PHENOTYPIC PATTERNS OF SIZE AND SHAPE IN NORTH AMERICAN KILLIFISH

BY

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DISSERTATION

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Abstract

Variation exists in multiple levels of organization, including between and within species, populations, and individuals. Fish are ideal organisms to explore questions related to the patterns of variation and the potential processes driving patterns. Fish occupy different types of habitats that vary in several important environmental parameters, including water velocity, predatory, prey, and spatial heterogeneity. Body size and shape (and associated characteristics such as fins) are great traits to focus on when exploring how fish may adapt to these different environmental pressures. Size and shape have strong effects on an individual's maneuverability in many species and, because of this, are believed to be targets for selection. Populations occupying different habitats have shown differences in body size and shape, although species differ in the direction and magnitude of the variation and not all species show a difference between habitat types.

The following chapters demonstrate just some of the multitude of complex questions that can be addressed by studying phenotypic differences across habitats in fish. Several such questions that are explored are: To what extent are there consistent differences in body shape and size between lentic and lotic habitats across different fish species? To what extent are there consistent differences in body shape and size between lake and stream habitats between populations within a species? What is the magnitude of variation in traits (fin shape) within a fish species? Is the phenotypic variation in body shape across populations attributable to genetic differentiation, phenotypic plasticity, or both?

Here, I examine phenotypic patterns in body size and shape at multiple levels of biological organization- from a family-wide comparison to a with-in species and even within-in a particular trait (the dorsal and anal fins). There are several broad findings. First, there are large

differences between males and females in body shape as well as in the shape of anal and dorsal fins, and these patterns appear to be robust to differences in flow regime. Some of these body shape differences may emerge simply due to the necessities of females producing eggs, but other shape differences cannot be attributed solely to these effects. For *F. notatus,* the unpaired fins (dorsal, anal, and perhaps caudal) differ between the sexes, and, again, the pattern is robust to habitat differences. The fact that dorsal and anal fin shape is tightly correlated along one axis, but that both males and females vary along that axis, suggests the possibility of interesting developmental constraints present. Second, there are differences in body size and shape between lentic and lotic habitats in *F. notatus*, and these patterns are somewhat generalizable to the entire fundulid family. Fish tend to be larger and deeper bodies with longer dorsal fin bases in lotic habitats than they are in lentic habitats. Finally, our analyses suggest that differences in shape are attributable to both genetics and plasticity as a function of water flow.

Academically, this research makes significant contributions to two major areas of research in evolutionary biology. First, because environmental factors (both biotic and abiotic) are what ultimately result in differential survival and/or reproductive success, this research elucidates some potential causes of adaptive evolution in natural populations which is often poorly understood. The only way to fully understand natural selection is through a detailed knowledge of the ecology and biology of organisms. Second, this work helps to clarify the role of the environment in determining body size and shape in fishes. While there has been significant interest in this topic by other researchers, there is seems to be very little consistency in how different environmental factors influence body size and shape. There is a clear need to for additional research in new species in order to develop a more thorough understanding of when a particular factor should be important.

In addition to its scholarly value, this work may offer practical, applied knowledge. As climate change progresses, extreme weather events such as spring floods and summer draughts are expected to increase in frequency. Stream fish of all sorts will be faced with the dual problem of countering extreme water flow at some points in time while having to accommodate a lack of water flow at other times. The hope is that this work provides insights as to how fish, in particular killifish (many species of which are widely distributed and abundant in nature, thus making them ecologically important, will deal with these challenges.

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Chapter 1: General Overview

Variation exists in multiple levels of organization, including between and within species, populations, and individuals. Fish are ideal organisms to explore questions related to the patterns of variation and the potential processes driving patterns. Fish occupy different types of habitats that vary in several important environmental parameters. Aquatic habitats can vary greatly in water velocity ranging from still/standing water habitats such as lakes and ponds (termed lentic habitats) to fast moving rivers and streams (termed lotic habitats). Because water velocity directly alters an individual's ability to maintain its position (Gee 1977) and its buoyancy (Beaver and Gee 1988), behaviors such as foraging (Webb 1984, Asaeda *et al.* 2005, Piccolo *et al.* 2008), mating (Nicoletto 1996), and predator evasion (Taylor and McPhail 1985) are all potentially affected. Water flow also influences the potential prey, competitors, and predators with which an individual encounters (Aadland 1993, Jackson *et al.* 2001, Pouilly *et al.* 2006).

Body size and shape (and associated characteristics such as fins) are great traits to focus on when exploring how fish may adapt to the various environment pressures. Size and shape have strong effects on an individual's maneuverability in many species and, because of this, are believed to be targets for selection (Walker 1997, Fraser *et al.* 2011). Changes in flow regime can also impact a fish's body size and shape (Webb 1984, Pakkasmaa and Piironen 2001, Langerhans 2008, Sagnes and Statzner 2009). It is often predicted that individuals in high water velocities should be narrower and have shallower bodies ("more streamlined") compared to those in low velocities (Langerhans 2008), but these general predictions are not universal (Pakkasmaa and Piironen 2001, McGuigan *et al.* 2003, Neves and Monteiro 2003, Krabbenhoft *et al.* 2009). Additionally, life-history theory predicts that increases in size-selective predation will cause populations to evolve a smaller size at maturity (Charlesworth 1980, Stearns 1992).

The following chapters demonstrate just some of the multitude of complex questions that can be addressed by studying phenotypic differences across habitats in fish. Several such questions that are explored are: To what extent are there consistent differences in body shape and size between lentic and lotic habitats across different fish species? To what extent are there consistent differences in body shape and size between lake and stream habitats between populations within a species? What is the magnitude of variation in traits (fin shape) within a fish species? Is the phenotypic variation in body shape across populations attributable to genetic differentiation, phenotypic plasticity, or both?

In addressing these questions, this research makes significant contributions to two major areas of research in evolutionary biology. First, because environmental factors (both biotic and abiotic) are what ultimately result in differential survival and/or reproductive success, this research elucidates some potential causes of adaptive evolution in natural populations which MacColl (2011) notes is often poorly understood (see also Wade and Kalisz 1990, Kawekci and Ebert 2004). Endler (1986) similarly mentions that the only way to fully understand natural selection is through a detailed knowledge of the ecology and biology of organisms. Second, this work helps to clarify the role of the environment in determining body size and shape in fishes. While there has been significant interest in this topic by other researchers (Pakkasmaa and Piironen 2001, McGuigan *et al*. 2003, Neves and Monteiro 2003, Langerhans 2008, Krabbenhoft *et al*. 2009), there is seems to be very little consistency in how different environmental factors influence body size and shape. There is a clear need to for additional research in new species in order to develop a more thorough understanding of when a particular factor should be important.

Here, I examine phenotypic patterns in body size and shape at multiple levels of biological organization. In Chapter 2, I analyze broad patterns in body size/shape between lentic and lotic habitats as well as sex differences between males and females across 34 species of North American killifish (family Fundulidae). Phenotypic differences between habitats within species were present. The largest effect was seen on body depth, where shallower bodies were more common in lentic habitats. Body size also differed where fish were larger in lotic habitats than in lentic habitats. Strong patterns in sexually dimorphic body shape were also present, particular with regard to dorsal fin position.

I followed up this family wide study with an investigation into the among-population within-species patterns in body size/shape in a single species, the blackstripe topminnow (*Fundulus notatus*) (chapter 3). *Fundulus notatus* is widely abundant in Illinois and (most importantly for this study) is found commonly in both lake and stream habitats. I asked whether there were robust patterns between lentic and lotic habitats that were consistent across drainages. I also examined sex-specific differences in body size/shape. I again found differences between lentic and lotic habitats, but this varied somewhat with drainage. One-year old fish were generally larger from lotic habitats, but this was only the case for 3 out of the 4 drainages that I sampled. Lotic fish also had longer dorsal fin bases than did lentic fish. The sexes also differed in body shape where males had longer dorsal and anal fin bases than did females.

In chapter 4, I delved into a more in-depth analysis of dorsal and anal fins. Previous reports had suggested that males and females differ in the size/shape of the unpaired fins. In chapter 3, we had simply used calipers to measure the length of the fin base (i.e. the length of the section where the fin connects with the body). Here, I sought to examine the actual shape of the dorsal and anal fins. The allure of these fins is that they are multi-functional. That is, they are used in multiple biological endeavors. Both dorsal and anal fins are used in swimming/maneuvering in the water column. Males also use dorsal and anal fins in displays towards females and competing males. Anal fins also function in directing the movement of eggs and sperm.

In this study, I removed (i.e. cut off) the dorsal and anal fins from the preserved fish from chapter 3 and imaged them. As with chapter 3, I found large differences between the sexes, particularly in one year-old fish. Males had larger dorsal and anal fins that were more pointed than those of females. There was little effect of lentic/lotic habitats on anal/dorsal fin shape. Most interesting was the finding that dorsal and anal fin angle (but not size) was tightly correlated across males and females. The pattern held even when considering each sex separately. This suggests that the shape development of these two fins may be linked.

In chapter 5, I returned to the question of whether the differences in size/shape between lentic and lotic habitats are attributable to genetic differentiation (i.e. different habitats select for different size/shape attributes) or phenotypic plasticity (i.e. constantly battling current alters growth trajectories) or some combination of the two. To do this, I created families of fish from stream and lake parents and then raised the offspring in either lentic (no flow) or lotic (flow present) rearing environments. For both size and shape, there was an interaction present between parental habitat (genetics) and rearing environment (plasticity). The interaction occurred because lake offspring were plastic in development, but stream fish were not. In the case of body shape, both the genetic and plastic effects "produced the right pattern" in that both the genetic effect and the plastic effect mirrored the pattern found in nature. For lake fish, dorsal fin bases were larger when they were reared in the lotic environment but smaller when reared in the lentic environment. Stream fish, regardless of the rearing environment, had large dorsal fin bases. In terms of body size, the pattern was mixed. Phenotypic plasticity among the lake fish produced the right pattern in that fish raised in the lotic environment were bigger than the fish raised in the lentic environment. However, on average, lake fish were larger than stream fish. Chapter 3 shows the opposite pattern.

Exactly why fish from lotic environments are larger than fish from lentic environments (patterns shown in chapters 2 and 3) is unclear, but it is not due to simple genetic differentiation among populations. Streams and lakes differ in a number of attributes that could potentially result in these size differences. Size differences could arise due to differences in food availability (assuming there is more food in streams which equates to bigger fish), differences in growing season (water temperatures rise sooner in streams than in lakes), or differences in size-selective predation where large individuals are more likely to be preyed upon in lakes. These hypotheses are readily testable and empirically tractable.

What broader lessons can we take from this body of work? First, there are large differences between males and females in body shape as well as in the shape of anal and dorsal fins, and these patterns appear to be robust to differences in flow regime. Some of these body shape differences may emerge simply due to the necessities of females producing eggs, but other shape differences cannot be attributed solely to these effects. For *F. notatus,* the unpaired fins (dorsal, anal, and perhaps caudal) differ between the sexes, and, again, the pattern is robust to habitat differences. The fact that dorsal and anal fin shape is tightly correlated along one axis, but that both males and females vary along that axis, suggests the possibility of interesting developmental constraints present. Second, there are differences in body size and shape between lentic and lotic habitats in *F. notatus*, and these patterns are somewhat generalizable to the entire fundulid family. Fish tend to be larger and deeper bodies with longer dorsal fin bases in lotic habitats than they are in lentic habitats. Finally, our analyses suggest that differences in shape are attributable to both genetics and plasticity as a function of water flow. In addition to its academic value, we hope that this work offers practical, applied knowledge. As climate change progresses, extreme weather events such as spring floods and summer draughts are expected to increase in frequency. Stream fish of all sorts will be faced with the dual problem of countering extreme water flow at some points in time while having to accommodate a lack of water flow at other times. The hope is that this work provides insights as to how fish, in particular killifish (many species of which are widely distributed and abundant in nature, thus making them ecologically important (Foster 1967; Atmar and Stewart 1972; Fleeger et al. 2008)), will deal with these challenges.

References

Aadland L. P. 1993. Stream habitat types: their fish assemblages and relationship to flow. *North American Journal of Fisheries Management* **13:** 790-806.

Asaeda T., T. K. Vu and J. Manatunge. 2005. Effects of flow velocity on feeding behavior and microhabitat selection of the Stone Moroko *Pseudorasbora parva*: A trade-off between feeding and swimming costs. *Transactions of the American Fisheries Society* **134:** 537-547.

Atmar G. L. and K. W. Stewart. 1972. Food, feeding selectivity, and ecological efficiencies of *Fundulus notatus* (Cyprinodontidae). *American Midland Naturalist* **88:** 76–89.

Beaver B. J. and J. H. Gee. 1988. Role of water current and related variables in determining buoyancy in the sticklebacks *Culaea inconstans* and *Pungitius pungitius*. *Canadian Journal of Zoology* **66:** 2006-2014.

Charlesworth B. 1980. *Evolution in age-structured populations*. Cambridge: Cambridge University Press.

Endler J. 1986. *Natural Selection in the Wild*. Princeton: Princeton University Press.

Fleeger J. W., D. S. Johnson, K. A. Galvan, and L. A. Deegan. 2008. Top-down and bottom-up control of infauna varies across the saltmarsh landscape. *Journal of Experimental Marine Biology and Ecology* **357:** 20–34.

Foster N. R. 1967. Comparative studies on the biology of killifishes (Pisces, Cyprinodontidae). PhD Dissertation, New York: Cornell University.

Fraser D. J., L. K. Weir, L. Bernatchez, M. M. Hansen, and E. B. Taylor. 2011. Extent and scale of local adaptation in salmonid fishes: review and meta-analysis. *Heredity* **106:** 404-420.

Gee J. H. 1977. Effects of size of fish, water temperature, and water velocity on buoyancy alteration by fathead minnows, *Pimephales promelas*. *Comparative Biochemistry and Physiology A: Physiology* **56:** 503-508.

Jackson D. A. P. R. Peres-Neto, and J. D. Olden. 2001. What controls who is where in freshwater fish communities - the roles of biotic, abiotic, and spatial factors. *Canadian Journal of Fisheries and Aquatic Science.* **58:** 157-170.

Kawecki T. J. and D. Ebert. 2004. Conceptual issues in local adaptation. *Ecology Letters* **7:** 1225-1241.

Krabbenhoft T. J., M. L. Collyer, and J. M. Quattro. 2009. Differing evolutionary patterns underlie convergence on elongate morphology in endemic fishes of Lake Waccamaw, North Carolina. *Biological Journal of the Linnean Society* **98:** 636-645.

Langerhans R. B. 2008. Predictability of phenotypic differentiation across flow regimes in fishes. *Integrative and Comparative Biology* **48:** 750-768.

MacColl A. D. C. 2011. The ecological causes of evolution. *Trends in Ecology and Evolution* **26:** 514-522.

McGuigan K., C. E. Franklin, C. Moritz, and M. W. Blows. 2003. Adaptation of rainbow fish to lake and stream habitats. *Evolution* **57:** 104-118.

Neves F. M. and L. R. Monteiro. 2003. Body shape and size divergence among populations of *Poecilia vivipara* in coastal lagoons of south-eastern Brazil. *Journal of Fish Biology* **63:** 928-941.

Nicoletto P. F. 1996. The influence of water velocity on the display behavior of male guppies, *Poecilia reticulata*. *Behavioral Ecology* **7:** 272-278.

Pakkasmaa S. and J. Piironen. 2001. Water velocity shapes juvenile salmonids. *Evolutionary Ecology* **14:** 721-730.

Piccolo J. J, N. F. Hughes, and M. D. Bryant. 2008. Water velocity influences prey detection and capture by drift-feeding juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead (*Oncorhynchus mykiss irideus*). *Canadian Journal of Fisheries and Aquatic Sciences* **65:** 266-275.

Pouilly M., S. Barrera, and C. Rosales. 2006. Changes of taxonomic and trophic structure of fish assemblages along an environmental gradient in the Upper Beni watershed (Bolivia). *Journal of Fish Biology* **68:** 137-156.

Sagnes P. and B. Statzner. 2009. Hydronamic abilities of riverine fish: a functional link between morphology and velocity use. *Aquatic Living Resources* **22:** 79-91.

Stearns S.C. 1992. *The Evolution of Life Histories*. New York: Oxford University Press.

