed no significant differences in shape between individuals from Lake Erie and the two Niagara River populations (Table 2). Niagara River populations were significantly different from one another, although the Goodall's F value was relatively small. Importantly, the analyses point to Lake Ontario being the most different with respect to body shape (Table 2). Lake Ontario emerald shiners were significantly different from the Lake Erie population and from both the upper and lower Niagara River populations. These comparisons yielded the highest Goodall's F values in the analysis (Table 2).

When the CVA was performed by site, Lake Erie and Niagara River populations clustered relatively close along the first canonical variate (CV1), while the Lake Ontario population diverged along this axis (Figure 5). The upper and lower Niagara River populations diverged along the second canonical axis (CV2). The "Jackknife Groupings" test across the four sites demonstrated that individuals were grouped more accurately by site than by habitat type. When grouping by site, 62.50% of individuals were correctly assigned, with an expected random rate of correct assignment of 25.46% (Table 3). Lake Erie and Niagara River populations displayed small differences in mean shape (Figure 6). However, the mean shape of Lake Ontario emerald shiners was not as deep-bodied, displayed a smaller caudal area, and had a slightly

larger head with a more downturned mouth (Figure 6). Together, these results suggest that emerald shiner populations from Lake Erie and the Niagara River were similar in shape, while the mean shape of the Lake Ontario population differed from the other three sites.

DISCUSSION

I investigated morphological differentiation between lake- and river-dwelling *Notropis atherinoides* in the lower Great Lakes basin. I found a small degree of distinction between the lake and river populations. However, grouping individuals by site seemed to better explain the shape variation observed here. When emerald shiner shape was analyzed by site, the results showed that divergence between lake populations was inconsistent. Specifically, Lake Erie emerald shiners displayed a shape more similar to both upper and lower Niagara River individuals than to emerald shiners from Lake Ontario. Franssen et al. (2013a) evaluated shape differences between stream and reservoir populations of *N. atherinoides* in northwest Mississippi. Similar to the current study, this group also observed inconsistent divergence in lotic populations. That is, only two of the three reservoir populations experienced consistent divergence from their respective stream populations. As a result, they too found relatively little habitat differentiation between stream and reservoir emerald shiner populations. They suggested that gene flow may restrict morphological divergence, although they had not yet collected genetic data.

Intraspecific variation may be seen as a balance between gene flow and local adaptation (Hendry et al. 2002). The emerald shiner populations in this study have shown a lack of genetic structuring (P. Michalak, personal communication). This suggests that panmixia may have constrained phenotypic divergence between the emerald shiner populations of Lake Erie and the

Niagara River. That is, the level of gene flow between these sites may have been stronger than potential selecting forces of local adaptation. Conversely, the environmental regimes in Lake Ontario may have induced a shift in body shape in the emerald shiners at that site. It is possible that the morphological differences found in the Lake Ontario emerald shiners are a result of phenotypic plasticity (Crispo 2008). This mechanism has previously been suggested when emerald shiners displayed morphometric divergence under gene flow. Young (2001) found that stream populations of emerald shiners diverged from reservoir populations, although population genetic data suggested that the populations are panmictic. This seems plausible, as experimental rearing of blacktail shiners (*Cyprinella venusta*), a small-bodied cyprinid, in flowing water demonstrated that some cyprinids are capable of exhibiting developmental plasticity in response to flow velocity (Franssen et al. 2013b).

However, the findings of the current study suggest that flow regime is not the best explanation for the observed pattern of divergence. The steady-unsteady swimming model predicts that populations inhabiting lakes will display a more robust form than river populations, due to differences in flow. This robust form is typically characterized by a deeper body, a larger caudal area and a smaller anterior region. This pattern of divergence is widely observed in cyprinid populations inhabiting lentic waters (Haas et al. 2010; Franssen 2011; Cureton and Broughton 2014). However, in the current study, individuals from Lake Erie and the Niagara River displayed a more robust mean body shape than the population from Lake Ontario. These findings oppose the predictions of the steady-unsteady swimming model. It seems that water velocity is not always the strongest predictor of variation in fish body shape (Haas et al. 2015). In some fish species, populations with fusiform shape can be found inhabiting lentic environments, while their more robust conspecifics inhabit lotic environments (McGuigan et al.

2003; Hendry et al. 2006). Further, this pattern is found across broad phylogenetic and ecological guilds, including a number of cyprinids (Krabbenhoft et al. 2009; Franssen et al. 2013a; Franssen et al. 2013b).

Abiotic factors other than flow regime have been linked to divergence in fish body shape. For example, recent studies have demonstrated the importance of the relationship between oxygen regime and fish form. In hypoxic environments, populations of the African cyprinid *Barbus neumayeri* display larger gills and larger heads than populations in normoxic waters (Langerhans et al. 2007). Further, rearing the Egyptian mouthbrooder (*Pseudocrenilabrus multicolor*) in hypoxic environments increases gill size, directly affecting head shape (Crispo and Chapman 2011). Interestingly, in the current study, emerald shiners collected from Lake Ontario displayed larger anterior regions than individuals from the other sites. Emerald shiners may be capable of exhibiting a plastic response (i.e., developmentally flexible) to low oxygen levels. Indeed, if the oxygen regime is unstable at this nearshore Lake Ontario site, it would be beneficial for these fish to possess such plastic traits, allowing them to cope with such fluctuations (Crispo 2008). However, it seems unlikely that a creek mouth that drains into Lake Ontario would experience any substantial periods of anoxia. Therefore, is doubtful that oxygen regime had a substantial effect on the shape of the fish analyzed here.

Biotic regimes, such as prey and predator communities, are known to influence the divergence of fish body shape between populations. For instance, the prey regime (i.e., the types and variety of prey items available) may play a role in driving divergence between planktivorous fish populations. There is a well-established link between fish morphology and foraging success (Skúlason and Smith 1995; Svanbäck and Eklöv 2004). This is seen in a number of cyprinids, as body shape is a primary factor in explaining intraspecific variation in drift-feeding success

(Rincón et al. 2007). Specifically, deeper-bodied individuals with more upturned mouths are more successful at drift foraging than more fusiform individuals (Rincón et al. 2007). This seems paradoxical, as drift feeding is associated with faster flowing water, in which a more streamlined body is energetically favorable (Blake 2004). Therefore, there appears to be a tradeoff between the energetic costs of sustained swimming and the benefits of drift feeding success. The role of morphological plasticity in response to prey regime is not well understood, but such a fitness tradeoff suggests that adaptation may play a large role. It is possible that the emerald shiners in this study from Lake Erie and the Niagara River experience a prey community that is more dominated by drifting zooplankton, while the emerald shiners I collected from Lake Ontario may not.

The steady-unsteady swimming model provides predictions about how predator densities influence the divergence of body shape between intraspecific fish populations. This model predicts that a predator-dense environment will select for a deeper body, which is associated with higher burst speeds in fast-start performance, a trait that increases predator evasion (Langerhans and Reznick 2010). Conversely, an environment with few predators will likely select for a more streamlined form (Langerhans and Reznick 2010), which improves steady swimming activities such as resource acquisition (Domenici 2003). For instance, populations of western mosquitofish (*Gambusia affinis*) that inhabit predator-dense environments typically display deeper bodies than populations living in environments that are free of predatory fish (Langerhans et al. 2004). These more robust individuals demonstrated higher burst speeds than their more fusiform counterparts, a trait associated with the unsteady swimming mode (Langerhans et al 2004). Conversely, these more fusiform individuals outperformed deeper-bodied individuals in prolonged swimming trials, demonstrating greater steady swimming abilities (Langerhans 2009). This morphological

response has been shown to be plastic in some cyprinids. Plasticity can be displayed through developmental processes of larvae and juvenile and through inducible changes in adults. For instance, adult crucian carp (*Carassius carassius*) undergo a plastic shift to deeper bodies in environments where they encounter predators (Brönmark and Miner 1992). This predator-induced change in body shape is accompanied by an increase in muscle mass and an improved escape response (Domenici et al. 2008). Similarly, adult goldfish (*Carassius auratus*) display an inducible and reversible shift to deeper bodies when exposed to predator odors (Chivers et al. 2008). Additionally, these deeper-bodied individuals have better survival rates in experimental encounters with yellow perch (*Perca flavescens*). Lastly, red shiners (*Cyprinella lutrensis*) display develop deeper bodies when experimentally reared in the presence of predatory fish than when not reared with a predator, despite the shape of the parental red shiner (Franssen 2011). This demonstrates not only that the shapes of some cyprinids can be predicted based on the presence or absence of predators, but also that there may be a developmentally plastic component to this response. Clearly, predator density has a strong influence over the body shape of fish across a number of taxa. The results of the current study showed that, on average, Lake Erie and Niagara River emerald shiners possessed deeper bodies than those from Lake Ontario. It is possible that the habitats of Lake Erie and the Niagara River have a predator regime that differs from that of Lake Ontario. Specifically, predator-dense environments in Lake Erie and the Niagara River may be a leading environmental factor driving the divergence toward deeper bodies.

To date, most studies on the influence of predators on the shape of prey fish have been binary, investigating the effect of presence versus absence of a single predator species. Little attention, however, has been paid to the effect of various fish predators (i.e., piscivorous fish,

birds, insects, etc.) on prey fish body shape. Undoubtedly, piscivores across taxa exhibit diverse modes of prey capture, which may induce diverse responses in prey body shape. It may be possible that the Lake Ontario site in this study contains an ecological guild of piscivores that differs in taxonomic composition from the sites in Lake Erie and along the Niagara River. That is, the emerald shiners taken from Lake Ontario may, for example, encounter higher levels of piscivorous birds than the emerald shiners from the other study sites. Future studies examining these higher trophic levels may lend insight into this issue.

In the current study, populations of lake- and river-dwelling *Notropis atherinoides* displayed inconsistent differences in body shape. This divergence occurred in the face of gene flow (P. Michalak, personal communication), which suggests that phenotypic plasticity may have played a role in the observed morphological divergence. However, local adaptation has been shown to occur in the presence of gene flow (Fitzpatrick et al. 2015). It is possible that the genetic markers used to analyze the genetic structuring of emerald shiners collected from Lake Erie, Niagara River, and Lake Ontario may not be representative of the quantitative traits that I used to assess phenotypic differentiation. Additionally, gene flow may facilitate adaptation by increasing diversity among these populations (Slatkin 1987; Crispo 2008). Ultimately, the relative contributions of genetic variation and phenotypic plasticity to the morphological divergence found here is inconclusive. Further studies, such as experimental rearing of emerald shiners, would shed light on this problem.

The direction of morphological divergence found in this study contrasted with the predictions of the steady-unsteady swimming model regarding differing water velocities. It was expected that emerald shiners from lakes would display a more robust form than those from the river. This pattern has previously been seen in emerald shiners inhabiting differing flow regimes

(Young 2001). However, emerald shiners taken from Lake Erie and the Niagara River displayed a more robust average form than those taken from Lake Ontario. These findings suggest that flow regime likely had little effect of shape divergence between these groups. Differences in oxygen may be able to drive this pattern of divergence, however it is unlikely that the Lake Ontario site experiences prolonged anoxic states. Differing prey regimes have previously been shown to drive morphological divergence. Another likely explanation for the morphological patterns seen here is differences in predatory regimes. Still, based on the data here, it is inconclusive which environmental factors are responsible for this divergence. As Bookstein (1991) points out, morphometric studies are not so much interested in the forms themselves, but in their associations, causes, and effects. Therefore, further analyses such as experimental rearing, predator surveys, diet analysis, or stable isotope analysis would shed light on the factors driving phenotypic differentiation between emerald shiner populations in this system. Ultimately, a better understanding of how emerald shiners interact with the biotic and abiotic regimes of the Erie-Ontario corridor would provide insight on the issue.

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Table 1. Jackknife grouping results showing the number of individuals assigned to the correct and incorrect *a priori* habitat group. The first column holds the *a priori* groups with sample sizes, and the subsequent columns show the number of individuals assigned to each group as determined by the CVA. Given group sizes, the expected random rate of correct assignments is 50.89 %. Correct assignments (i.e., Lake-Lake and River-River) are shown in boldface.

	Lake	River
Lake (N = 60)	**34**	26
River (N = 60)	18	**42**
	76 correct assignments out of 120 (63.33 %)	

Table 2. Site-wise comparisons, with Goodall's F on the upper right and the associated non-parametric p-values on the bottom left of the table. Lake Ontario (LO) fish differ most in shape compared to fish from the other three sites. Fish from the upper (UN) and lower (LN) Niagara River sites also differed in shape.

	LE	**UN**	**LN**	**LO**
LE		1.85	1.87	10.07
UN	0.0667		2.39	9.13
LN	0.0756	0.0189		6.88
LO	0.0011	0.0011	0.0011	

Table 3. Jackknife grouping results showing the number of individuals assigned to the correct and incorrect *a priori* site group. The first column holds the *a priori* groups with sample sizes, and the subsequent columns show the number of individuals assigned to each group as determined by the CVA. Given group sizes, the expected random rate of correct assignments is 25.46 %. Correct assignments (i.e., LE-LE, UN-UN, etc.) are shown in boldface.

	LE	UN	LN	LO
LE (N = 30)	**17**	7	6	0
UN (N = 30)	6	**15**	7	2
LN (N = 30)	5	4	**21**	0
LO (N = 30)	1	6	1	**22**
75 correct assignments out of 120 (62.5 %)				