Taylor E. B. and J. D. McPhail. 1985. Variation in body morphology among British Columbia populations of coho salmon, *Oncorhynchus kisutch*. *Canadian Journal of Fisheries and Aquatic Sciences* **42:** 2020-2028.

Wade M. J. and S. Kalisz. 1990. The causes of natural selection. *Evolution* **44:** 1947-1955.

Walker J. A. 1997. Ecological morphology of lacustrine three-spine stickleback *Gasterosteus aculeatus* L. (Gasterosteridae) body shape. *Biological Journal of the Linnean Society* **61:** 3-50.

Webb P. W. 1984. Form and function in fish swimming. *Scientific American* **251:** 58-68

Chapter 2: Comparison of body size and shape across the Fundulidae family

Abstract

Body size and body shape have important fitness consequences for fish and other organisms and understanding what drives patterns in size and shape is a primary goal of ecomorphology studies. To truly understand patterns of body size and shape and the potential processes driving it, the effects of sex and habitat should be examined across multiple species, ideally in a phylogenetic framework to consider evolutionary relatedness. Here, we explore the patterns of body size and shape across an entire family (the Fundulidae family). Our goal was to examine how sex and aspects of the environment affect morphological variation. We found substantial variation in both size and shape both within and among the various species of Fundulidae. Those species that differed between habitats showed a consistent pattern where fish in lotic habitats had greater body depth and greater overall size than fish in lentic habitats. Sex had large, consistent patterns across the phylogeny with respect to dorsal fin position. Interestingly, most species did not show differences in *multiple* aspects of shape as a result of habitat or sex. Determining why these patterns appear in some species but not others requires further work knowing whether these differences are due to genetic differentiation and/or phenotypic plasticity, but such studies offer the potential to enhance our understanding of the underlying genetic architecture that drives sexual dimorphism and habitat-based population divergence in body size/shape within and among species.

Introduction

Phenotypic variation is ubiquitous in nature. Many taxa show substantial variation in morphological features, including coloration, ornamentation, body size, and body shape. The utilization of these traits in research can offer substantial insights into the evolutionary history of organisms and understanding what drives the patterns of these traits is a primary goal of ecomorphology studies.

In fish, variation in body size and/or shape have important fitness consequences for an organism through impacts on a variety of activities such as prey capture (Rincón *et al*. 2007), predator avoidance (Brönmark and Miner 1992, Domenici and Blake 1997, Eklov and Svanback 2006) and reproductive success (Foster *et al*. 1992). Sex-specific differences in body size and shape are common (Parker 1992, Georga and Koumoundouros 2010). These typically arise due to the demands placed on egg production in females. Similarly, many studies have documented patterns in size or shape across different habitats (Brinsmead and Fox 2002, McGuigan *et al*. 2003, Keeley *et al.*, 2005, Welsh *et al*. 2013). Variation in body size or shape may represent local adaptation, where natural selection acts to favor different suites of traits that maximize fitness in different environments (Williams 1966, Kawecki and Ebert 2004). Local adaptation is important because it can fuel population differentiation and could even potentially lead to speciation (Schluter 2001).

The literature on body size and shape contains inconsistent and conflicting patterns of differences across species, habitats and/or sexes. For instance, Langerhans (2008) developed and tested a model about phenotypic differences based on flow regimes. He found strong support for a prediction that individuals in high water velocities should be narrower and have shallower bodies (“more streamlined”) compared to those in low velocities. However, several studies have found either the opposite pattern or no effect of water velocity on body shape (Pakkasmaa and Piironen 2001, McGuigan *et al*. 2003, Neves and Monteiro 2003, Krabbenhoft *et al*. 2009, Carlson and Lauder 2011). These incongruent patterns may be a result of the limited focus of past studies. Many studies of size or shape tend to concentrate on one species across multiple habitats (Keeley *et al*. 2005) or compare multiple (often unrelated) species found in one particular habitat (Krabbenhoft *et al*. 2009).

Patterns of body size and/or shape vary based on what specific aspect of the habitat is driving the differences observed. According to theory, individuals in high water velocities should be narrower and have shallower bodies ("more streamlined") compared to those in low velocities (Langerhans 2008), but these general predictions are not always upheld (Pakkasmaa and Piironen 2001, McGuigan *et al*. 2003, Neves and Monteiro 2003, Krabbenhoft *et al*. 2009). Smaller and deeper bodied species tend to occupy slower moving water (Wikramanayake 1990). Within a lotic habitat, different positions within the water column can differ with respect to water velocity (Westenbroek 2006). As such, species that occupy different parts of the water column may also differ in morphology (Aleev 1969, Felley 1984). Aleev (1969) noted that a dorsal fin closer to the center of gravity allows for more maneuverability because it acts more like a rudder whereas one further back toward the caudal fin would act more like a stabilizer.

In addition to water velocity, predators can drive phenotypic differentiation in body size/shape. Predation may favor body size/shape that aids either in anti-predator movements or that precludes consumption by the predator (i.e. predators can't get prey in their mouths) (Webb 1984a,b, Brönmark and Miner 1992, Hendry *et al*. 2006). However, similar to the influence of water velocity on body size and shape, predation does not always result in morphological differences (Young *et al*. 2011).

To truly understand patterns of body size and shape and the potential processes driving it, the effects of sex and habitat should be examined across multiple species. Ideally, these studies would also consider phylogenetic effects of theses species, as patterns in body size and shape may reflect evolutionary relatedness (Guill *et al.* 2003). Closely related species may more closely resemble each other simply because of a shared common ancestor. Conversely, species not as closely related may resemble each other because traits may arise independently multiple times.

Here, we explore the patterns of body size and shape across an entire family (the Fundulidae family). Our goal was to examine how sex and aspects of the environment affect morphological variation. We examined environmental influences both by comparing lentic and lotic habitats and by exploring patterns based on position in the water column. When possible, we also analyzed our data in a phylogenetic framework.

Methods

Study System: The Fundulidae family is a group of 41 species across four genera, *Fundulus*, *Lucania*, *Adinia*, and *Leptolucania*, with *Fundulus* accounting for the vast majority of the species. These fish are native mainly to North America and islands in the Caribbean with a few ranging as far south as Mexico, but some have also recently become invasive in Europe (Bernardi *et al.* 1995, Gisbert and Lopez 2007). Both molecular and morphological analyses indicate that *Fundulus* is not monophyletic (Wiley 1986, Whitehead 2010). *Lucania, Leptolucania,* and *Adinia* occur within the genus *Fundulus.*

Data Collection: Museum specimens of every species from the Fundulidae family were borrowed from one or more museum collections (Illinois Natural History Survey, American

Museum of Natural History, Texas Natural History Collection, Florida Museum of Natural History, and National Museum of Natural History). To ensure all species belonging to this family were included, species composition of the family was verified using the Catalog of Fishes (Eschmeyer 2010).

The right side of each specimen was photographed using an 8 megapixel Nikon COOLPIX 8700 digital camera on a background consisting of 1 millimeter by 1 millimeter grids. The sex of each specimen was then determined based on sexually dimorphic black coloration patterns using data from one or more sources (Welsh in review). In many of these species, juveniles have a similar color pattern as adult females. We therefore classified individuals as adults based on the size at which male and female sexually dimorphic coloration was discernible for each species.

As specimens were collected from a variety of habitats over a large geographic range, we decided to broadly group the habitats as either "lentic" (still water habitats, such as lakes, ponds, and swamps) or "lotic" (moving water habitats, such as streams, creeks, and rivers) based on the locality information available from the museum records. While these terms reflect water velocity, we do not mean to imply that water velocity is the only difference between the habitats that could be driving any habitat-specific patterns. Those habitats that either did not fit either of those categories (such as oceans, bayous, tidal areas, and others) or could not confidently be determined because of lack of detail in museum records were omitted. In total, we had approximately 1,300 specimens from 36 species that fit our criteria (known sex from either lentic or lotic habitat) (Table 2.1).

Geometric morphometric techniques using the TPS software series (http://life.bio.sunysb.edu/morph) were used to determine body size and shape (reviewed in

Rohlf and Marcus 1993, Zelditch *et al.* 2012). Geometric morphometrics allows for comparisons of size and shape independent of each other based on anatomical landmarks defined by Cartesian coordinates. We used a series of 32 landmarks (12 homologous Type I, 20 "sliding" semi-landmarks) that were digitally placed on the right side of the body of the fish (Figure 2.1) using tpsDig (v2.14). Specimens where one or more of these landmarks were obscured were excluded from the analyses. Type I landmarks are placed on distinctive, homologous features of the fish (i.e. center of the eye, anterior base of dorsal fin, etc.) whereas semi-landmarks are used to cover large regions of the body where there are no distinctive features (curves, large areas without homologous features, etc.) (Bookstein 1997, Mitteroecker and Gunz 2009).

Assessing Size and Shape

A Generalized Procrustes analysis (GPA) was performed in tpsRelw (v1.49) to remove variation in landmark configurations that is due to differences in factors unrelated to shape, such as position and orientation of the specimens (Rohlf and Slice 1990). This produced measures of size (centroid size) and shape (partial warp scores and uniform components) that are independent of each other. Thin plate splines in tpsRelw were used to visualize the shape changes that occurred along each principal component axis.

Centroid size was used as the measure of body size. A principal components analysis using the partial warp scores and uniform components in a covariance matrix was used as a measure of body shape. We only considered the first four principal components because individually they accounted for at least 5% of the variation and together they explained over 85% of the variation between species.

Analyses

Our first goal was to determine the effects of sex, habitat, and their interaction on body size and shape for each species using analysis of variance (ANOVA). As mentioned previously, only samples that could be confidently sexed (i.e. no juveniles) and from habitats that could be classified (lentic or lotic) were included. For both size (centroid size) and shape (PC scores), we considered the effects of habitat, sex, species and all of their interactions. Habitat, sex, and the habitat*sex interaction were tested over their interaction with sex (i.e sex tested over sex*species, habitat over habitat*species, etc.). The error degrees of freedom were generated using a Satterthwaite approximation, which incorporates a weighted average of the interaction with species and the mean square error (Sattherwaite, 1946). We conducted the analyses for all 39 species in which we had samples of at least one sex from at least one habitat type and then on a reduced data set that only those 21 species in which we had samples of both sexes from both habitats (see Table 2.1). Because many species have populations in both habitats, we could not statistically analyze habitat or sex in a phylogenetic framework, but, rather, qualitatively assessed the effect of phylogeny by referencing the patterns to the phylogenetic tree. The phylogeny used was the combined nuclear and mitochondrial tree created by Whitehead (2010) (see Figure 1A in that reference).

Because many of our results showed interactions with species (see Results), we performed follow-up ANOVAs at the within-species level. Separately for each species, we performed a two-way ANOVA that included the effects of sex, habitat, and the interaction between them. For these separate ANOVAs, we tested over the total number of individuals and not by using a Sattherwaite approximation.

Our second goal was to determine whether body size or shape varied due to the position in the water column that the species is typically found in. As water velocity of different parts of the water column is only expected to differ substantially in moving water environments, we only considered populations in the lotic flow regime. To do this, we first obtained information about their position in the water column. This came primarily from published records (field guides, journal articles, and dissertations), although some information was obtained from more informal means, such as websites from the North American Native Fishes Association (NANFA), the department of natural resources of various states, and personal communications with aquarium hobbyists. We then classified each species into one of two mutually exclusive categories: surface or beneath the surface (hereafter referred to "surface" or "beneath", respectively) (Table 2.1). There were many species in which this information could not be confidently obtained, but we were ultimately able to assign this for 22 species.

The relationship between position in the water column and body size or shape was analyzed at the species level first using a phylogenetic ANOVA (Garland *et al.* 1993). The Phylogenetic ANOVA works by creating a null distribution for the F-statistic based on Brownian motion simulation on the phylogeny. Thus, the p-value is obtained by comparing the observed test-statistic to simulated test-statistics after an arbitrarily large number of simulations (in this case, 10,000). In other words, a significant phylogenetic p-value for a factor (PC1, PC2, PC4, or Centroid Size) indicates that a consideration of the phylogeny is important when analyzing that particular factor. Any significant phylogenetic p-values were then followed up with a phylogenetic post-hoc test between the groups using a bonferroni adjustment. Factors with non-significant phylogenetic p-values were analyzed with a standard ANOVA and any significant results from this were followed up with standard post-hoc comparisons. Phylogenetic ANOVAs and post-hoc tests were conducted in R (v. 2.15.1) using the “phylANOVA” in the “phytools” package. All other analyses were conducted using SAS (v. 9.3).

Results

The first four principal components together explained 85.3% of the variation in shape between the species, however only PC 1, 2, and 4 were biologically meaningful. PC3 explained 10.4% of the variation but corresponded to preservation artifact. With fish, preservation artifact is indicated by the characteristic “U”-shaped bending where the head and tail bend in one direction and the middle of the body bend in the other (see Wund *et al.* 2008). This was subsequently confirmed by using the “unbend specimen” module in tpsUtil, which essentially removed PC3 (i.e. PC4 became PC3 after applying this module).

Shape change along each Principal Component Axis

PC1 explained 48.5% of the variation and was related to dorsal fin position (Figure 2.2A). A more positive PC1 score indicated a more posteriorly-positioned dorsal fin (i.e. closer to the caudal fin) while a more negative score indicated a dorsal fin closer to the middle of the body. PC2 accounted for 20.9% and was related to body depth (Figure 2.2B). Individuals ranged from deep bodied (more positive PC2 value) to shallow bodied. PC4 explained 5.5% of the variation and was related to head position (Figure 2.2C). Individuals ranged from a slightly more upturned mouth and eye (more positive PC4 value) to more centrally located mouth and eye.

Species Analyses- Body Shape

Dorsal fin position (PC1) was strongly sexually dimorphic whereas habitat had strong effects on body depth (PC2) (Table 2.2). Because these results were qualitatively the same whether we considered all 39 species or only those 21 species that had both males and females in both habitats (Table 2.2), we only show results for those 21 species. Females had a smaller PC1 value (i.e. a dorsal fin closer to the caudal fin) than males (Figure 2.3). Dorsal fin position also showed an interaction between habitat and species, where most species that showed a difference between habitats had a more posteriorly-positioned dorsal fin (more positive PC1 score) in lentic habitats (Figure 2.4). For body depth, there was a habitat effect, but this varied based on species (as evidenced by the interaction between habitat and species). For those species that showed a difference in depth between habitats, most had more positive PC2 values (deeper bodies) in lotic habitats (Figure 2.5). There were also an interaction between sex and species, such that most species that showed an effect had females with more positive PC2 values (deeper bodies) than males (Figure 2.5). Head position showed no effect of sex or habitat, but did there was an interaction between habitat and species. For those species that showed a habitat effect, most had a more positive PC4 value (more upturned mouth/eye) in lentic than lotic (Figure 2.6).

When we considered each species separately, these results were generally the same (Supplemental Table 2.1). Dorsal fin position (PC1) showed a clear and consistent pattern of sexual dimorphism. Sex had a significant effect in 28 out of 34 species for PC1, and in all females had a smaller PC1 value (i.e. a dorsal fin closer to the caudal fin) than males. There were also smaller effects of sex on body depth (PC2) and head position (PC4) (3 species each). The largest effect of habitat was seen on body depth (PC2). Of the eleven species that showed an effect of habitat on body depth (PC2), nine had shallower bodies in lentic habitats. Smaller

Patterns also emerged for dorsal fin position (PC1) and head position (PC4). Of the eight species that showed a change in dorsal fin position (PC1) as a function of habitat, seven had a dorsal fin closer to the caudal fin in lotic habitats. For the seven species that showed differences in head position (PC4) between habitats, five had a slightly more centrally located mouth and eye in lotic habitats.

Species varied in the extent to which they were sexually dimorphic and/or variable between lentic and lotic habitats. Aside from the strong sex effect on PC1, the majority of species did not show an effect of sex or habitat. For those species that did show a significant effect, very few were significantly different for multiple aspects of shape (i.e. multiple PC axes). One species (*Fundulus dispar*) varied as a function of habitat in all three components of shape and none varied as a function of sex in all three. Seven species (*Adinia xenica*, *Fundulus majalis*, *Fundulus seminolis*, *Fundulus zebrinus*, *Leptolucania ommata*, *Lucania goodei*, and *Lucania parva*) differed between habitats in two aspects of shape and only five (*Adinia xenica*, *Fundulus diaphanus*, *Fundulus dispar*, *Fundulus pulvereus*, and *Lucania goodei*) showed sexual dimorphism in two of the three shape components.