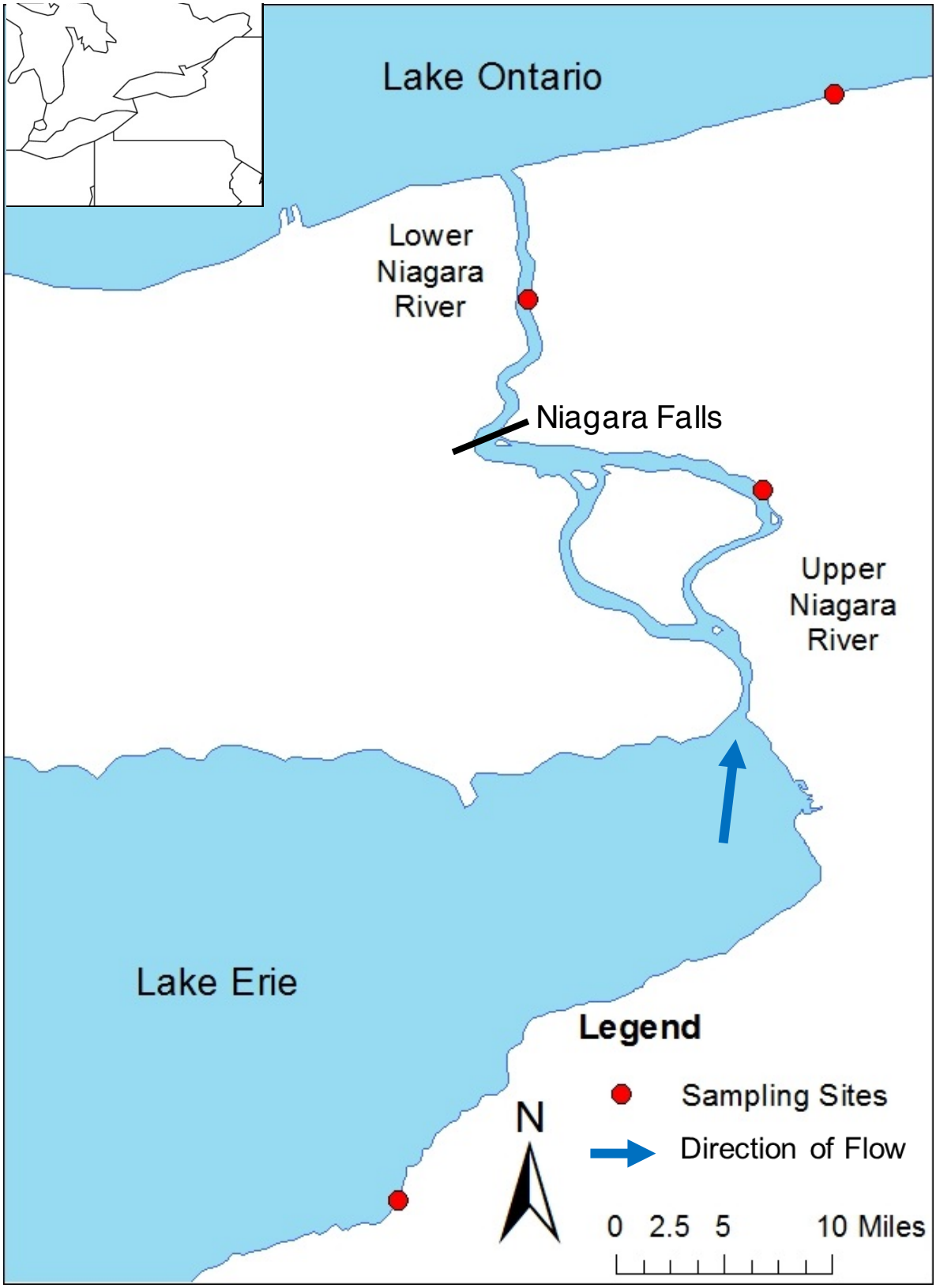
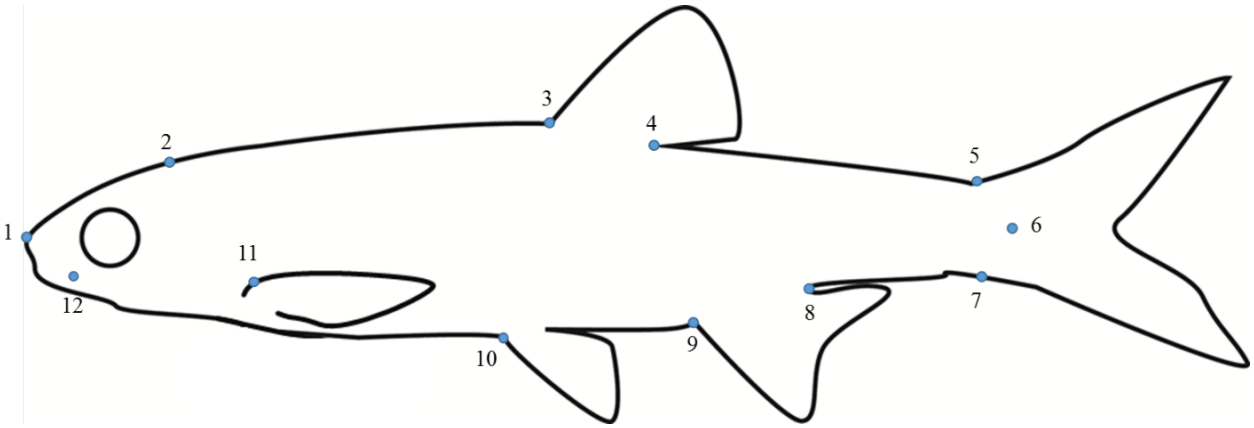


Figure 1. Map of four sampling locations from Lake Erie, upper Niagara River, lower Niagara River, and Lake Ontario.



1. Anterior tip of upper jaw
2. Left-rear notch of the skull immediately lateral to the dorsal midline
3. Origin of dorsal fin
4. Insertion of dorsal fin
5. Dorsal base of caudal fin membrane
6. Posterior-most tip of caudal peduncle at lateral midline
7. Ventral base of caudal fin membrane
8. Insertion of anal fin
9. Origin of anal fin
10. Origin of pelvic fin
11. Origin of pectoral fin
12. Posterior edge of angular (lower jaw) bone

Figure 2. Outline of an emerald shiner and the location and description of 12 homologous landmarks used in the geometric morphometrics analysis (landmarks adapted from Young 2001).

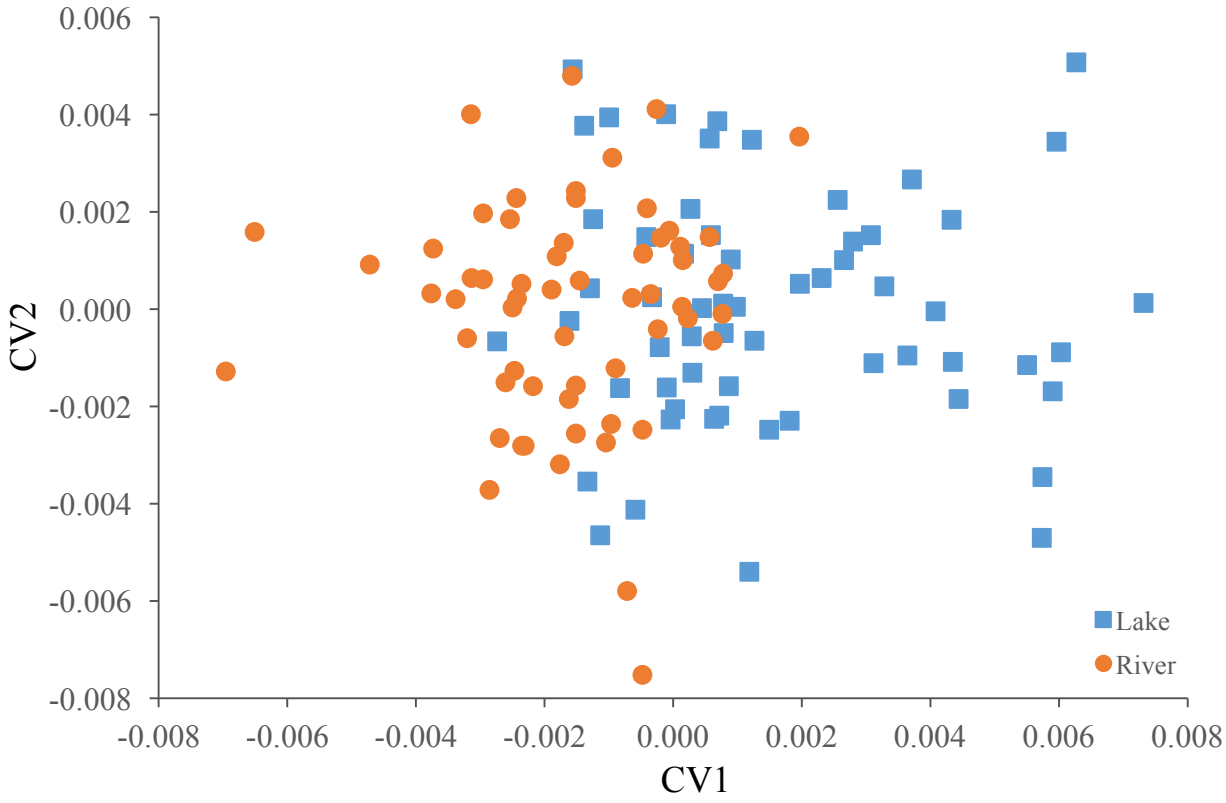


Figure 3. Canonical variates analysis of *Notropis atherinoides* inhabiting two different habitats. (a) Scatter plot of the first and second canonical variates scores of emerald shiners inhabiting lakes (blue squares) and rivers (orange circles).

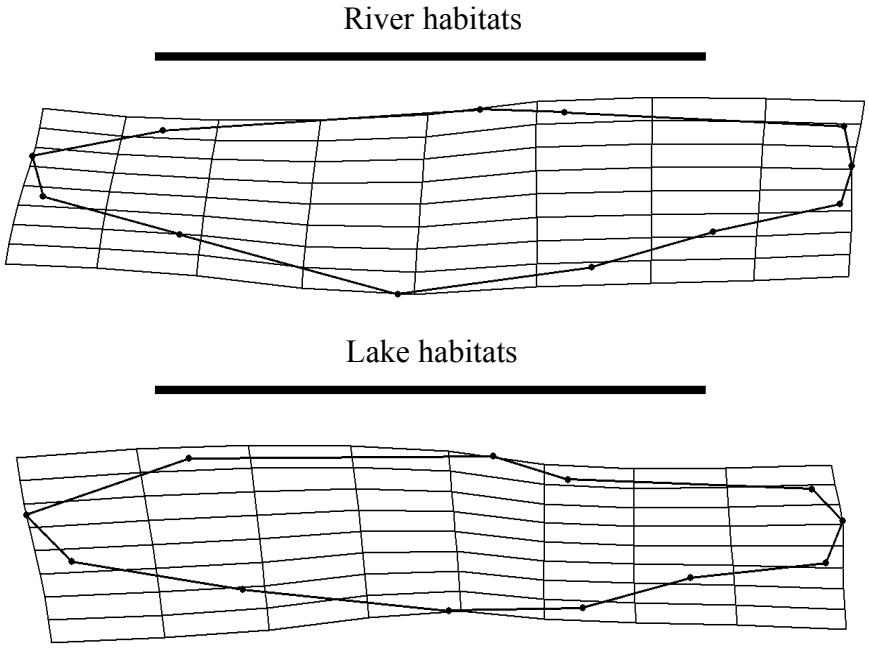


Figure 4. Mean shapes of *Notropis atherinoides* collected from river habitats and from lake habitats. Visualizations are magnified 10X to aid in visualizing differences in shape between habitat types. River emerald shiners were deeper-bodied, possessed larger caudal regions, and had smaller heads with more upturned mouths.

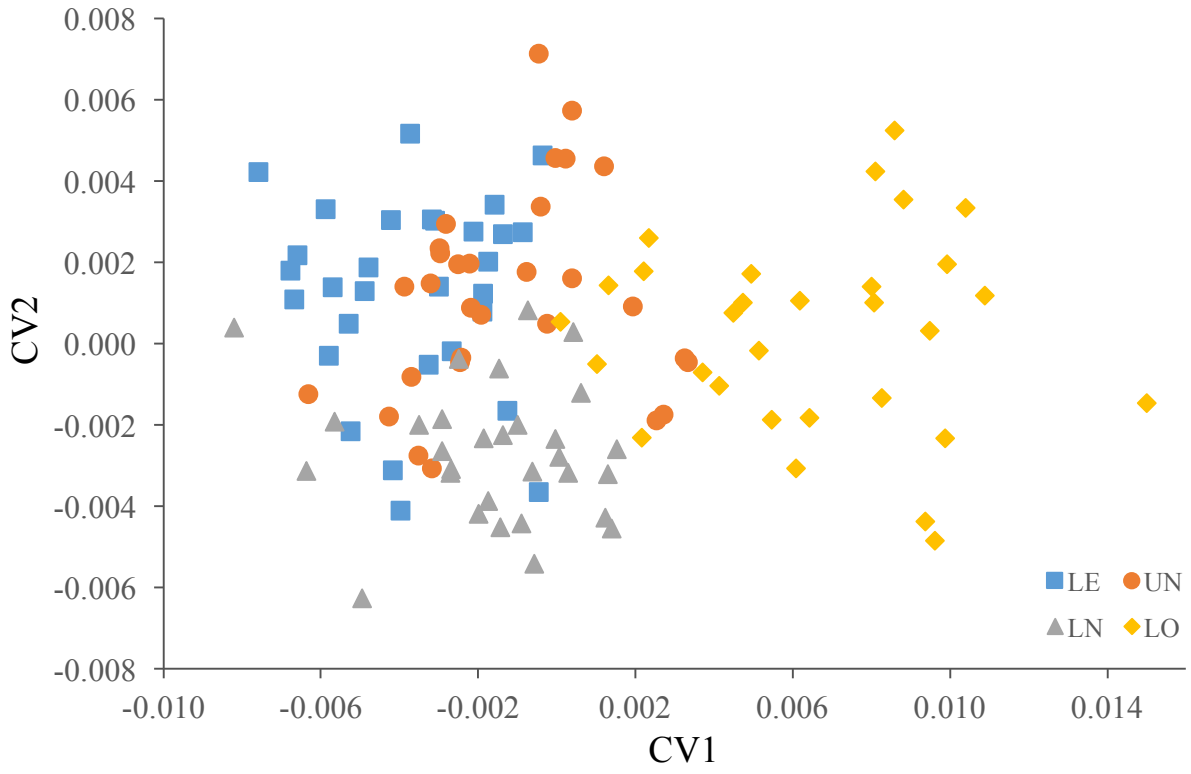


Figure 5. Canonical variates analysis of four different populations of *Notropis atherinoides*. Scatter plot of the first and second canonical variates scores of emerald shiners from Lake Erie (squares), the upper Niagara River (circles), the lower Niagara River (triangles), and Lake Ontario (diamonds). CV1 separates Lake Ontario (LO) emerald shiners from the remaining three sites, while the upper (UN) and lower (LN) Niagara River emerald shiners diverge along CV2.