Position in the water column

The relationship between dorsal fin position (PC1) and position in the water column showed a phylogenetic signal (Table 2.3). Species found at the surface of the water column had a dorsal fin located closer to the middle of the body (more negative PC1 values) than those found beneath the surface (phylogenetic post-hoc $p = 0.0051$) (Figure 2.7). Dorsal fin position was the only aspect of size or shape that showed a relationship with position in the water column.

Species Analyses- Body Size

Body size varied as a function of lentic versus lotic habitats (Table 2.2). As with body shape, this habitat-specific difference in size was qualitatively the same whether considering all 39 species or only the 21 species with both sexes in both habitats and, so, we only discuss the latter. Habitat differences in body size exist, such that individuals in lotic habitats were larger than those in lentic habitats (Figure 2.8). When analyzed on the within-species level, the results were qualitatively the same. Individuals from lentic habitats were consistently smaller than those from lotic habitats in all eight species that showed an effect (Supplement Table 2.1).

Discussion

We documented substantial variation in body size/shape both as a function of habitat and sex. The unique aspect of this study is that many species of killifish occur in both lentic and lotic habitats. Hence, we were able to consider habitat and sex simultaneously across an entire family. We were also able to document whether variation among species was attributable to location within the water column. We discuss these findings below.

Lentic versus Lotic Habitats

Body size differed as a function of habitat. Individuals from lentic habitats had a smaller body size than lotic habitats. This is consistent with the pattern in standard length observed between lake and stream populations of *Fundulus notatus* (Welsh *et al.* 2013). However, this pattern is not true for all fish species of fish (Moser *et al*. 2012). Water velocity can impact body size through growth, although different species respond differently (Davison 1997, Grünbaum *et al.* 2008, Fischer-Rousseau *et al.* 2010).

Concerning body shape, species-specific variation existed in all aspects of shape (dorsal fin position, body depth, and head position). Generally, individuals in lotic habitats had a more centrally located dorsal fin, greater body depth, and more centrally located mouth and eye. A dorsal fin further from the center of gravity (which in these species is close to the middle of the body) serves to act as a stabilizer and aid in sustained swimming (Aleev 1969, Rincón *et al.* 2007). Thus, this position would assist individuals living in lotic habitats to deal with the challenges of constant water flow, such as maintaining themselves around favorable microhabitats (e.g. breeding substrate, food resources, etc.). The results of body depth is in opposition to Langerhans (2008), who predicted that individuals in habitats with higher water velocities should have shallower bodies in order to reduce energy expenditure due to drag (i.e. they are more "streamlined"). However, this prediction does not hold in other species where it has been explored (Pakkasmaa and Piironen 2001, McGuigan *et al.* 2003, Neves and Monteiro 2003, Krabbenhoft *et al.* 2009). It could be that the increased body depth allows for great muscle mass, thus offsetting any cost to drag that these organisms face, as all aspects of the body, including the caudal peduncle, got deeper (and the caudal peduncle is the site of a lot of muscle in fish). The reason for the difference in mouth and eye is not clear.

Effects Due to Sex

Sexual dimorphism in dorsal fin position was present. Females had dorsal fins that were located more posteriorly than males. This may reflect differences in the role of the dorsal fin during courtship and reproduction in these species. During courtship, males in many of these species engage in actions such as circling around the female, both in the context of a mating display and as a way of directing her to a male's territory or suitable spawning substrate, whereas females do not engage in such behaviors (Foster 1967). This likely requires a lot more maneuverability, which would be better aided by a more centrally located dorsal fin (Aleev 1969). Similarly, the males of some species exhibit territorial behavior during the breeding season and these actions likely require more maneuverability, as males will engage in sigmoidal displays in the presence of other males (Foster 1967). This may also at least partly explain the pattern in sexual dimorphism in body depth. Sex differences in body depth were mediated by the species, but most species showing a difference had females with deeper bodies than males. Trade-offs between body depth and swimming performance exist, such that deeper bodies result in greater drag and, so, should be more favorable for short bursts of swimming (Webb 1984a,b, Blake 2004). Thus, if males engage in more swimming than females (perhaps due to courtship and male-male competition activities mentioned previously) then this could explain this pattern.

Position in the Water Column

The effects of lentic/lotic habitats could not be analyzed in a phylogenetic framework because many species had populations in both habitats. This was not the case for position in the water column. Indeed, dorsal fin position (PC1) showed a phylogenetic signal due to the fact that species in the starhead topminnow clade (*F. blairae – F. olivaceous*) were so similar. Despite the phylogenetic signal, there were still strong effects of water column position on PC1. Phylogenetic post-hoc tests to consider this non-independence revealed that species found at the surface of the water had a dorsal fin located closer to the middle of the body than those species found deeper (beneath the surface). The dorsal fin aids in stabilization and maneuverability (Drucker and Lauder 2001, Standen and Lauder 2007, Chadwell and Ashley-Ross 2012) and a dorsal fin closer to the middle of the body is likely to be more effective for stability and sustained swimming (Aleev 1969, Rincón *et al*. 2007). The shift documented here- a more centrally located dorsal fin beneath the surface- may reflect differences in water velocity, as it is often the water somewhere beneath the surface that has the fastest flow (Westenbroek 2006). Thus, better stability and sustained swimming ability would be more beneficial for species facing higher water velocities.

For body size, there was a non-phylogenetic trend toward species in the deepest part of the water column being larger than those from either the surface or middle depth. This reflects a general pattern among fish where larger individuals/species are generally found deeper in the water, for many potential reasons (more/diverse prey, thermal tolerances, etc).

Why do only some species respond?

As mentioned above, many of the interactions with sex and habitat were mediated by the interaction with species. While there is often a general pattern, there are still many species that either show no difference or, less commonly, are opposite to the overall pattern. Determining why these lentic/lotic patterns appear in some species but not others requires knowing whether these differences are due to genetic differentiation, phenotypic plasticity, or some combination of the two. If the patterns are largely genetic in nature, then our results suggests that (1) something prevents local adaptation to lentic/lotic habitats or (2) there are multiple ways to deal with the demands of lentic/lotic environments, or (3) there are confounding environmental variables that obscure patterns attributable to lentic/lotic environments.

For example, high levels of gene flow between habitat types can prevent local adaptation as can a lack of genetic variation. Alternatively, animals may rely on behavioral traits such as choosing areas of low flow in lotic habitats thus allowing themselves to live in an effectively lentic habitat even when occurring in flowing water. Another possibility is that there may be genuine confounding elements of the environment. For instance, water velocity and predation may act in opposing manners. An increase in body depth, as observed here, might be a way of overcoming gape-limited predators (Brönmark and Miner 1992). Indeed, the majority of predatory freshwater fish species are gape-limited (Zaret 1980). However, if a deeper body produces greater drag, then fish in high water velocity sites may simultaneously experience selection for reducing drag (shallower body depth) and overcoming gape-limited predators (deeper body depth).

Another possibility is that differences in body shape/size reflect phenotypic plasticity where growth patterns differ based on whether individuals develop in lentic or lotic habitats (Grünbaum *et al*. 2007, Fischer-Rousseau *et al*. 2010). If phenotypic plasticity is largely responsible for the patterns in lentic/lotic morphology within species, then the question arises as to why some species are plastic and others are not. There have been recent calls for studies examining the roles of genetic and environmental variation in generating patterns in body size/shape across populations and species (Langerhans 2008). Future work exploring these possibilities would enhance our understanding of the underlying genetic architecture that drives sexual dimorphism and habitat-based population divergence in body size/shape within and among species.

References

Aleev Y. G. 1969. Functional and gross anatomy in fish. *Israel Program for Scientific Translations* (available from U.S. Dep. Commer, Nat. Tech. Inf. Serv., Springfield, Va., as TT67-51391).

Bernardi G. C., M. Fernandez-Delgado, Gomez-Chiarri, and D. A. Powers. 1995. Origin of a

Spanish population of *Fundulus heteroclitus* inferred by cytochrome-b sequence analysis. *Journal of Fish Biology* **47:** 737–740.

Blake R. W. 2004. Fish functional design and swimming performance. *Journal of Fish Biology* **65**: 1193–1222.

Bookstein F. L. 1997. Landmark methods for forms without landmarks: morphometrics of group differences in outline shape. *Medical Image Analysis* **1:** 225-243.

Brinsmead J. and M. G. Fox. 2002. Morphological variation between lake- and stream-dwelling rock bass and pumpkinseed populations. *Journal of Fish Biology* **61:** 1619–1638.

Brönmark C. and J. G. Miner. 1992. Predator-induced phenotypical change in body morphology in Crucian Carp. *Science* **258:** 1348-1350.

Carlson R. L. and G. V. Lauder. 2011. Escaping the flow: boundary layer use by the darter *Etheostoma tetrazonum* (Percidae) during benthic station holding. *Journal of Experimental Biology* **214:** 1181-1193.

Chadwell B. A. and M. A. Ashley-Ross. 2012. Musculoskeletal morphology and regionalization within the dorsal and anal fins of Bluegill Sunfish (*Lepomis macrochirus*). *Journal of Morphology* **273:** 405-422.

Charlesworth B. 1980. *Evolution in age-structured populations*. Cambridge: Cambridge Press.

Conover D. O. and S. B. Munch. 2002. Sustaining fisheries yields over evolutionary time scales. *Science* **297:** 94-96.

Davison W. 1997. The effects of exercise training on teleost fish, a review of recent literature. *Comparative Biochemistryand Physiology A: Molecular and Integrative Physiology* **117A:** 67–75.

Domenici P. and R. Blake. 1997. The kinematics and performance of fish fast-start swimming. *Journal of Experimental Biology* **200:** 1165-1178.

Drucker E. G. and G. V. Lauder. 2001. Locomotor function of the dorsal fin in teleost fishes: experimental analysis of wake forces in sunfish. *Journal of Experimental Biology* **204:** 2943-2958.

Eklov P. and R. Svanback. 2006. Predation risk influences adaptive morphological variation in fish populations. *American Naturalist* **167:** 440-452.

Eschmeyer W. N. (ed.) Catalog of Fishes electronic version (26 August 2010). http://research.calacademy.org/ichthyology/catalog/fishcatmain.asp

Felley J. D. 1984. Multivariate identification of morphological-environmental relationships with the Cyprinidae (Pisces). *Copeia* **1984:** 442-455.

Fischer-Rousseau L., K. P. Chu, and R. Cloutier. 2010. Developmental plasticity in fish exposed to a water velocity gradient: a complex response. *Journal of Experimental Zoology B–Molecular and Developmental Evolution* **314B:** 67–85.

Foster N. R. 1967. Comparative studies on the biology of killifishes (Pisces, Cyprinodontidae). PhD Dissertation, Cornell University.

Foster S. A. 1992. The phenotypic integration of life history and morphology- an example from 3-spined stickleback, *Gasterosteus aculeatus* L. *Journal of Fish Biology* **41** (Supplement B)**:** 21-35.

Garland T. Jr., A. W. Dickerman, C. M. Janis, and J. A. Jones. 1993. Phylogenetic analysis of covariance by computer simulation. *Systematic Biology* **42:** 265-292.

Georga I. and G. Koumoundouros. 2010. Thermally induced plasticity of body shape in adult Zebrafish *Danio rerio* (Hamilton, 1822). *Journal of Morphology* **271:** 1319-1327.

Gisbert E. and M. A. Lopez. 2007. First record of a population of the exotic mummichog *Fundulus heteroclitus* (L., 1766) in the Mediterranean Sea basin (Ebro River delta). *Journal of Fish Biology* **71:** 1220–1224.

Grünbaum T., R. Cloutier, P. M. Mabee, and N. R. Le Francois. 2007. Early developmental plasticity and integrative responses in arctic charr (*Salvelinus alpinus*): effects of water velocity on body size and shape. *Journal of Experimental Zoology* **308B:** 396–408.

Guill J. M., D. C. Heins, and C. S. Hood. 2003. The effects of phylogeny on interspecific body shape variation in Darters (Pisces: Percidae). *Systematic Biology* **52:** 488-500.

Hendry A. P., M. L. Kelly, M. T. Kinnison, and D. N. Reznick 2006. Parallel evolution of the sexes? Effects of predation and habitat features on the size and shape of wild guppies. *Journal of Evolutionary Biology* **19:** 741-754.

Kawecki T. J. and D. Ebert. 2004. Conceptual issues in local adaptation. *Ecology Letters* **7:** 1225-1241.

Keeley E. R., E. A. Parkinson, and E. B. Taylor. 2005. Ecotypic differentiation of native rainbow trout (*Oncorhynchus mykiss*) populations from British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences* **62:** 1523-1539.

Kitano J., S. Mori, and C. L. Peichel. 2012. Reduction of sexual dimorphism in stream-resident forms of three-spined stickleback *Gasterosteus aculeatus*. *Journal of Fish Biology* **80:** 131-146.

Kneib R. T. 1978. Habitat, diet, reproduction, and growth of the spotfin killifish, *Fundulus luciae*, from a North Carolina salt marsh. *Copeia* **1978:** 164-168.

Krabbenhoft T. J., M. L. Collyer, and J. M. Quattro. 2009. Differing evolutionary patterns underlie convergence on elongate morphology in endemic fishes of Lake Waccamaw, North Carolina. *Biological Journal of the Linnean Society* **98:** 636-645.

Langerhans R. B. 2008. of phenotypic differentiation across flow regimes in fishes. *Integrative and Comparative Biology* **48:** 750-768.

Langerhans R. B. and A. M. Makowicz. 2009. Shared and unique features of morphological differentiation between predator regimes in *Gambusia caymanensis*. *Journal of Evolutionary Biology* **22:** 2231-2242.

Larimore R. W. and Bayley P. B. 1996. The fishes of Champaign County, Illinois, during a century of alterations of a prairie ecosystem. *Illinois Natural History Survey Bulletin* **35:** 53-183.

McGuigan K., C. E. Franklin, C. Moritz, and M. W. Blows. 2003. Adaptation of rainbow fish to lake and stream habitats. *Evolution* **57:** 104-118.

Mitteroecker P. and P. Gunz. 2009. Advances in geometric morphometrics. *Evolutionary Biology* **36:** 235-247.

Moser D., M. Roesti, and D. Berner. 2012. Repeated lake-stream divergence in stickleback life history within a central European lake basin. *PLoS ONE* **7:** e50620.

Neves F. M. and L. R. Monteiro. 2003. Body shape and size divergence among populations of *Poecilia vivipara* in coastal lagoons of south-eastern Brazil. *Journal of Fish Biology* **63:** 928-941.

Pakkasmaa S. and J. Piironen. 2001. Water velocity shapes juvenile salmonids. *Evolutionary Ecology* **14:** 721-730.

Parker G. A. 1992. The evolution of sexual size dimorphism in fish. *Journal of Fish Biology* **41 (**Supplement B)**:** 1–20.

Prunier R., K. E. Holsinger, and J. E. Carlson. 2012. The effect of historical legacy on adaptation: do closely related species respond to the environment in the same way? *Journal of Evolutionary Biology* **25:** 1636–1649.

Pyron M. 1996. Sexual size dimorphism and phylogeny in North American minnows. *Biological Journal of the Linnean Society* **57:** 327-341.

Rincón P. A., M. Bastir, and G. D. Grossman. 2007. Form and Performance: Body Shape and Prey-Capture Success in Four Drift-Feeding Minnows. *Oecologia* **152:** 345-355.

Rohlf F. J. and L. F. Marcus. 1993. A revolution in morphometrics. *Trends in Ecology and Evolution* **8:** 129-132.

Rohlf F. J. and D. E. Slice. 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Zoology* **39:** 40-59.

Ross S. T. 2001. Inland Fishes of Mississippi. Mississippi Department of Wildlife, Fisheries, and Parks, Mississippi.

Satterthwaite F. W. 1946. An approximate distribution of estimates of variance components. *Biometrics Bulletin* **2:** 110-114.

Schluter D. 2001. Ecology and the origin of species. *Trends in Ecology and Evolution* **16:** 372-380.

Shute P. W., D. G. Lindquist, and J. R. Shute. 1983. Breeding behavior and early life history of the waccamaw killifish, *Fundulus waccamensis*. *Environmental Biology of Fishes* **8:** 293-300.