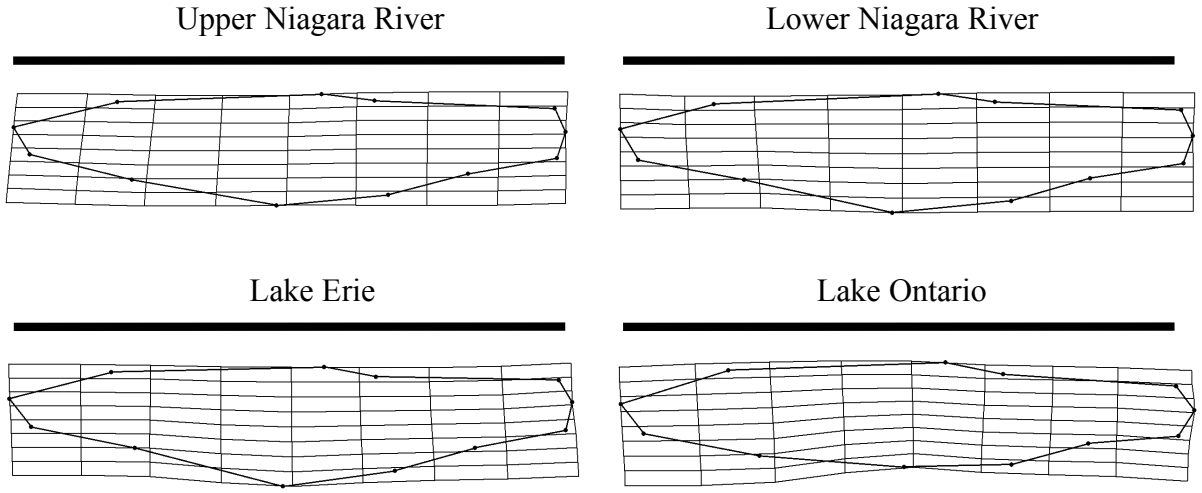


Figure 6. Mean shapes of *Notropis atherinoides* collected from four sites: the upper and lower Niagara River, Lake Erie, and Lake Ontario. Visualizations are magnified 3X to aid in visualizing differences in shape between sites.

Appendix A: Seasonal Variation in Shape of Emerald Shiners

In the current study, *Notropis atherinoides* were collected during three sampling events over the course of 2015. The goal was to obtain a sample that would be representative of the populations in this system. However, seasonal variation in shape may preclude the analysis of shape variation as it relates to differences in environmental factors across space. For instance, the second sampling event overlapped with peak spawning of emerald shiners (personal observation). Typically, emerald shiners do not exhibit sexual dimorphism except during spawning periods (Flittner 1964). Specifically, female emerald shiners exhibit enlarged abdominal cavities due to an increase in gonad size. Also, in the third sample, the lower Niagara River and the Lake Ontario populations contained young of the year (YOY) emerald shiners, while the samples from the Lake Erie and upper Niagara River sites contained only age-1 individuals. This may be problematic, as some cyprinids undergo changes in shape through ontogenetic allometry (Bravi et al. 2013). Therefore, these YOY may skew the average shape of fish from these two sites. Additionally, a few individuals from the third Lake Ontario sample displayed lesions and swollen abdominal cavities that suggest they may have been diseased. These external symptoms may directly affect the shape of these individuals.

I assessed seasonal variation in emerald shiners to determine if individuals from all three sampling events can be pooled for an analysis of habitat differentiation. I analyzed the shape of individuals from four sites across the summer and autumn of 2015. As mentioned in the main text, emerald shiners were collected during three sampling events from the upper Niagara River (UN), lower Niagara River (LN), Lake Erie (LE), and Lake Ontario (LO). Sample dates are as follows: LE on 19 June, 27 July, and 6 October; UN on 24 June, 29 July, and 5 October; LN on 2 July, 30 July, and 5 October; LO on 17 June, 30 July, and 13 October. As previously mentioned,

individuals were sorted into the following age groups, based on size (R. Snyder, personal communication): age 0 (< 60 mm), age 1 (60-84 mm), and age 2+ (> 85 mm). Age-1 individuals were the target size range for the geometric morphometric analysis. When 30 age-1 individuals were not available, samples were supplemented first with age-2+ individuals and then with age-0 individuals if needed. To perform this analysis, I grouped emerald shiners based on sampling event. Individuals from the first sampling event at each site were pooled to make the "early season" sample. Similarly, the second and third samples for each site were pooled to form the "mid-season" and "late season" samples, respectively.

Geometric morphometric analyses were performed using the same methods described in the main text. A single factor MANOVA was performed with 900 permutations. A canonical variates analysis and a "Jackknife Groupings" test were performed. All statistical analyses were performed using CVAGen. The program tpsRegr was used to obtain mean shapes for the three groups. If there are differences between the mean shapes of emerald shiners from each sample, then samples should not be pooled for other analyses.

The MANOVA demonstrated that there was a seasonal effect on shape of emerald shiners from the lower Great Lakes system ($F = 9.62$, $p < 0.001$). The "Jackknife Groupings" test found that individuals were grouped into the correct sampling group 67.13% of the time, with an expected random rate of correct assignment of 33.38 % (Table A1). The early season sample had the highest rate of correct assignment, with 70%. The CVA showed that emerald shiners grouped together rather well by sampling event, although there was some overlap (Figure A1). Divergence between fish from the early season sample and the two later samples occurred primarily along the first canonical variate. On the other hand, divergence between fish from mid-season sample and the late season sample was not as strong and occurred mostly along the

second canonical variate. Pairwise analyses of the three samples showed that they were all significantly different from one another ($p = 0.0011$, Table A2). However, Goodall's F values showed that fish from the mid- and late season samples resembled each other more than they resembled individuals from the early sample. Mean shapes of fish from the three seasonal samples also demonstrated that individuals from the mid- and late season samples appeared to have similar shapes (Figure A2). Fish from the mid and late samples displayed relatively deeper bodies than individuals from the first sample. This may be explained by the spawning condition of females in the mid-season sample, and perhaps by the effects of diseases on certain individuals in the late season sample.

These data suggest that emerald shiners from the three sampling events should not be pooled for geometric morphometrics analysis of habitat differentiation. There was no obvious environmental factor that may have influenced the individuals from the early sample in a way that may preclude an analysis of habitat differentiation. However, the possible effect that spawning may have on mid-season females and the presence of young of the year and potentially diseased individuals in the late season makes their use in further analyses questionable. Moreover, the early sample displayed greater variation and seemed to have diverged from the other two samples. This suggests that the early season sample was more representative of the emerald shiner populations that were analyzed here. Therefore, the analyses of habitat differentiation and site-wise comparisons in this study only included individuals from the early season.

Table A1. Jackknife grouping results showing the number of individuals assigned to the correct and incorrect *a priori* sample group. The first column holds the *a priori* groups with sample sizes, and the subsequent columns show the number of individuals assigned to each group as determined by the CVA. Given group sizes, the expected random rate of correct assignments is 33.38 %. Correct assignments (i.e., Early Season-Early Season, Mid-Season-Mid-Season, etc.) are shown in boldface.

	Early Season	Mid-Season	Late Season
Early Season (N = 120)	**84**	15	21
Mid-Season (N = 120)	12	**80**	28
Late Season (N = 120)	19	23	**77**
241 correct assignments out of 360 (67.13 %)			

Table A2. Pairwise comparisons of the three sampling events, with Goodall's F values on the upper right, and the associated non-parametric p-values on the bottom left of the table. There were significant differences in shape between fish sampled early in the season, mid-season, and late in the season.

	Early Season	**Mid-Season**	**Late Season**
Early Season		14.02	8.75
Mid-Season	0.0011		5.37
Late Season	0.0011	0.0011	

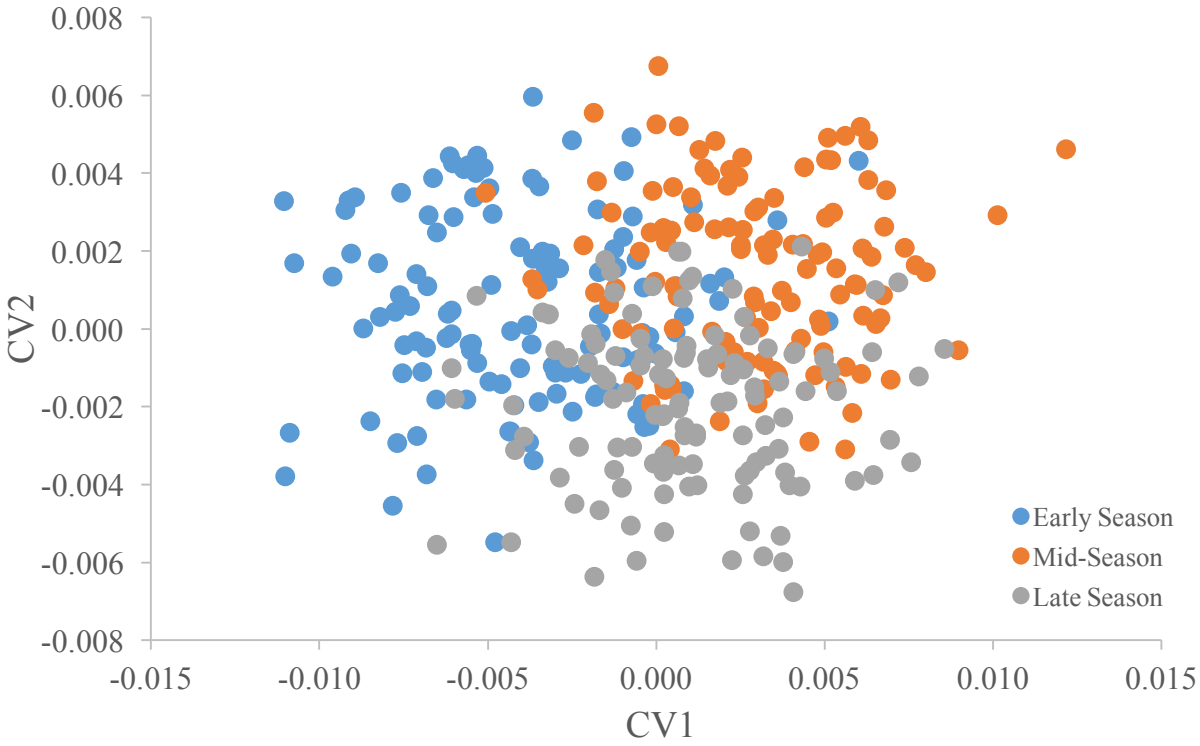
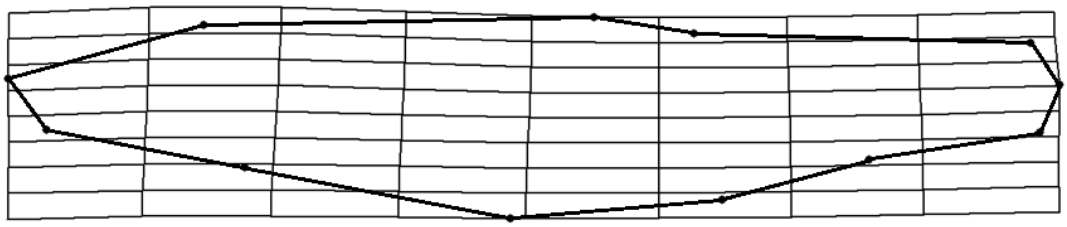
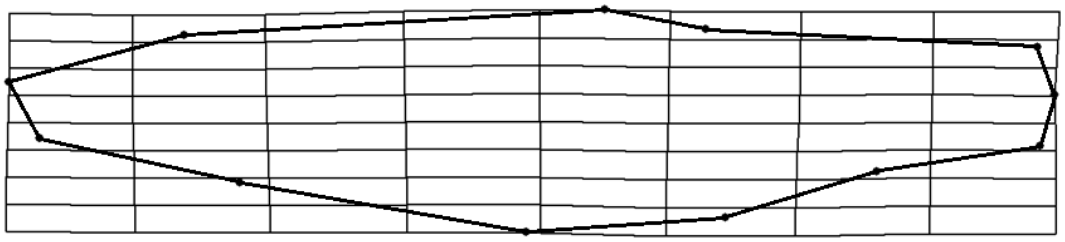


Figure A1. Scatter plot of the first and second canonical variates scores of *Notropis atherinoides* from three sampling periods across the summer and autumn of 2015. The early season sample is represented by the blue circles, the mid-season sample is represented by the orange circles, and the late season sample is represented by the grey circles. The results indicate that the average shape of the emerald shiners collected in this study changed as the sampling season progressed.

(a) Early



(b) Mid



(c) Late

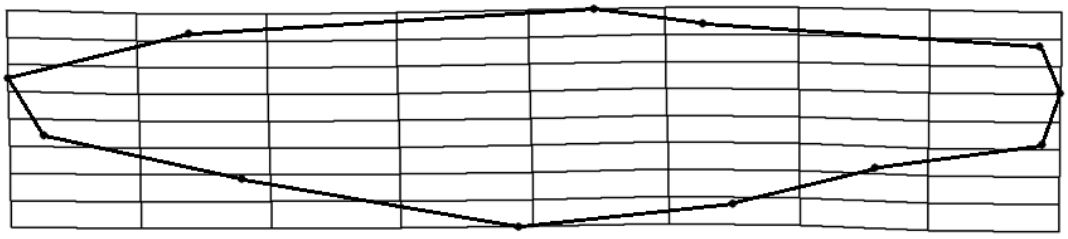


Figure A2. Mean shapes of *Notropis atherinoides* from three sampling events: (a) early season, (b) mid-season, (c) late-season. Visualizations are magnified 3X to aid in visualizing differences in shape between age classes.

Appendix B: Shape Analysis of Emerald Shiners from Three Age Classes

Some cyprinids undergo changes in shape through ontogenetic allometry (Bravi et al. 2013). This suggests that different age classes for these species should not be pooled for geometric morphometric analyses. Previous work has shown that emerald shiner size classes 40-59 mm, 60-84 mm, and > 85 mm align with 1 yr, 2 yr, and 3 yr age classes respectively (R. Snyder, personal communication). In this analysis I used these size ranges as a proxy for age. To determine the size range to be used in the current analysis of habitat differentiation in *Notropis atherinoides*, I compared the shapes of individuals from these three age classes.

I tried to collect at least 20 emerald shiners from each size range from a local bait shop. Due to a lack of age-3 individuals, I analyzed 20 individuals from the following size classes: 40-59 mm, 60-79 mm, and > 80 mm. These individuals were preserved in 70% formalin for two weeks and then photographed on their lateral left side.

Geometric morphometric analyses were performed using the same methods described in the main text. To statistically analyze the samples, a canonical variate analysis and a "Jackknife Groupings" test were performed. All statistical analyses were performed using CVAGen. The program tpsRegr was used to obtain mean shapes for the two groups.

The CVA showed that emerald shiners from these three age classes have distinct shapes from one another. Divergence between the three age classes occurs primarily along the first canonical variate, and there is very little overlap. The age-1 class groups to the far left, age-3 individuals cluster to the right, and age-2 class generally occupy an intermediate position (Figure B1). The "Jackknife Groupings" test found that individuals grouped into the correct age class 75% of the time (Table B1). Based on Goodall's F-statistics, there were significant differences in shape among all three age classes (Table B2). Mean shapes of the three age classes show that

age-1 had much more streamlined middle regions, whereas age-3 individuals typically had much more robust forms (Figure B2). On the other hand, age-2 emerald shiners appeared to have a form that was intermediate.

These data suggest that geometric morphometric analyses of habitat differentiation should control for age. That is, only a single age class should be analyzed in the current study. The CVA and the mean shapes showed that age-2 emerald shiners had a shape that was intermediate to the other two age classes analyzed. Additionally, this age class is typically the most abundant age class throughout the year (personal observation). Therefore, age-2 emerald shiners were the target age class for the current analysis of habitat differentiation.