Standen E. M. and G. V. Lauder. 2007. Hydrodynamic function of dorsal and anal fins in brook trout (*Salvelinus fontinalis*). *The Journal of Experimental Biology* **210:** 325-339.

Stearns S. C. 1992. *The evolution of life histories*. Oxford Univ. Press, Oxford, U.K.

Webb P. W. 1984a. Body form, locomotion and foraging in aquatic vertebrates. *American Zoologist* **24:** 107-120.

Webb P. W. 1984b. Form and function in fish swimming. *Scientific American* **251:** 58-68.

Welsh D. P., M. Zhou, S. M. Mussmann, L. G. Fields, C. L. Thomas, S. P. Pearish, S. L. Kilburn, J. L. Parker, L. R. Stein, J. A. Bartlett, C. R. Bertram, T. J. Bland, K. L. Laskowski, B. C. Mommer, X. Zhuang, R. C. Fuller. 2013. The effects of age, sex, and habitat on body size and shape of the blackstripe topminnow, *Fundulus notatus* (Cyprinodontiformes: Fundulidae) (Rafinesque 1820). *Biological Journal of the Linnean Society* **108:** 784-789.

Westenbroek S. M. 2006. Estimates of shear stress and measurements of water levels in the lower Fox River near Green Bay, Wisconsin. USGS *Scientific Investigations Report* 2006-5226.

Whitehead A. 2010. The evolutionary radiation of diverse osmotolerant physiologies in killifish (*Fundulus* sp.). *Evolution* **64:** 2070-2085.

Wikramanayake E. D. 1990. Ecomorphology and biogeography of a tropical stream fish assemblage: evolution of assemblage structure. *Ecology* **71:** 1756-1764.

Wiley E. O. 1986. A study of the evolutionary relationships of *Fundulus* topminnows (Teleostei: Fundulidae). *American Zoologist* **26:** 121-130.

Williams G. C. 1966. Adaptation and Natural Selection. Princeton University Press, Princeton.

Wund M. A., J. A. Baker, B. Clancy, J. L. Golub, and S. A. Foster. 2008. A test of the "flexible stem" model of evolution: ancestral plasticity, genetic accommodation, and morphological divergence in the Threespine Stickleback radiation. *The American Naturalist* **172:** 449-462.

Young M. J., Simmons L. W., Evans J. P. 2011. Predation is associated with variation in colour pattern, but not body shape or colour reflectance, in a rainbowfish (*Melanotaenia australis*). *Journal of Animal Ecology* **80:** 183-191.

Zaret T. M. 1980. *Predation and freshwater communities*. Yale University Press, New Haven, Connecticut.

Zelditch M. L., D. L. Swiderski, and H. D. Sheets. 2012. *Geometric morphometrics for Biologists: A Primer*. 2nd edition. Academic Press, Massachusetts.

Figures

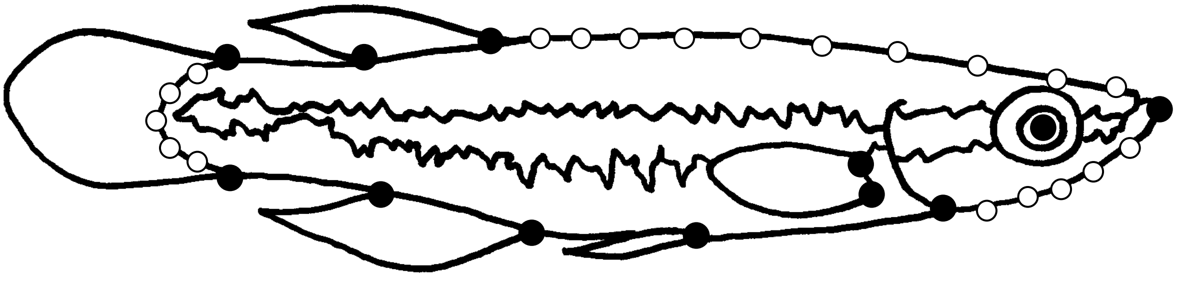

Figure 2.1. Landmarks used for geometric morphometric analysis. Black dots are Type I landmarks (12 in total) and white dots are semi-landmarks (20 in total).

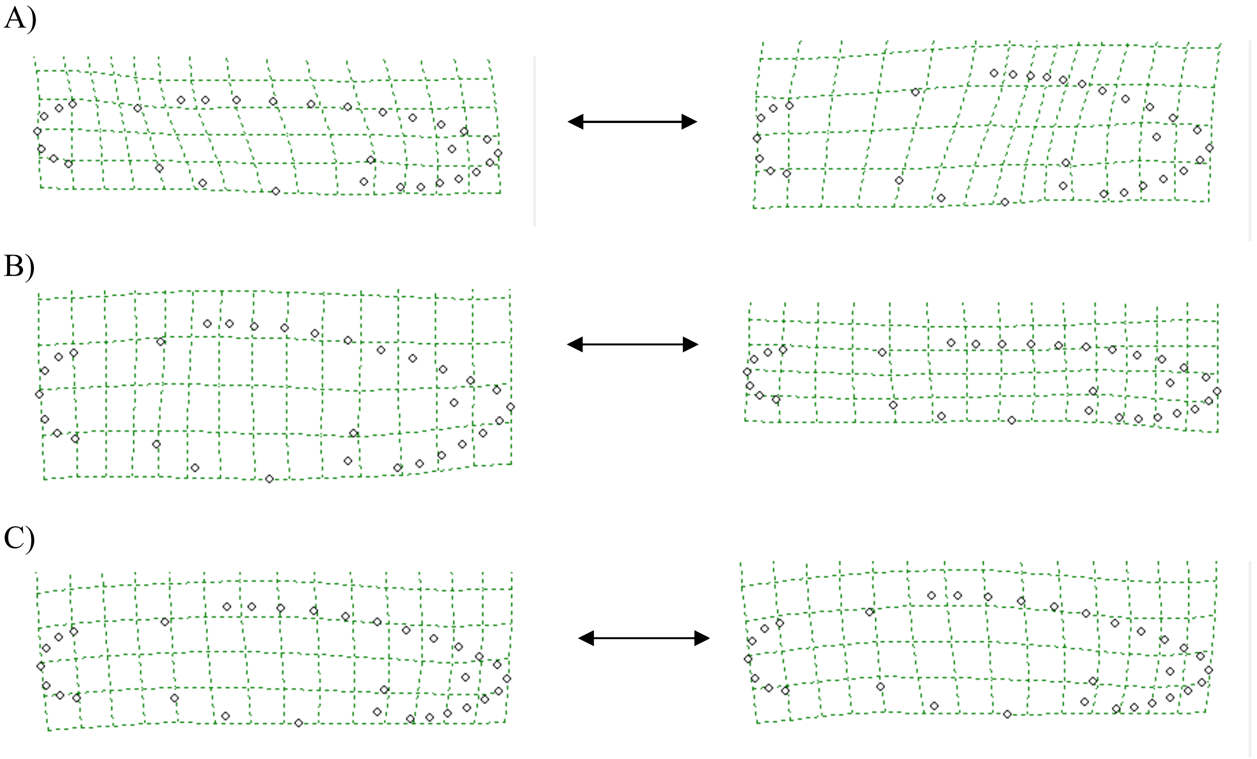

Figure 2.2. Thin plate spline representations of body shape changes along A) PC1, B) PC2, and C) PC4. Representations on the left side of the arrow indicate positive PC scores and those on the right side indicate negative PC scores.

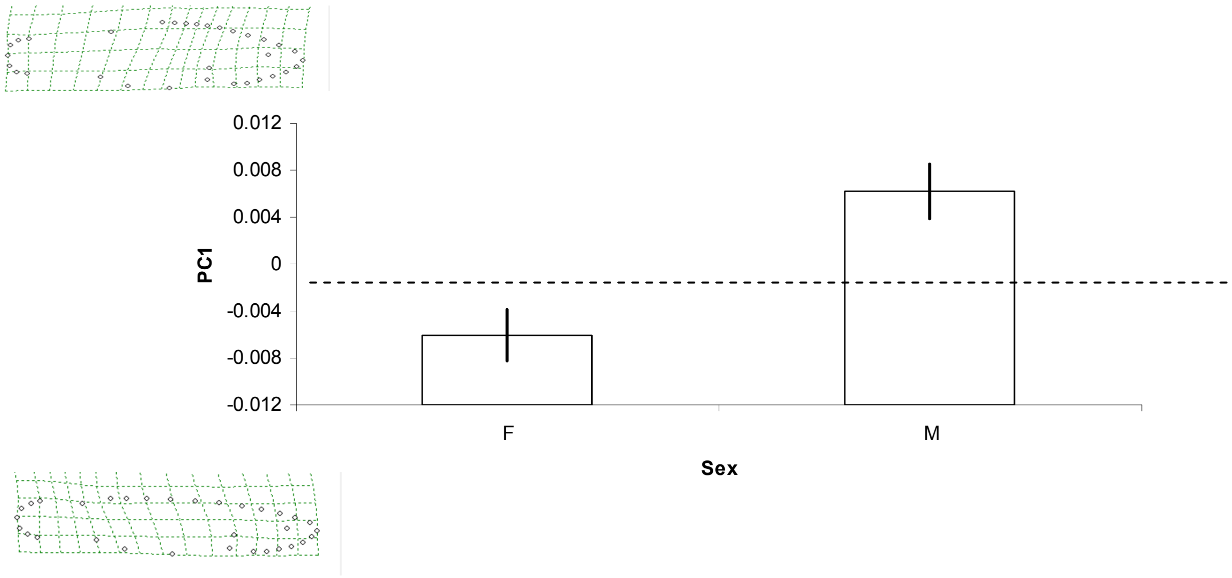

Figure 2.3. PC1 (dorsal fin position) as a function of sex. Error bars are ± 1 standard error.

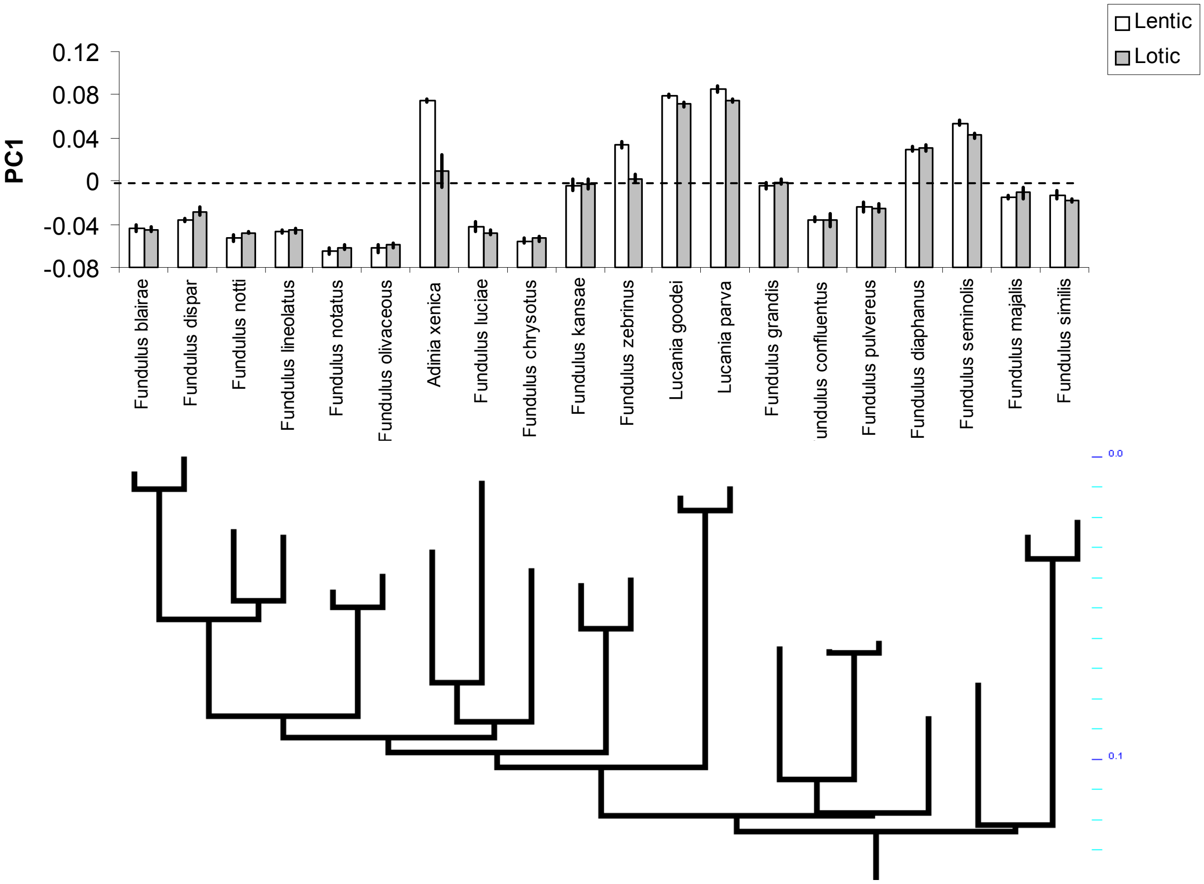

Figure 2.4. PC1 (dorsal fin position) as a function of the interaction between species and habitat. Error bars are ± 1 standard error. Please note that 1 species, *Leptolucania ommata*, is not shown because it's location on the tree is not known.

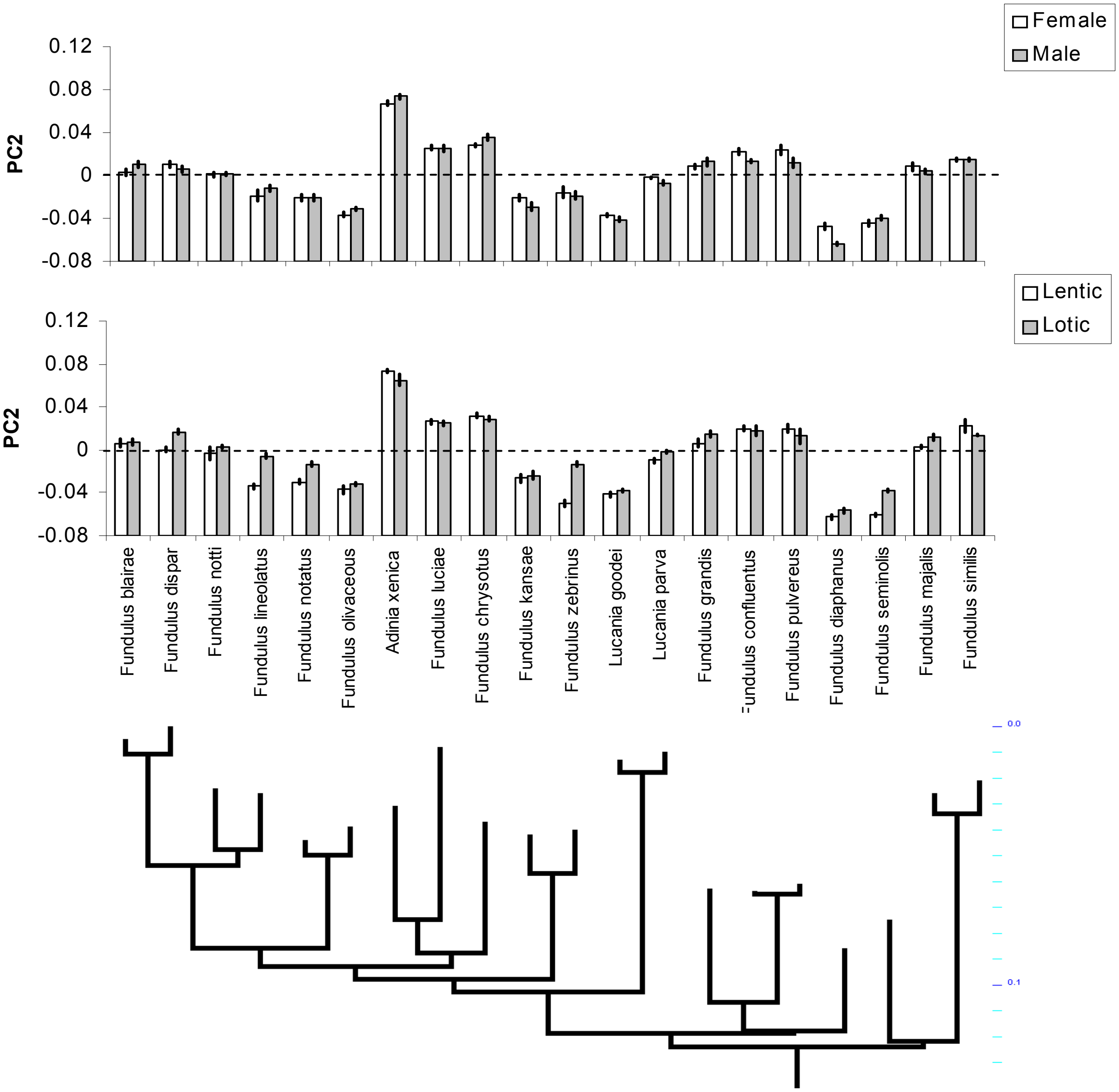

Figure 2.5. PC2 (body depth) as a function of the interaction between species and sex (top graph) and species and habitat (bottom graph). Error bars are ± 1 standard error. Please note that 1 species, *Leptolucania ommata*, is not shown because it's location on the tree is not known.

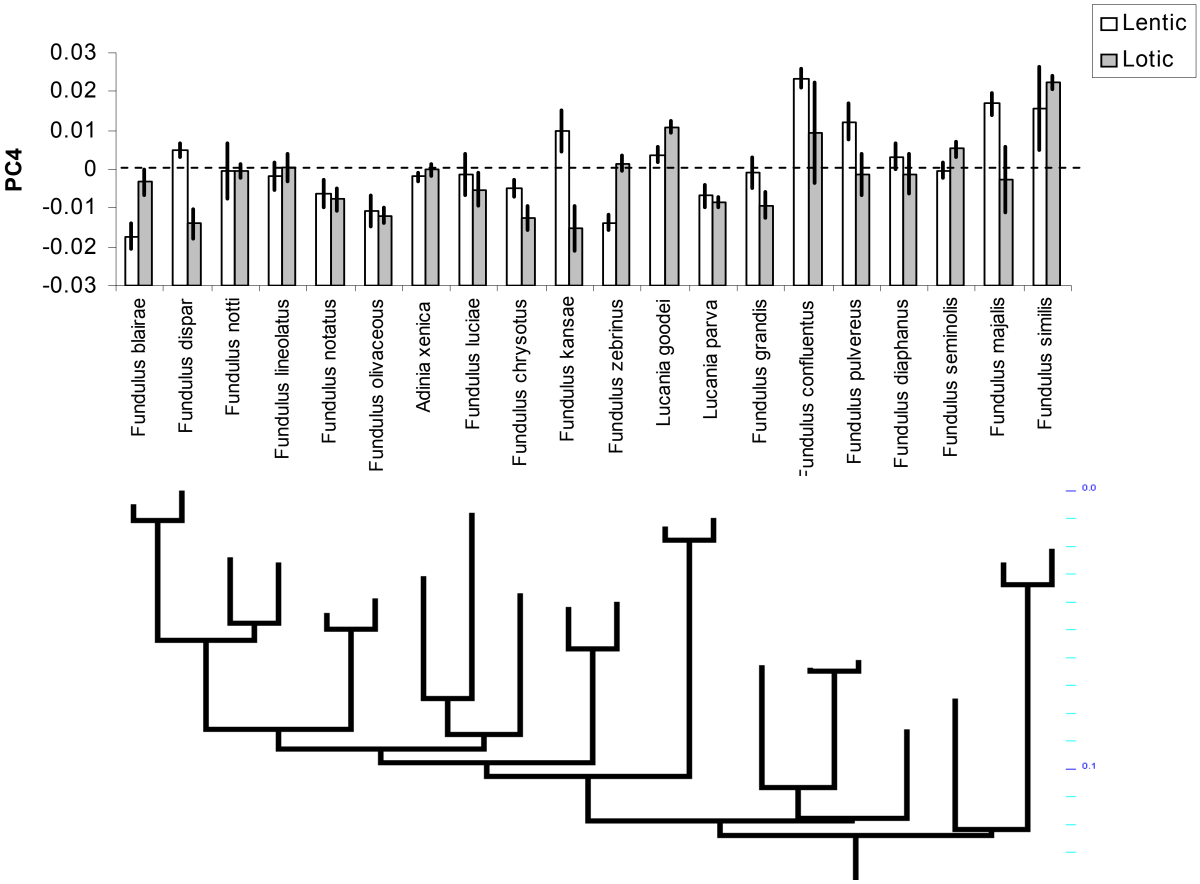

Figure 2.6. Head position as a function of the interaction between habitat and species. Error bars are ± 1 standard error. Please note that 1 species, *Leptolucania ommata*, is not shown because it's location on the tree is not known.

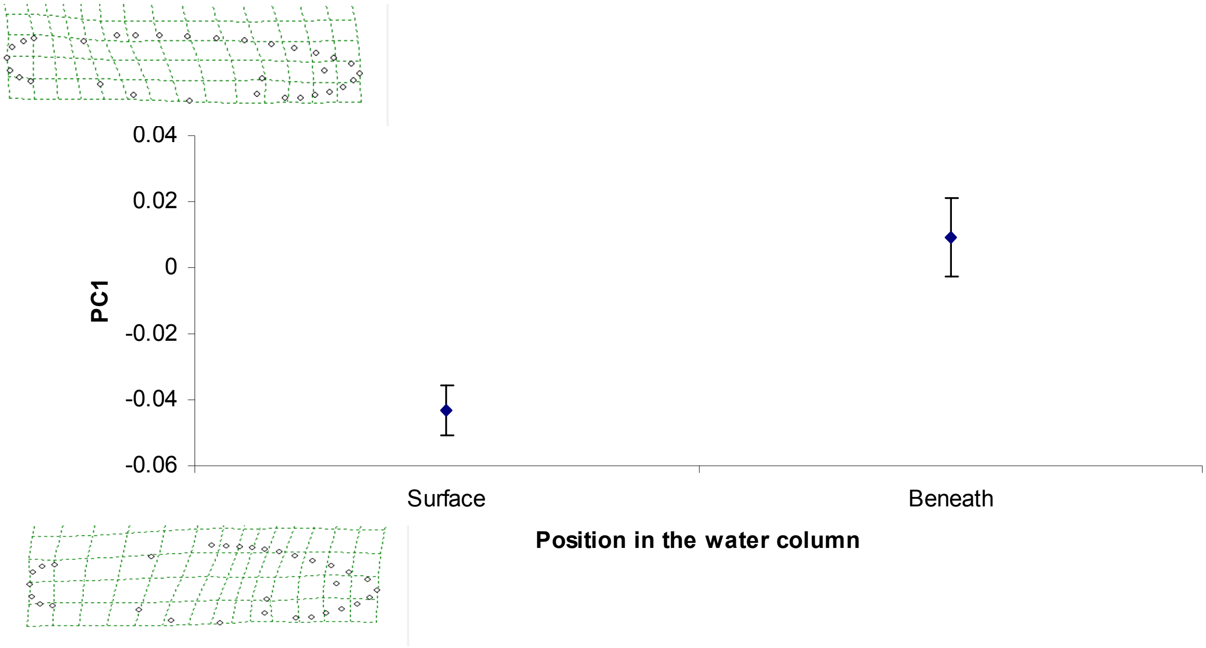

Figure 2.7. Dorsal fin position as a function of position in the water column. Error bars are ± 1 standard error.

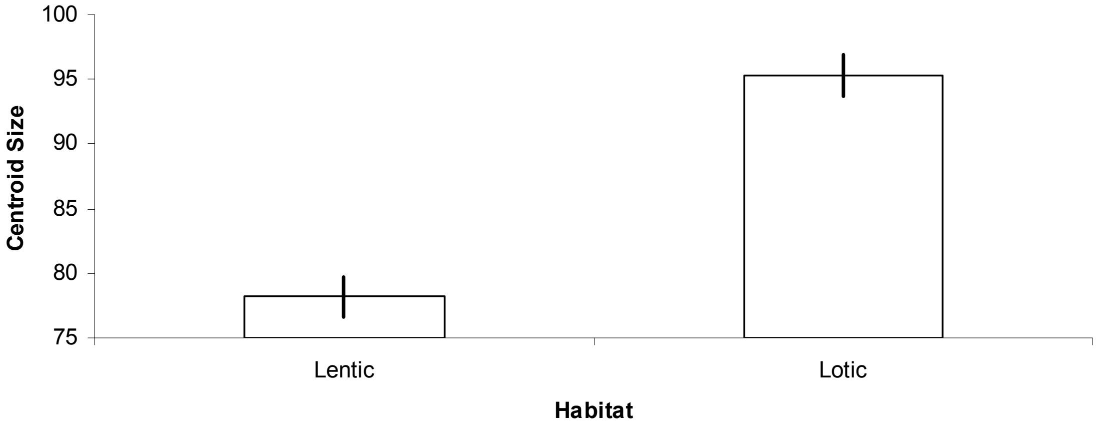

Figure 2.8. Body size as a function of habitat. Error bars are ± 1 standard error.

Tables

Table 2.1. Species, sample sizes, and classification of position in the water column used in the analyses. Position in the water column was divided into two mutually exclusive categories (see Methods for further details). Asterisks identify those 21 species with both sexes in both habitats.

Species	**Habitat**		**Sex**		**Position in Water Column**
	Lentic	**Lotic**	**Female**	**Male**	
*Adinia xenica**	79	24	38	65	surface
Fundulus bermudae	20	0	8	12	surface
Fundulus bifax	0	7	5	2	N/A
*Fundulus blairae**	16	27	20	23	N/A
Fundulus catenatus	0	27	8	19	beneath
*Fundulus chrysotus**	26	19	30	15	surface
Fundulus cingulatus	0	27	18	9	beneath
*Fundulus confluentus**	38	10	29	19	N/A
*Fundulus diaphanus**	20	11	7	24	beneath
*Fundulus dispar**	29	23	24	28	surface
Fundulus escambiae	2	4	3	3	surface
Fundulus euryzonus	0	21	10	12	surface
*Fundulus grandis**	18	21	20	19	N/A
Fundulus grandissimus	0	2	2	0	N/A
Fundulus heteroclitus	0	19	10	9	N/A
Fundulus jenkinsi	10	3	9	4	N/A
Fundulus julisia	0	4	2	2	N/A

Table 2.1 (cont.)

*Fundulus kansae**	20	8	11	17	beneath
Fundulus lima	0	2	1	1	beneath
*Fundulus lineolatus**	14	28	19	23	surface
*Fundulus luciae**	12	32	23	21	beneath
*Fundulus majalis**	18	6	8	16	N/A
*Fundulus notatus**	24	27	24	27	surface
*Fundulus notti**	10	51	31	30	surface
*Fundulus olivaceous**	12	38	20	30	surface
*Fundulus pulvereus**	17	10	12	15	N/A
Fundulus rathbuni	0	26	13	13	N/A
Fundulus relictus	57	0	18	39	N/A
Fundulus rubrifrons	0	21	5	16	N/A
Fundulus sciadicus	5	22	8	19	surface
*Fundulus seminolis**	15	74	44	45	beneath
*Fundulus similis**	5	62	53	15	beneath
Fundulus stellifer	0	20	12	8	beneath
Fundulus waccamensis	12	0	8	4	N/A
*Fundulus zebrinus**	4	30	14	20	N/A
*Leptolucania ommata**	24	19	24	19	N/A
*Lucania goodei**	23	47	36	34	beneath
Lucania interioris	22	0	12	10	N/A
*Lucania parva**	27	78	67	38	beneath

Table 2.2. ANOVA results for all 39 species or just the 21 species in which there were samples of both males and females from both lentic and lotic habitats for A) PC1, B) PC2, C) PC4, and D) Centroid Size. Significant factors are in **bold**.

A)

	39 Species			21 Species		
Factor	MS	F	p	MS	F	p
Species	**0.0598**	**$F_{38,1309}$ = 297.30**	**<0.0001**	**0.0963**	**$F_{20,1006}$ = 412.21**	**<0.0001**
Sex	**0.0288**	**$F_{1,57.06}$ = 72.56**	**<0.0001**	**0.0207**	**$F_{1,23.218}$ = 35.09**	**<0.0001**
Habitat	0.0053	$F_{1,24.343}$ = 2.31	0.1416	0.0057	$F_{1,20.545}$ = 1.80	0.1941
Sex*Habitat	0.0002	$F_{1,24.173}$ = 0.45	0.5084	0.0002	$F_{1,24.804}$ = 0.51	0.4822
Sex*Species	0.0005	$F_{37,20.297}$ = 1.16	0.3643	0.0007	$F_{20,20}$ = 1.47	0.1989
Habitat*Species	**0.0033**	**$F_{23,21.32}$ = 7.58**	**<0.0001**	**0.0038**	**$F_{20,20}$ = 8.40**	**<0.0001**
Sex*Habitat*Species	**0.0005**	**$F_{20,1309}$ = 2.24**	**0.0013**	**0.0005**	**$F_{20,1006}$ = 1.95**	**0.0076**
Error	0.0002			0.0002		

B)

	39 Species			21 Species		
Factor	MS	F	p	MS	F	p
Species	**0.0266**	**$F_{38,1309}$ = 177.60**	**<0.0001**	**0.0378**	**$F_{20,1006}$ = 270.26**	**<0.0001**
Sex	0.000006	$F_{1,61.843}$ = 0.02	0.8834	0.0002	$F_{1,23.292}$ = 0.54	0.4693
Habitat	**0.0048**	**$F_{1,25.846}$ = 5.72**	**0.0244**	**0.0053**	**$F_{1,20.955}$ = 4.80**	**0.0398**
Sex*Habitat	0.00008	$F_{1,30.957}$ = 0.55	0.4644	0.0001	$F_{1,30.336}$ = 0.57	0.4546
Sex*Species	**0.0003**	**$F_{37,20.987}$ = 2.33**	**0.0213**	**0.0004**	**$F_{20,20}$ = 2.93**	**0.0101**
Habitat*Species	**0.0012**	**$F_{23,23.321}$ = 8.54**	**<0.0001**	**0.0013**	**$F_{20,20}$ = 9.84**	**<0.0001**
Sex*Habitat*Species	0.0001	$F_{20,1309}$ = 0.91	0.5713	0.0001	$F_{20,1006}$ = 0.95	0.5181
Error	0.0001			0.0001		

Table 2.2 (cont.)

C)

	39 Species			21 Species		
Factor	MS	F	p	MS	F	p
Species	**0.0019**	**$F_{38,1309}$ = 8.12**	**<0.0001**	**0.0027**	**$F_{20,1006}$ = 11.49**	**<0.0001**
Sex	0.0001	$F_{1,66.156}$ = 0.30	0.5873	0.0003	$F_{1,24.192}$ = 0.66	0.4243
Habitat	0.0013	$F_{1,29.306}$ = 1.96	0.1718	0.0014	$F_{1,22.398}$ = 1.93	0.1788
Sex*Habitat	0.000002	$F_{1,25.49}$ = 0.01	0.9391	0.000006	$F_{1,25.739}$ = 0.02	0.8957
Sex*Species	0.0004	$F_{37,20.516}$ = 1.08	0.4384	0.0005	$F_{20,20}$ = 1.35	0.2556
Habitat*Species	**0.0009**	**$F_{23,21.72}$ = 2.17**	**0.0373**	**0.0008**	**$F_{20,20}$ = 2.31**	**0.0341**
Sex*Habitat*Species	**0.0004**	**$F_{20,1309}$ = 1.73**	**0.0237**	**0.0004**	**$F_{20,1006}$ = 1.64**	**0.0368**
Error	0.0002			0.0002		

D)

	39 Species			21 Species		
Factor	MS	F	p	MS	F	p
Species	**20626.02**	**$F_{38,1309}$ = 36.49**	**<0.0001**	**24946.39**	**$F_{20,1006}$ = 45.54**	**<0.0001**
Sex	671.41	$F_{1,61.094}$ = 0.69	0.4107	1872.24	$F_{1,24.459}$ = 1.81	0.1912
Habitat	**7056.86**	**$F_{1,32.339}$ = 6.12**	**0.0188**	**6862.31**	**$F_{1,23.243}$ = 5.01**	**0.0351**
Sex*Habitat	1207.80	$F_{1,23.808}$ = 0.98	0.3327	1215.44	$F_{1,23.675}$ = 0.99	0.3295
Sex*Species	1238.08	$F_{37,20.364}$ = 0.91	0.6146	1144.28	$F_{20,20}$ = 0.83	0.6583
Habitat*Species	1431.11	$F_{23,21.207}$ = 1.08	0.4344	1552.96	$F_{20,20}$ = 1.13	0.3951
Sex*Habitat*Species	**1385.11**	**$F_{20,1309}$ = 2.45**	**0.0004**	**1376.59**	**$F_{20,1006}$ = 2.51**	**0.0003**
Error	565.32			547.74		

Table 2.3. P-value for standard and phylogenetic ANOVA based on location in the water column (surface or beneath). Significant values ($p < 0.05$) are highlighted in **bold**.