Table B1. Jackknife grouping results showing the number of individuals assigned to the correct and incorrect *a priori* age class. The first column holds the *a priori* groups with sample sizes, and the subsequent columns show the number of individuals assigned to each group as determined by the CVA. Given group sizes, the expected random rate of correct assignments is 33.39 %. Correct assignments (i.e., Age 1 – Age 1, Age 2 – Age 2, etc.) are shown in boldface.

	Age 1	Age 2	Age 3
Age 1 (N = 20)	**15**	5	0
Age 2 (N = 20)	3	**13**	4
Age 3 (N = 20)	1	2	**17**
	45 correct assignments out of 60 (75.00 %)		

Table B2. Pairwise comparisons of three age classes, with Goodall's F values on the upper right, and the associated non-parametric p-values on the bottom left of the table. There were significant differences in shape among all three age classes examined in this study.

	Age 1	**Age 2**	**Age 3**
Age 1		2.78	13.65
Age 2	0.0078		5.67
Age 3	0.0011	0.0011	

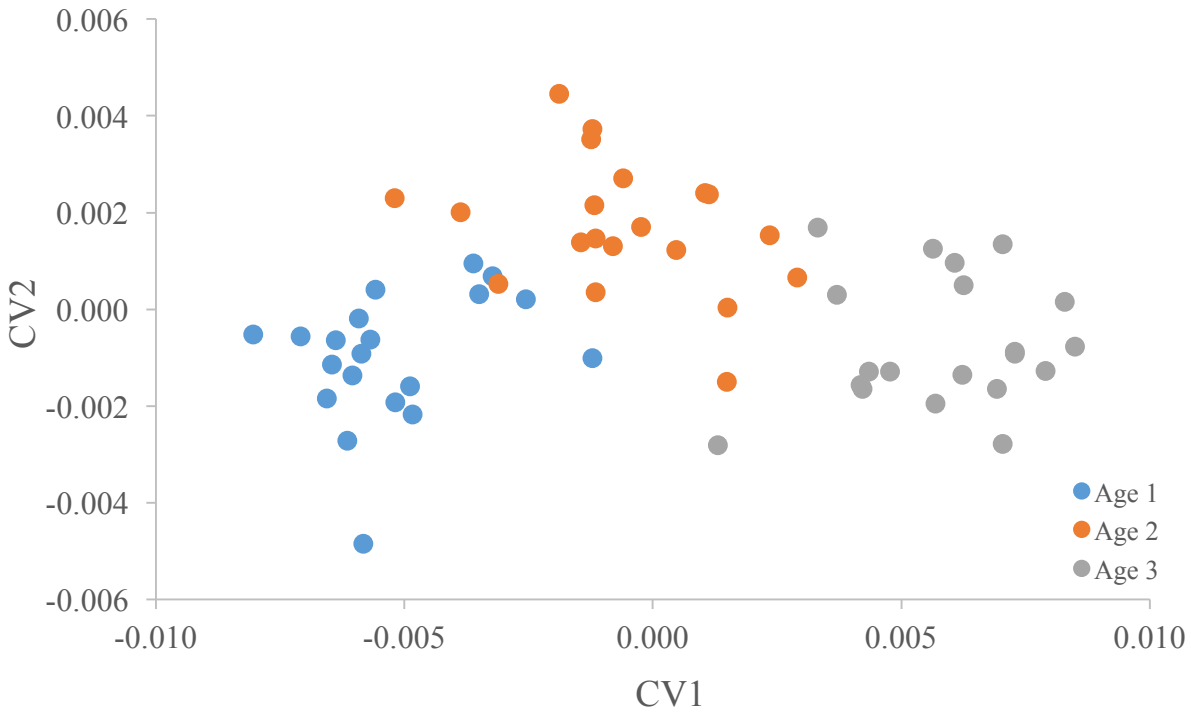
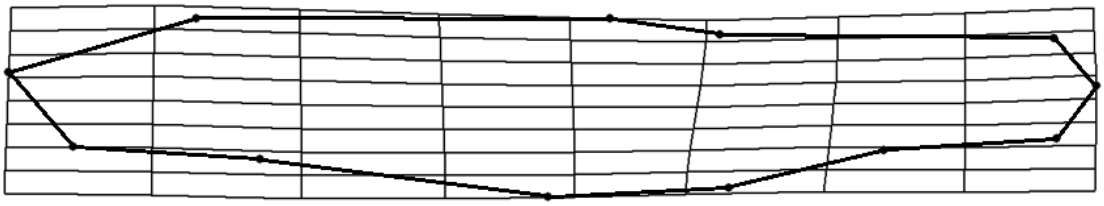
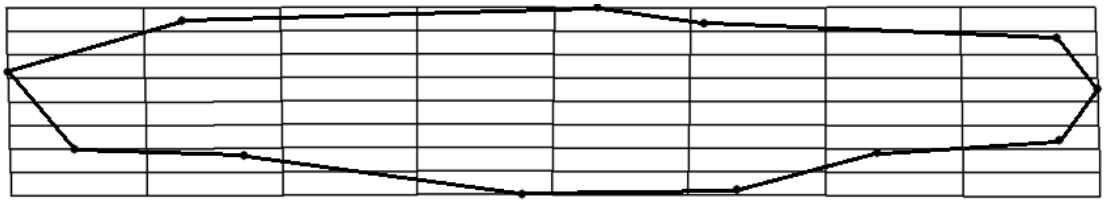


Figure B1. Canonical variates analysis of *Notropis atherinoides* from three different age classes. (a) Scatter plot of the first and second canonical variates scores of emerald shiners that are age 1 (blue circle), age 2 (orange circles), and age 3 (grey circle).

(a) Age 1



(b) Age 2



(c) Age 3

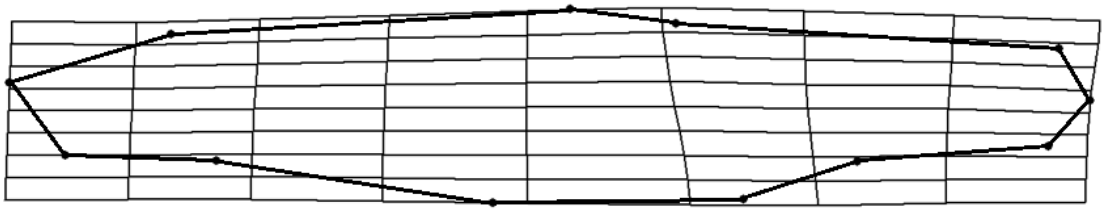


Figure B2. Mean shapes of *Notropis atherinoides* of three age classes: (a) age 1, (b) age 2, (c) age 3. Visualizations are magnified 3X to aid in visualizing differences in shape between age classes. Age 1 emerald shiners had relatively streamlined body forms, age 3 individuals were more robust, and age 2 individuals showed intermediate body shapes.

Appendix C: Effect of Preservation on Shape of Emerald Shiners

When analyzing fish for differences in shape, it is ideal to analyze individuals that are fresh (i.e., not preserved; Berbel-Filho et al. 2013). However, photographing each individual fish the same day they were collected is not always feasible. Standardized preservation of these fish provides an alternative. Although preservation distorts the true shape of fish, short-term fixation in ethanol is the best option when necessary (Berbel-Filho et al. 2013). To assess the effects of short-term preservation of emerald shiners in ethanol, I compared the geometric shapes of fresh *Notropis atherinoides* to those that were stored in 95% ethanol for two weeks.

A sample of 30 age-2 emerald shiners were collected from the upper Niagara River and photographed on their left lateral side that same day. All individuals were then preserved for 14 days in 95% ethanol and photographed a second time in the same way. Geometric morphometric analyses were performed on both groups using the same methods described in the main text. A single factor MANOVA was performed with 900 permutations. A canonical variates analysis and a "Jackknife Groupings" test were performed. All statistical analyses were performed using CVAGen. The program tpsRegr was used to obtain mean shapes for the two groups.

The MANOVA demonstrated that there is a significant effect of preservation ($F = 11.96$, $p = 0.001$). The CVA showed that, based on shape, emerald shiners were grouped into the two treatments with no overlap (Figure C1). Further, individuals were grouped into the correct treatment group 93.33% of the time (Table C1). Divergence occurs entirely along the first canonical variate, with a complex change in shape in response to preservation (Figure 1). These data show that preservation by 95% ethanol does change the shape of age-2 emerald shiners from the Niagara River. Ideally, geometric morphometric analyses of habitat differentiation in emerald shiners would be performed using fresh fish. However, the logistics of the analyses make this

difficult. Therefore, in the current analysis of habitat differentiation, samples were preserved in a standardized manner. All samples were preserved in 95% ethanol for 14 days. Additionally, the "Unbend Specimens" function of tpsUtil was used to correct for the bending associated with preservation.

Table C1. Jackknife grouping results showing the number of individuals assigned to the correct and incorrect *a priori* treatment group. The first column holds the *a priori* groups with sample sizes, and the subsequent columns show the number of individuals assigned to each group as determined by the CVA. Given group sizes, the expected random rate of correct assignments is 50.22 %. Correct assignments (i.e., Fresh-Fresh and Preserved-Preserved) are shown in boldface.

	Fresh	Preserved
Fresh (N = 30)	**27**	3
Preserved (N = 30)	1	**29**
	56 correct assignments out of 60 (93.33 %)	

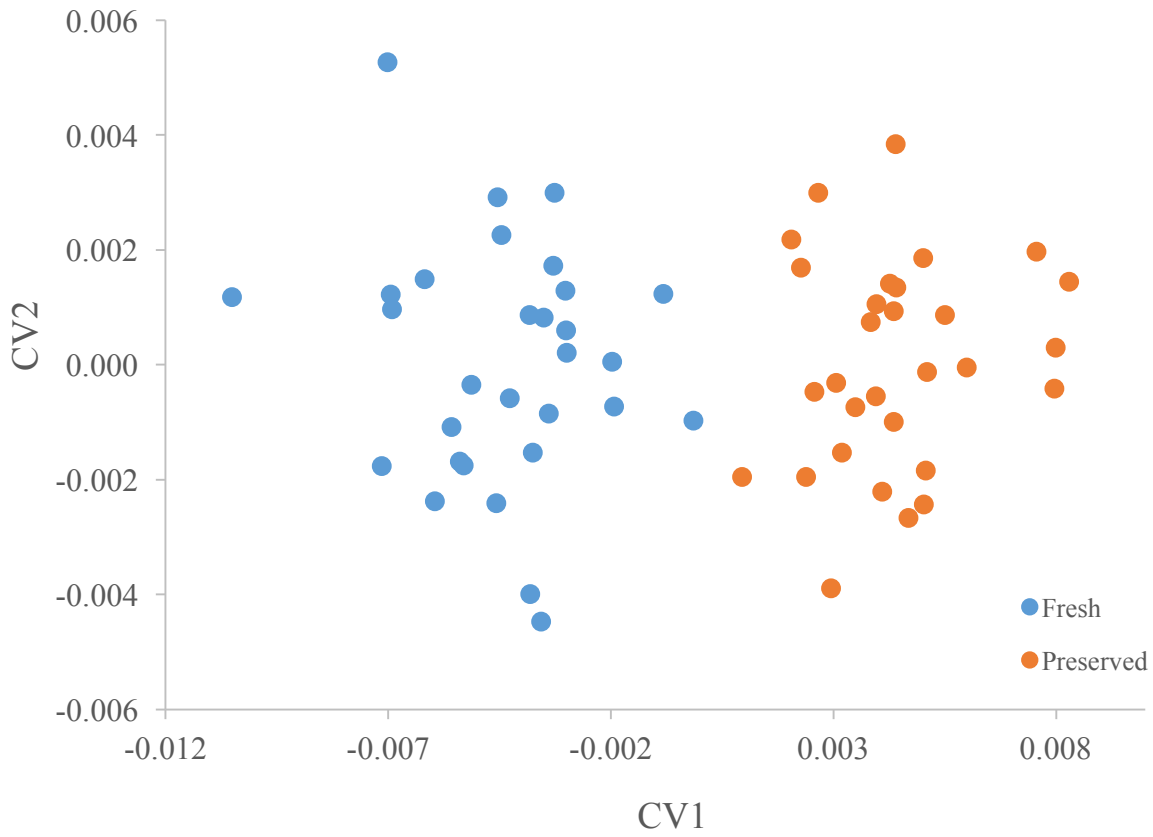
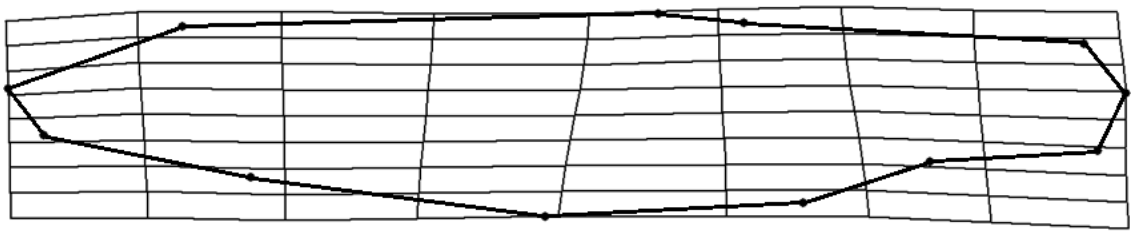


Figure C1. Canonical variates analysis of fresh and preserved *Notropis atherinoides*. (a) Scatter plot of the first and second canonical variates scores of emerald shiners that were fresh (blue circles) and preserved in 95% ethanol for 14 days (orange circles).

(a) Fresh



(b) Preserved

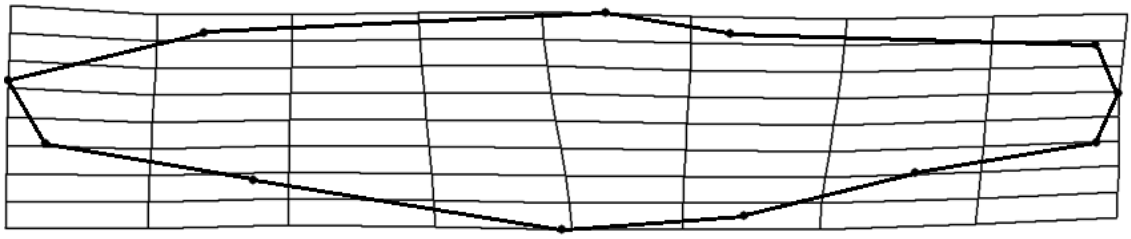


Figure C2. Mean shapes of *Notropis atherinoides* from two treatments: (a) Analyzed fresh, (b) analyzed following preservation for two weeks in 95% ethanol. Visualizations are magnified 3X to aid in visualizing differences in shape between age classes. A complex change in shape occurred in emerald shiners after two weeks in ethanol.