Factor	**Test statistic**	**Standard ANOVA**	**Phylogenetic ANOVA**
PC1	$F_{1,20} = 12.94$	**p = 0.0018**	**p = 0.0030**
PC2	$F_{1,20} = 0.42$	p = 0.5228	p = 0.5954
PC4	$F_{1,20} = 0.59$	p = 0.4527	p = 0.5215
Centroid Size	$F_{1,20} = 1.39$	p = 0.2516	p = 0.3387

Supplemental Table 2.1. F-values from analyses of variance for each species using adult specimens from 36 species for A) PC1, B) PC2, C) PC4, and D) Centroid Size. *Fundulus grandissimus* and *F. lima* are not included because no comparisons could be made (only 2 samples of each species). N/A is for those species either not in both habitats or did not have samples of both sexes from a habitat. Significant effects ($p < 0.05$) are highlighted in **bold**.

A)

Species	Sex	Habitat	Sex*Habitat
Adinia xenica	**$F_{1, 99} = 9.71$** **$p = 0.0024$**	**$F_{1, 99} = 56.15$** **$p < 0.0001$**	$F_{1, 99} = 0.94$ $p = 0.3346$
Fundulus bermudae	**$F_{1, 18} = 20.03$** **$p = 0.0003$**	N/A	N/A
Fundulus bifax	$F_{1,5} = 0.01$ $p = 0.9157$	N/A	N/A
Fundulus blairae	**$F_{1, 39} = 28.19$** **$p < 0.0001$**	$F_{1, 39} = 1.37$ $p = 0.2487$	$F_{1, 39} = 0.15$ $p = 0.7038$
Fundulus catenatus	**$F_{1, 25} = 36.55$** **$p < 0.0001$**	N/A	N/A
Fundulus chrysotus	**$F_{1, 41} = 16.11$** **$p = 0.0002$**	$F_{1, 41} = 1.41$ $p = 0.2419$	$F_{1, 41} = 0.33$ $p = 0.5677$
Fundulus cingulatus	$F_{1, 25} = 3.94$ $p = 0.0582$	N/A	N/A
Fundulus confluentus	$F_{1, 44} = 1.90$ $p = 0.1749$	$F_{1, 44} = 2.90$ $p = 0.0959$	**$F_{1, 44} = 13.49$** **$p = 0.0006$**
Fundulus diaphanus	**$F_{1, 27} = 38.03$** **$p < 0.0001$**	$F_{1, 27} = 0.05$ $p = 0.8297$	$F_{1, 27} = 0.40$ $p = 0.5300$
Fundulus dispar	**$F_{1, 48} = 60.36$** **$p < 0.0001$**	**$F_{1, 48} = 15.50$** **$p = 0.0003$**	**$F_{1, 48} = 39.44$** **$p < 0.0001$**
Fundulus escambiae	$F_{1, 3} = 1.42$ $p = 0.3195$	$F_{1, 3} = 0.89$ $p = 0.4141$	N/A
Fundulus euryzonus	**$F_{1, 20} = 4.70$** **$p = 0.0424$**	N/A	N/A
Fundulus grandis	**$F_{1, 35} = 14.49$** **$p = 0.0005$**	$F_{1, 35} = 0.41$ $p = 0.5279$	$F_{1, 35} = 0.54$ $p = 0.4686$
Fundulus heteroclitus	**$F_{1, 17} = 16.92$** **$p = 0.0007$**	N/A	N/A
Fundulus jenkinsi	**$F_{1, 10} = 8.06$** **$p = 0.0176$**	$F_{1, 10} = 1.20$ $p = 0.2994$	N/A
Fundulus julisia	**$F_{1, 2} = 48.82$** **$p = 0.0199$**	N/A	N/A
Fundulus kansae	**$F_{1, 24} = 34.12$** **$p < 0.0001$**	$F_{1, 24} = 0.25$ $p = 0.6239$	$F_{1, 24} = 4.22$ $p = 0.0510$
Fundulus lineolatus	$F_{1, 38} = 2.28$ $p = 0.1390$	$F_{1, 38} = 0.10$ $p = 0.7502$	$F_{1, 38} = 0.02$ $p = 0.8926$

Supplemental Table 2.1 (cont.)

Fundulus luciae	**$F_{1, 40} = 8.27$** **$p = 0.0064$**	$F_{1, 40} = 3.59$ $p = 0.0653$	$F_{1, 40} = 0.15$ $p = 0.6992$
Fundulus majalis	$F_{1, 20} = 0.00$ $p = 0.9949$	$F_{1, 20} = 0.98$ $p = 0.3331$	$F_{1, 20} = 0.34$ $p = 0.5636$
Fundulus notatus	**$F_{1, 47} = 11.75$** **$p = 0.0013$**	$F_{1, 47} = 1.25$ $p = 0.2698$	$F_{1, 47} = 3.84$ $p = 0.0559$
Fundulus notti	**$F_{1, 57} = 4.09$** **$p = 0.0477$**	$F_{1, 57} = 1.49$ $p = 0.2280$	$F_{1, 57} = 0.26$ $p = 0.6128$
Fundulus olivaceous	$F_{1, 46} = 0.12$ $p = 0.7347$	$F_{1, 46} = 0.52$ $p = 0.4727$	$F_{1, 46} = 0.03$ $p = 0.8567$
Fundulus pulvereus	**$F_{1, 23} = 5.65$** **$p = 0.0262$**	$F_{1, 23} = 0.11$ $p = 0.7396$	**$F_{1, 23} = 4.83$** **$p = 0.0384$**
Fundulus rathbuni	**$F_{1, 24} = 48.01$** **$p < 0.0001$**	N/A	N/A
Fundulus relictus	**$F_{1, 55} = 50.94$** **$p < 0.0001$**	N/A	N/A
Fundulus rubrifrons	$F_{1, 19} = 2.80$ $p = 0.1106$	N/A	N/A
Fundulus sciadicus	**$F_{1, 24} = 39.83$** **$p < 0.0001$**	**$F_{1, 24} = 4.46$** **$p = 0.0453$**	N/A
Fundulus seminolis	**$F_{1, 85} = 10.31$** **$p = 0.0019$**	**$F_{1, 85} = 4.03$** **$p = 0.0477$**	$F_{1, 85} = 0.26$ $p = 0.6091$
Fundulus similis	**$F_{1, 64} = 6.58$** **$p = 0.0127$**	$F_{1, 64} = 0.48$ $p = 0.4917$	$F_{1, 64} = 0.19$ $p = 0.6662$
Fundulus stellifer	**$F_{1, 18} = 20.95$** **$p = 0.0002$**	N/A	N/A
Fundulus waccamensis	**$F_{1, 10} = 14.02$** **$p = 0.0038$**	N/A	N/A
Fundulus zebrinus	**$F_{1, 30} = 19.53$** **$p = 0.0001$**	**$F_{1, 30} = 63.59$** **$p < 0.0001$**	**$F_{1, 30} = 9.07$** **$p = 0.0052$**
Leptolucania ommata	$F_{1, 39} = 1.83$ $p = 0.1836$	**$F_{1, 39} = 4.15$** **$p = 0.0484$**	$F_{1, 39} = 1.28$ $p = 0.2654$
Lucania goodei	**$F_{1, 66} = 4.05$** **$p = 0.0482$**	**$F_{1, 66} = 9.81$** **$p = 0.0026$**	$F_{1, 66} = 3.30$ $p = 0.0736$
Lucania interioris	**$F_{1, 20} = 7.69$** **$p = 0.0117$**	N/A	N/A
Lucania parva	**$F_{1, 101} = 81.22$** **$p < 0.0001$**	**$F_{1, 101} = 22.00$** **$p < 0.0001$**	$F_{1, 101} = 2.16$ $p = 0.1443$

Supplemental Table 2.1 (cont.)
B)

Species	**Sex**	**Habitat**	**Sex*Habitat**
Adinia xenica	**$F_{1, 99} = 6.94$** **$p = 0.0098$**	**$F_{1, 99} = 8.38$** **$p = 0.0047$**	**$F_{1, 99} = 4.17$** **$p = 0.0437$**
Fundulus bermudae	$F_{1, 18} = 0.29$ $p = 0.5958$	N/A	N/A
Fundulus bifax	$F_{1,5} = 0.55$ $p = 0.4929$	N/A	N/A
Fundulus blairae	$F_{1, 39} = 1.61$ $p = 0.2117$	$F_{1, 39} = 0.00$ $p = 0.9763$	$F_{1, 39} = 0.36$ $p = 0.5518$
Fundulus catenatus	$F_{1, 25} = 3.21$ $p = 0.0852$	N/A	N/A
Fundulus chrysotus	$F_{1, 41} = 4.03$ $p = 0.0514$	$F_{1, 41} = 1.92$ $p = 0.1728$	$F_{1, 41} = 0.20$ $p = 0.6547$
Fundulus cingulatus	$F_{1, 25} = 2.08$ $p = 0.1612$	N/A	N/A
Fundulus confluentus	$F_{1, 44} = 1.96$ $p = 0.1685$	$F_{1, 44} = 0.92$ $p = 0.3420$	$F_{1, 44} = 0.29$ $p = 0.5937$
Fundulus diaphanus	**$F_{1, 27} = 28.73$** **$p < 0.0001$**	$F_{1, 27} = 0.37$ $p = 0.5496$	$F_{1, 27} = 2.15$ $p = 0.1544$
Fundulus dispar	$F_{1, 48} = 1.07$ $p = 0.3068$	**$F_{1, 48} = 17.49$** **$p = 0.0001$**	$F_{1, 48} = 0.15$ $p = 0.7050$
Fundulus escambiae	$F_{1, 3} = 0.99$ $p = 0.3924$	$F_{1, 3} = 1.31$ $p = 0.3348$	N/A
Fundulus euryzonus	$F_{1, 20} = 0.07$ $p = 0.7958$	N/A	N/A
Fundulus grandis	$F_{1, 35} = 0.49$ $p = 0.4878$	$F_{1, 35} = 3.92$ $p = 0.0557$	$F_{1, 35} = 0.00$ $p = 0.9479$
Fundulus heteroclitus	$F_{1, 17} = 1.69$ $p = 0.2114$	N/A	N/A
Fundulus jenkinsi	$F_{1, 10} = 2.44$ $p = 0.1495$	**$F_{1, 10} = 16.26$** **$p = 0.0024$**	N/A
Fundulus julisia	$F_{1, 2} = 0.99$ $p = 0.4247$	N/A	N/A
Fundulus kansae	$F_{1, 24} = 1.34$ $p = 0.2589$	$F_{1, 24} = 1.23$ $p = 0.2777$	$F_{1, 24} = 1.17$ $p = 0.2896$
Fundulus lineolatus	$F_{1, 38} = 0.12$ $p = 0.7262$	**$F_{1, 38} = 40.58$** **$p < 0.0001$**	$F_{1, 38} = 0.82$ $p = 0.3714$
Fundulus luciae	$F_{1, 40} = 0.00$ $p = 0.9736$	$F_{1, 40} = 0.00$ $p = 0.9597$	$F_{1, 40} = 0.02$ $p = 0.8787$
Fundulus majalis	$F_{1, 20} = 3.85$ $p = 0.0638$	**$F_{1, 20} = 11.56$** **$p = 0.0028$**	$F_{1, 20} = 1.86$ $p = 0.1872$

Supplemental Table 2.1 (cont.)

Fundulus notatus	$F_{1, 47} = 0.04$ $p = 0.8453$	**$F_{1, 47} = 23.93$** **$p < 0.0001$**	$F_{1, 47} = 0.35$ $p = 0.5573$
Fundulus notti	$F_{1, 57} = 2.44$ $p = 0.1237$	$F_{1, 57} = 2.85$ $p = 0.0968$	$F_{1, 57} = 3.15$ $p = 0.0815$
Fundulus olivaceous	$F_{1, 46} = 3.88$ $p = 0.0548$	**$F_{1, 46} = 4.06$** **$p = 0.0498$**	$F_{1, 46} = 0.49$ $p = 0.4889$
Fundulus pulvereus	$F_{1, 23} = 3.58$ $p = 0.0713$	$F_{1, 23} = 0.17$ $p = 0.6879$	$F_{1, 23} = 0.65$ $p = 0.4277$
Fundulus rathbuni	$F_{1, 24} = 0.00$ $p = 0.9888$	N/A	N/A
Fundulus relictus	$F_{1, 55} = 0.16$ $p = 0.6923$	N/A	N/A
Fundulus rubrifons	$F_{1, 19} = 0.59$ $p = 0.4506$	N/A	N/A
Fundulus sciadicus	$F_{1, 24} = 0.61$ $p = 0.4429$	$F_{1, 24} = 0.00$ $p = 0.9621$	N/A
Fundulus seminolis	$F_{1, 85} = 1.61$ $p = 0.2086$	**$F_{1, 85} = 24.78$** **$p < 0.0001$**	$F_{1, 85} = 0.50$ $p = 0.4809$
Fundulus similis	$F_{1, 64} = 0.46$ $p = 0.4984$	$F_{1, 64} = 2.18$ $p = 0.1445$	$F_{1, 64} = 0.59$ $p = 0.4439$
Fundulus stellifer	$F_{1, 18} = 2.58$ $p = 0.1255$	N/A	N/A
Fundulus waccamensis	$F_{1, 10} = 0.00$ $p = 0.9817$	N/A	N/A
Fundulus zebrinus	$F_{1, 30} = 0.01$ $p = 0.9211$	**$F_{1, 30} = 28.40$** **$p < 0.0001$**	$F_{1, 30} = 0.89$ $p = 0.3533$
Leptolucania ommata	$F_{1, 39} = 0.02$ $p = 0.8977$	**$F_{1, 39} = 35.72$** **$p < 0.0001$**	$F_{1, 39} = 2.38$ $p = 0.1307$
Lucania goodei	**$F_{1, 66} = 5.43$** **$p = 0.0229$**	$F_{1, 66} = 2.71$ $p = 0.1042$	$F_{1, 66} = 1.51$ $p = 0.2241$
Lucania interioris	$F_{1, 20} = 0.47$ $p = 0.5015$	N/A	N/A
Lucania parva	$F_{1, 101} = 2.99$ $p = 0.0871$	**$F_{1, 101} = 7.54$** **$p = 0.0071$**	$F_{1, 101} = 0.02$ $p = 0.9020$

C)

Species	**Sex**	**Habitat**	**Sex*Habitat**
Adinia xenica	$F_{1, 99} = 2.41$ $p = 0.1235$	$F_{1, 99} = 0.70$ $p = 0.4060$	$F_{1, 99} = 0.03$ $p = 0.8553$
Fundulus bermudae	$F_{1, 18} = 0.93$ $p = 0.3470$	N/A	N/A

Supplemental Table 2.1 (cont.)

Fundulus bifax	$F_{1,5} = 0.09$ $p = 0.7805$	N/A	N/A
Fundulus blairae	$F_{1,39} = 0.16$ $p = 0.6946$	**$F_{1,39} = 5.12$** **$p = 0.0293$**	$F_{1,39} = 1.45$ $p = 0.2351$
Fundulus catenatus	$F_{1,25} = 3.46$ $p = 0.0745$	N/A	N/A
Fundulus chrysotus	$F_{1,41} = 2.37$ $p = 0.1313$	**$F_{1,41} = 6.14$** **$p = 0.0174$**	$F_{1,41} = 3.79$ $p = 0.0583$
Fundulus cingulatus	**$F_{1,25} = 10.81$** **$p = 0.0030$**	N/A	N/A
Fundulus confluentus	$F_{1,44} = 3.92$ $p = 0.0539$	$F_{1,44} = 0.05$ $p = 0.8284$	$F_{1,44} = 3.57$ $p = 0.0653$
Fundulus diaphanus	$F_{1,27} = 0.18$ $p = 0.6773$	$F_{1,27} = 1.44$ $p = 0.2408$	$F_{1,27} = 0.93$ $p = 0.3439$
Fundulus dispar	**$F_{1,48} = 23.05$** **$p < 0.0001$**	**$F_{1,48} = 54.29$** **$p < 0.0001$**	**$F_{1,48} = 7.30$** **$p = 0.0095$**
Fundulus escambiae	$F_{1,3} = 0.24$ $p = 0.6587$	$F_{1,3} = 0.09$ $p = 0.7891$	N/A
Fundulus euryzonus	$F_{1,20} = 0.89$ $p = 0.3574$	N/A	N/A
Fundulus grandis	$F_{1,35} = 0.10$ $p = 0.7575$	$F_{1,35} = 2.65$ $p = 0.1126$	$F_{1,35} = 0.03$ $p = 0.8726$
Fundulus heteroclitus	$F_{1,17} = 0.04$ $p = 0.8482$	N/A	N/A
Fundulus jenkinsi	$F_{1,10} = 0.58$ $p = 0.4644$	$F_{1,10} = 1.91$ $p = 0.1972$	N/A
Fundulus julisia	$F_{1,2} = 0.23$ $p = 0.6801$	N/A	N/A
Fundulus kansae	$F_{1,24} = 0.60$ $p = 0.4475$	**$F_{1,24} = 6.05$** **$p = 0.0215$**	$F_{1,24} = 2.85$ $p = 0.1046$
Fundulus lineolatus	$F_{1,38} = 0.06$ $p = 0.8023$	$F_{1,38} = 0.02$ $p = 0.8789$	$F_{1,38} = 0.03$ $p = 0.8551$
Fundulus luciae	$F_{1,40} = 0.01$ $p = 0.9253$	$F_{1,40} = 0.20$ $p = 0.6584$	$F_{1,40} = 0.22$ $p = 0.6404$
Fundulus majalis	$F_{1,20} = 1.27$ $p = 0.2736$	**$F_{1,20} = 7.58$** **$p = 0.0123$**	$F_{1,20} = 0.02$ $p = 0.8987$
Fundulus notatus	$F_{1,47} = 0.04$ $p = 0.8422$	$F_{1,47} = 0.48$ $p = 0.4936$	$F_{1,47} = 0.37$ $p = 0.5433$
Fundulus notti	$F_{1,57} = 3.17$ $p = 0.0802$	$F_{1,57} = 0.03$ $p = 0.8694$	$F_{1,57} = 3.63$ $p = 0.0619$
Fundulus olivaceous	$F_{1,46} = 2.01$ $p = 0.1625$	$F_{1,46} = 0.10$ $p = 0.7560$	$F_{1,46} = 0.16$ $p = 0.6891$
Fundulus pulvereus	**$F_{1,23} = 8.22$** **$p = 0.0087$**	**$F_{1,23} = 7.39$** **$p = 0.0122$**	$F_{1,23} = 1.02$ $p = 0.3227$

Supplemental Table 2.1 (cont.)

Fundulus rathbuni	$F_{1, 24} = 0.73$ $p = 0.4019$	N/A	N/A
Fundulus relictus	$F_{1, 55} = 3.27$ $p = 0.0761$	N/A	N/A
Fundulus rubrifons	$F_{1, 19} = 0.45$ $p = 0.5125$	N/A	N/A
Fundulus sciadicus	$F_{1, 24} = 2.61$ $p = 0.1193$	$F_{1, 24} = 0.49$ $p = 0.4900$	N/A
Fundulus seminolis	$F_{1, 85} = 0.89$ $p = 0.3470$	$F_{1, 85} = 2.09$ $p = 0.1522$	$F_{1, 85} = 0.55$ $p = 0.4595$
Fundulus similis	$F_{1, 64} = 0.90$ $p = 0.3466$	$F_{1, 64} = 0.99$ $p = 0.3230$	$F_{1, 64} = 0.41$ $p = 0.5235$
Fundulus stellifer	$F_{1, 18} = 1.20$ $p = 0.2868$	N/A	N/A
Fundulus waccamensis	$F_{1, 10} = 0.14$ $p = 0.7174$	N/A	N/A
Fundulus zebrinus	$F_{1, 30} = 0.31$ $p = 0.5813$	$F_{1, 30} = 2.03$ $p = 0.1648$	$F_{1, 30} = 0.02$ $p = 0.8807$
Leptolucania ommata	$F_{1, 39} = 2.84$ $p = 0.1000$	$F_{1, 39} = 1.18$ $p = 0.2836$	$F_{1, 39} = 0.70$ $p = 0.4086$
Lucania goodei	$F_{1, 66} = 0.10$ $p = 0.7531$	**$F_{1, 66} = 7.74$** **$p = 0.0070$**	$F_{1, 66} = 0.45$ $p = 0.5067$
Lucania interioris	$F_{1, 20} = 1.38$ $p = 0.2542$	N/A	N/A
Lucania parva	$F_{1, 101} = 1.45$ $p = 0.2307$	$F_{1, 101} = 0.94$ $p = 0.3347$	**$F_{1, 101} = 5.03$** **$p = 0.0272$**

D)

Species	**Sex**	**Habitat**	**Sex*Habitat**
Adinia xenica	$F_{1, 99} = 0.79$ $p = 0.3753$	**$F_{1, 99} = 40.90$** **$p < 0.0001$**	$F_{1, 99} = 0.42$ $p = 0.5166$
Fundulus bermudae	$F_{1, 18} = 2.43$ $p = 0.1364$	N/A	N/A
Fundulus bifax	$F_{1,5} = 0.76$ $p = 0.4226$	N/A	N/A
Fundulus blairae	$F_{1, 39} = 0.02$ $p = 0.8963$	$F_{1, 39} = 0.40$ $p = 0.5297$	$F_{1, 39} = 2.32$ $p = 0.1362$
Fundulus catenatus	**$F_{1, 25} = 4.37$** **$p = 0.0469$**	N/A	N/A
Fundulus chrysotus	$F_{1, 41} = 0.18$ $p = 0.6743$	**$F_{1, 41} = 22.53$** **$p < 0.0001$**	$F_{1, 41} = 0.54$ $p = 0.4682$

Supplemental Table 2.1 (cont.)

Fundulus cingulatus	$F_{1,25} = 3.60$ $p = 0.0694$	N/A	N/A
Fundulus confluentus	$F_{1,44} = 3.84$ $p = 0.0564$	$F_{1,44} = 0.55$ $p = 0.4613$	$F_{1,44} = 1.30$ $p = 0.2596$
Fundulus diaphanus	**$F_{1,27} = 66.51$** **$p < 0.0001$**	**$F_{1,27} = 6.05$** **$p = 0.0206$**	$F_{1,27} = 0.23$ $p = 0.6379$
Fundulus dispar	$F_{1,48} = 0.50$ $p = 0.4831$	**$F_{1,48} = 95.53$** **$p < 0.0001$**	$F_{1,48} = 0.30$ $p = 0.5873$
Fundulus escambiae	$F_{1,3} = 0.17$ $p = 0.7067$	$F_{1,3} = 9.40$ $p = 0.0547$	N/A
Fundulus euryzonus	$F_{1,20} = 0.43$ $p = 0.5215$	N/A	N/A
Fundulus grandis	$F_{1,35} = 2.98$ $p = 0.0931$	$F_{1,35} = 0.20$ $p = 0.6614$	**$F_{1,35} = 12.43$** **$p = 0.0012$**
Fundulus heteroclitus	$F_{1,17} = 1.23$ $p = 0.2821$	N/A	N/A
Fundulus jenkinsi	$F_{1,10} = 2.09$ $p = 0.1789$	$F_{1,10} = 4.54$ $p = 0.0589$	N/A
Fundulus julisia	$F_{1,2} = 0.01$ $p = 0.9204$	N/A	N/A
Fundulus kansae	$F_{1,24} = 3.64$ $p = 0.0685$	$F_{1,24} = 0.06$ $p = 0.8140$	$F_{1,24} = 0.14$ $p = 0.7131$
Fundulus lineolatus	$F_{1,38} = 3.16$ $p = 0.0834$	$F_{1,38} = 1.05$ $p = 0.3111$	$F_{1,38} = 0.08$ $p = 0.7844$
Fundulus luciae	$F_{1,40} = 1.73$ $p = 0.1958$	$F_{1,40} = 0.17$ $p = 0.6853$	**$F_{1,40} = 6.14$** **$p = 0.0175$**
Fundulus majalis	$F_{1,20} = 2.63$ $p = 0.1206$	$F_{1,20} = 2.44$ $p = 0.1337$	$F_{1,20} = 0.03$ $p = 0.8607$
Fundulus notatus	$F_{1,47} = 0.77$ $p = 0.3854$	**$F_{1,47} = 4.61$** **$p = 0.0369$**	$F_{1,47} = 2.85$ $p = 0.0979$
Fundulus notti	$F_{1,57} = 1.60$ $p = 0.2115$	$F_{1,57} = 2.09$ $p = 0.1538$	$F_{1,57} = 3.50$ $p = 0.0664$
Fundulus olivaceous	$F_{1,46} = 7.14$ $p = 0.0104$	$F_{1,46} = 0.52$ $p = 0.4740$	$F_{1,46} = 0.85$ $p = 0.3614$
Fundulus pulvereus	$F_{1,23} = 1.06$ $p = 0.3132$	$F_{1,23} = 0.72$ $p = 0.4051$	$F_{1,23} = 1.61$ $p = 0.2170$
Fundulus rathbuni	$F_{1,24} = 0.05$ $p = 0.8198$	N/A	N/A
Fundulus relictus	**$F_{1,55} = 8.74$** **$p = 0.0046$**	N/A	N/A
Fundulus rubrifons	$F_{1,19} = 0.00$ $p = 0.9482$	N/A	N/A

Supplemental Table 2.1 (cont.)

Fundulus sciadicus	$F_{1, 24} = 0.50$ $p = 0.4876$	$F_{1, 24} = 1.16$ $p = 0.2929$	N/A
Fundulus seminolis	$F_{1, 85} = 0.67$ $p = 0.4140$	**$F_{1, 85} = 9.67$ $p = 0.0026$**	$F_{1, 85} = 0.52$ $p = 0.4743$
Fundulus similis	$F_{1, 64} = 0.03$ $p = 0.8722$	$F_{1, 64} = 1.38$ $p = 0.2451$	$F_{1, 64} = 0.03$ $p = 0.8722$
Fundulus stellifer	$F_{1, 18} = 0.10$ $p = 0.7503$	N/A	N/A
Fundulus waccamensis	$F_{1, 10} = 0.85$ $p = 0.3793$	N/A	N/A
Fundulus zebrinus	$F_{1, 30} = 0.80$ $p = 0.3771$	$F_{1, 30} = 2.81$ $p = 0.1042$	$F_{1, 30} = 0.00$ $p = 0.9611$
Leptolucania ommata	**$F_{1, 39} = 5.07$ $p = 0.0301$**	**$F_{1, 39} = 38.43$ $p < 0.0001$**	$F_{1, 39} = 0.48$ $p = 0.4944$
Lucania goodei	$F_{1, 66} = 1.18$ $p = 0.2822$	$F_{1, 66} = 0.04$ $p = 0.8381$	**$F_{1, 66} = 6.01$ $p = 0.0168$**
Lucania interioris	$F_{1, 20} = 0.00$ $p = 0.9772$	N/A	N/A
Lucania parva	**$F_{1, 101} = 8.58$ $p = 0.0042$**	**$F_{1, 101} = 7.21$ $p = 0.0085$**	$F_{1, 101} = 1.33$ $p = 0.2511$

Chapter 3: Comparison of body size and shape in *Fundulus notatus*[1]

Abstract

Lake and stream habitats pose a variety of challenges to fishes due to differences in variables such as water velocity, habitat structure, prey community, and predator community. These differences can cause divergent selection on size/shape. Here, we measured sex, age, length, and eight different morphological traits of the blackstripe topminnow, *Fundulus notatus*, from nineteen lake and stream populations across four river drainages in central Illinois. Our goal was to determine whether body size and shape differed consistently between lake and stream habitats across drainages. We also considered the effects of age and sex as they may affect size and morphology. We found large differences in body size of age 1 topminnows where stream fish were generally larger than lake fish. Body shape mainly varied as a function of sex. Adult male topminnows had larger morphological traits (with the exception of body width) than females, in particular, longer dorsal and anal base lengths. Subtle effects of habitat were present. Stream fish had a longer dorsal fin base than lake fish. These phenotypic patterns may be the result of genetic and/or environmental variation. As these lakes are human-made, the observed differences, if genetic, would have had to occur relatively rapidly (within about 100 years).

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Introduction

Lake and stream habitats differ in many ecologically important characteristics including both biotic (predator and prey assemblages) and abiotic (spatial complexity and water velocity) factors (Jackson *et al*. 2001). Rivers/streams have a faster water velocity, are generally more heterogeneous habitats (Eadie *et al*. 1986) and contain lower predator abundances (Larimore and Bayley 1996) than lakes. Studies comparing lakes and streams have found differences in fish body size and shape (Hendry *et al*. 2002, Collin and Fumagalli 2011). The general prediction is that individuals will be more "streamlined" in river/stream habitats (Langerhans 2008), but there is evidence that this is not universally true (Brinsmead and Fox 2002, McGuigan *et al*. 2003). Differences in size and shape may also arise from intrinsic factors, such as sex or age (Parker 1992, Simonovic *et al*. 1999, Hendry *et al.* 2006, Spoljaric and Reimchen 2011).

Our goal was to explore body size and shape differences of the blackstripe topminnow, *Fundulus notatus*, between lake and stream populations across two ages and both sexes. We also examine the consistency of such patterns by utilizing populations from four different river drainages. Drainage was found to be important in determining the body shape of *F. notatus* in another study, although that study was only conducted in rivers (Schaefer *et al.* 2011). Gene flow is likely higher between populations within a drainage than between populations across drainages and, thus, examining patterns across multiple drainages allows for an assessment of the generality and consistency of the pattern. Consistent correlations between an organism's phenotype and their environment provide potential clues as to the adaptive significance of these traits (Reznick and Travis 1996).

Materials and Methods

A total of 572 blackstripe topminnows were collected with seines and dipnets from eight sites (four lakes and four streams) in 2008 and seventeen (seven lakes and ten streams) in 2010, of which five sites were sampled in both years. The data from the two years were analyzed together. The sites were chosen so that at least one lake and one stream were from each of 4 different river drainage systems in central Illinois (Table 3.1, Supplemental Figure 3.1).

Prior to measurement, fish were euthanized with an overdose of MS-222, preserved in formalin, and then stored in 70% ethanol. Following the methods of Grünbaum *et al*. (2007), fish were measured for body size (standard length) and eight standard linear morphometric measurements: body height, body width, caudal peduncle height, dorsal fin base, anal fin base, head height, head width, and eye diameter (Supplemental Figure 3.2). All measurements were made to the nearest 0.1 mm using Vernier calipers. Fish were aged to the nearest year using growth rings on their scales observed under light microscopy (DeVries and Frie 1996). Sex was determined visually by examining lateral stripe patterning and fin ray length (Carranza and Winn 1954).

Statistical Analysis

Individuals were classified into age classes corresponding to the number of full years they completed (i.e. age 0 are those individuals less than 1 year of age, age 1 are individuals over 1 but less than 2, etc.). We used a Chi-square analysis to test for differences in the age distribution as a function of habitat as well as to test for differences in the distribution of the sexes between the two habitat types.

We analyzed size and shape separately. For all analyses, we only considered individuals in age classes 0 and 1 because many populations only contained these two ages (see Table 3.1). To examine patterns in size, we compared body size between age-classes and sexes using a general linearized model. To examine patterns in shape, we regressed each of the 8 morphometric traits on body size and then analyzed the residuals in a principal components analysis using the correlation matrix. We focused on the first four principal components because together they accounted for almost 90% of the overall variation (Table 3.2).

For both the size and shape analyses, the main terms were tested using either population or sex*population as the level of replication. We modeled the effects of habitat, drainage, sex, habitat*drainage, and population nested within the interaction of habitat*drainage. We consider drainage and population(habitat*drainage) to be random. We analyzed the data separately for each age class and only included those populations that had at least 2 individuals of both sexes for a particular age. All analyses were performed in SAS V.2 (Cary, NC). All raw data have been deposited in Dryad (Dryad data repository doi:10.5061/dryad.j7th2).

Results

Age and Body Size

Lakes consisted of 59% age 0 individuals, 39% age 1, and 2% age 2, while streams consisted of 57% age 0, 37% age 1, 5% age 2, and 1% age 3 individuals (Table 3.1). The age distribution did not differ between habitat types, regardless of whether we excluded age 3 (X_2^2 = 3.8332, p = 0.1471) or combined ages 2 and 3 into one age class (X_2^2 = 5.4417, p = 0.0658). The latter analysis trended toward a pattern where lakes were shifted towards a younger age-distribution and streams towards an older distribution. The number of individuals of each sex did not differ between habitat types, as both lakes and streams consisted of 56% males and 44% females (X_1^2 = 0.0391, p = 0.8432).

Body size did not differ between the two sexes, but did differ among habitat types and drainages (Table 3.3). For age class 0, body size differed among habitat types, but the pattern depended on drainage, such that stream fish were larger in the Vermilion and Kaskaskia drainages and lake fish were larger in the Embarras and Sangamon drainages ($F_{3,8}$ = 2.19, p = 0.0138). In age class 1, stream fish were significantly larger than lake fish ($F_{1,8}$ = 7.86, p = 0.0228) (Figure 3.1).

Body Shape

The first four principal components together accounted for 88.5% of the variation (Table 3.2). The first principal component accounted for 61.5% of the variation. All traits with the exception of body width loaded positively onto PC1. In age class 1, males were more "robust" (i.e. had more positive PC scores) than females ($F_{1,8}$ = 7.69, p = 0.0223) (Figure 3.2). The drainages which flow into the Ohio River (Vermillion and Embarras) had more robust age 1 fish than those which flow into the Mississippi River ($F_{3,8}$ = 8.56, p = 0.0066) (Table 3.4).

PC2 was positively associated with body and head width (Table 3.2). We found no effect of habitat, drainage, sex, or any of their interactions for either age class (Table 3.5).

PC3 was positively associated with eye diameter and negatively associated with dorsal fin base length (Table 3.2). Lake individuals had larger PC3 scores (i.e. larger eye diameter and smaller dorsal fin base length) than stream individuals in both age 0 ($F_{1,8}$ = 15.03, p = 0.0043) and age 1 ($F_{1,8}$ = 5.69, p = 0.0435). For age class 1, females had larger eyes and smaller dorsal fin base length than males ($F_{1,8}$ = 5.32, p = 0.0454) (Table 3.6).

PC4 was positively associated with anal and dorsal fin base lengths and negatively associated with head width (Table 3.2). For age class 1, males had greater PC4 values (i.e. larger anal and dorsal fin base lengths and smaller head widths) than females ($F_{1,8} = 14.09$, $p = 0.0047$) (Table 3.7).

Discussion

Our goals were to explore body size and shape differences of the blackstripe topminnow, *Fundulus notatus*, between lake and stream populations across two ages and both sexes and to examine the consistency of such patterns. Body size was driven by habitat, but differences in shape primarily resulted from sexual dimorphism. These differences mainly appeared in the later age class and were largely consistent across the four river drainages. We discuss these results in more detail below.

Adult (age class 1) stream fish were generally larger than adult lake fish. This may be explained by differences in predation and/or water velocity between lakes and streams. The lake sites sampled for this study are often stocked with predatory game fish (Larimore and Bayley 1996). High predation on larger fish/adults would select for smaller size at maturation (Charlesworth 1980, Conover and Munch 2002). Water velocity also affects body size through growth, although different species respond differently (Davison 1997, Bhagat *et al*. 2006, Grünbaum *et al*. 2008, Fischer-Rousseau *et al*. 2010).

There was an effect of sex on three of the four principal component axes, suggesting that there is substantial sexual dimorphism in body shape of topminnows, particularly in the older age class. This is not surprising, as sexual dimorphism in body shape is common among many fish species (Parker 1992). In general, male topminnows have larger traits, in particular greater anal and dorsal fin base lengths, than females. Differences in the fins had previously been qualitatively described and were attributed to the sex-specific roles of these fins in courtship and spawning (Carranza and Winn 1954).

There was an effect of habitat on body shape, where individuals from lakes had a larger eye diameter and smaller dorsal fin base length than those from streams. Dorsal fins aid maneuvering and stabilization in the water column (Standen and Lauder 2007) and this is presumably more important for fish facing higher water velocities. However, there is interspecific variation in the use of the dorsal fin under different swimming speeds (Drucker and Lauder 2005) and, thus, it is unknown whether the differences observed here are related to water velocity or some other environmental difference between lakes and streams. Interpreting the eye pattern is also difficult, as some fish have large eyes when they occur in clear water for picking zooplankton out of the water column (Huber *et al*. 1997) while others have larger eyes when living in low light environments (Warrant and Locket 2004). Either of these scenarios could apply to lake topminnows, as they spend most of their time at the water surface but overwinter in deeper water (Carranza and Winn 1954).

It is unknown whether these phenotypic patterns are the result of genetic and/or environmental variation. Because all of our lake sites are human-made and less than 100 years old, the observed differences among populations within drainages, if genetic, would have had to develop fairly quickly. Impounding streams to form lakes is starting to be recognized as a potentially important driver of morphological change (Haas *et al*. 2010, Franssen 2011). Our study was not designed to explicitly test this, as only four of our lake sites were created through impoundments, however rapid evolution has been documented in many taxa (Thompson 1998, Simberloff *et al*. 2000, Reznick and Ghalambor 2005) including another cyprinodontiform (Collyer *et al*. 2007).

References

Bhagat Y., M. G. Fox, and M. T. Ferreira. 2006. Morphological differentiation in introduced pumpkinseed *Lepomis gibbosus* (L.) occupying different habitat zones in Portuguese reservoirs. *Journal of Fish Biology* **69** (Supplement C)**:** 79-94.

Brinsmead J. and M. G. Fox. 2002. Morphological variation between lake- and stream-dwelling rock bass and pumpkinseed populations. *Journal of Fish Biology* **61:** 1619-1638.

Carranza J. and H. E. Winn. 1954. Reproductive behavior of the blackstripe topminnow, *Fundulus notatus*. *Copeia* **1954:** 273-278.

Charlesworth B. 1980. *Evolution in age-structured populations*. Cambridge: Cambridge Press.

Collin H. and L. Fumagalli. 2011**.** Evidence for morphological and adaptive genetic divergence between lake and stream habitats in European minnows (*Phoxinus phoxinus*, Cyprinidae). *Molecular Ecology* **20:** 4490-4502.

Collyer M. L., C. A. Stockwell, D. C. Adams, and M. H. Reiser. 2007. Phenotypic plasticity and contemporary evolution in introduced populations: evidence from translocated populations of white sands pupfish (*Cyprinodon tularosa*). *Ecological Research* **22:** 902-910

Conover D. O. and S. B. Munch. 2002. Sustaining fisheries yields over evolutionary time scales. *Science* **297:** 94-96.

Davison W. 1997. The effects of exercise training on teleost fish, a review of recent literature. *Comparative Biochemistry and Physiology A: Molecular and Integrative Physiology* **117A:** 67-75.

DeVries D. R. and R. Frie. 1996. Growth and age estimation. In: Murphy BR, Willis DW, eds *Fisheries Technique*. Maryland: American Fisheries Society, pp. 483-508.

Drucker E. G. and G. V. Lauder. 2005. Locomotor function of the dorsal fin in rainbow trout: kinematic patterns and hydrodynamic forces. *The Journal of Experimental Biology* **208:** 4479-4494.

Eadie J. M., T. A. Hurly, R. D. Montgomerie, and K. L. Teather. 1986**.** Lakes and rivers as islands: speies-area relationships in the fish faunas of Ontario. *Environmental Biology of Fishes* **15:** 81-89.

Fischer-Rousseau L., K. P. Chu, and R. Cloutier. 2010. Developmental plasticity in fish exposed to a water velocity gradient: a complex response. *Journal of Experimental Zoology B- Molecular and Developmental Evolution* **314B:** 67-85.

Franssen N. R. 2011. Anthropogenic habitat alteration induces rapid morphological divergences in a native stream fish. *Evolutionary Applications* **4:** 791-804.

Grünbaum T., R. Cloutier, and N. R. Le Francois. 2008. Positive effects of exposure to increased water velocity on growth of newly hatched Arctic charr, *Salvelinus alpinus* L. *Aquaculture Research* **39:** 106-110.

Grünbaum T., R. Cloutier, P. M. Mabee, and N. R. Le Francois. 2007. Early developmental plasticity and integrative responses in arctic charr (*Salvelinus alpinus*): Effects of water velocity on body size and shape. *Journal of Experimental Zoology* **308B:** 396-408.

Haas T.C., M. J. Blum, and D. C. Heins. 2010. Morphological responses of a stream fish to water impoundment. *Biology Letters* **6:** 803-806.

Hendry A.P., M. L. Kelly, M. T. Kinnison, and D. N. Reznick. 2006. Parallel evolution of the sexes? Effects of predation and habitat features on the size and shape of wild guppies. *Journal of Evolutionary Biology* **19:** 741-754.

Hendry A.P., E. B. Taylor, and J. D. McPhail. 2002. Adaptive divergence and the balance between selection and gene flow: lake and stream stickleback in the Misty system. *Evolution* **56:** 1199-1216.

Huber R., M. J. vanStaaden, L. S. Kaufman, and K. F. Liem. 1997. Microhabitat use, trophic patterns, and the evolution of brain structure in African cichlids. *Brain Behavior and Evolution* **50:** 167-182.

Jackson D.A., P. R. Peres-Neto, and J. D. Olden. 2001. What controls who is where in freshwater fish communities - the roles of biotic, abiotic, and spatial factors. *Canadian Journal of Fisheries and Aquatic Sciences* **58:** 157-170.

Langerhans R. B. 2008. Predictability of phenotypic differentiation across flow regimes in fishes. *Integrative and Comparative Biology* **48:** 750-768.

Larimore R. W. and P. B. Bayley. 1996. The fishes of Champaign County, Illinois, during a century of alterations of a prairie ecosystem. *Illinois Natural History Survey Bulletin* **35:** 53-183.

McGuigan K., C. E. Franklin, C. Moritz, and M. W. Blows. 2003. Adaptation of rainbow fish to lake and stream habitats. *Evolution* **57:** 104-118.

Pakkasmaa S. and J. Piironen. 2001. Water velocity shapes juvenile salmonids. *Evolutionary Ecology* **14:** 721-730.

Parker G. A. 1992. The evolution of sexual size dimorphism in fish. *Journal of Fish Biology* **41** (Supplement B)**:** 1-20.

Peres-Neto P. R. and P. Magnan. 2004. The influence of swimming demand on phenotypic plasticity and morphological integration: a comparison of two polymorphic charr species. *Oecologia* **140:** 36-45.

Reznick D. N. and C. K. Ghalambor. 2005. Selection in nature: experimental manipulations of natural populations. *Integrative and Comparative Biology* **45:** 456-462.

Reznick D.N. and J. Travis. 1996. The empirical study of adaptation in natural populations. In: Rose MR, Lauder GV, eds. *Adaptation*. New York: Academic Press, pp. 243-290.

Satterthwaite F. W. 1946. An approximate distribution of estimates of variance components. *Biometrics Bulletin* **2:** 110-114.

Schaefer J., D. Duvernell, and B. Kreiser. 2011**.** Shape variability in topminnows (*Fundulus notatus* species complex) along the river continuum. *Biological Journal of the Linnean Society* **103:** 612-621.

Simberloff D., T. Dayan, C. Jones, and G. Ogura. 2000. Character displacement and release in the small Indian mongoose, *Herpestes javanicus*. *Ecology* **81:** 2086-2099.

Simonovic P. D., P. Garner, E. A. Eastwood, V. Kovac, and G. H. Copp. 1999**.** Correspondence between ontogeneticshifts in morphology and habitat use in minnow *Phoxinus phoxinus*. *Environmental Biology of Fishes* **56:** 117-128.

Spoljaric M. A. and T. E. Reimchen. 2011. Habitat-specific trends in ontogeny of body shape in stickleback from coastal archipelago: potential for rapid shifts in colonizing populations. *Journal of Morphology* **272:** 590-597.

Standen E. M. and G. V. Lauder. 2007. Hydrodynamic function of dorsal and anal fins in brook trout (*Salvelinus fontinalis*). *The Journal of Experimental Biology* **210:** 325-339.

Thompson J. N. 1998. Rapid evolution as an ecological process. *Trends in Ecology and Evolution* **13:** 329-332.

Warrant E. J. and N. A. Locket. 2004. Vision in the deep sea. *Biological Reviews* **79:** 671-712.

Figures

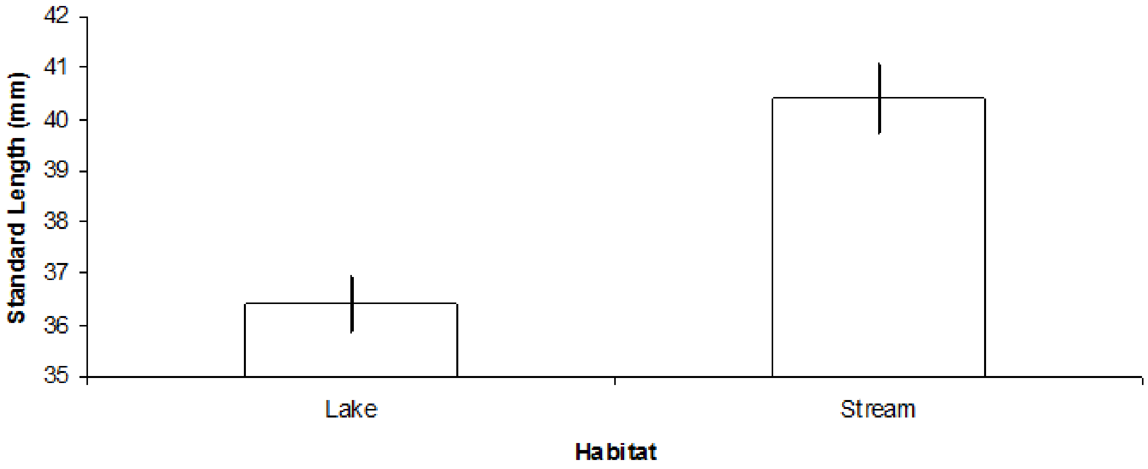


Figure 3.1. Standard length of fish from age class 1 as a function of habitat. Error bars are ± 1 standard error.

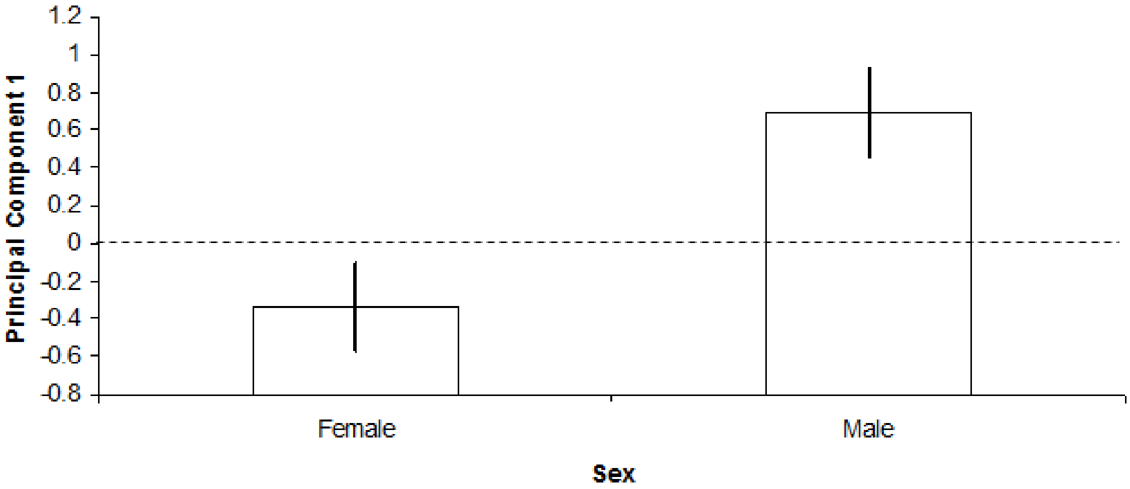
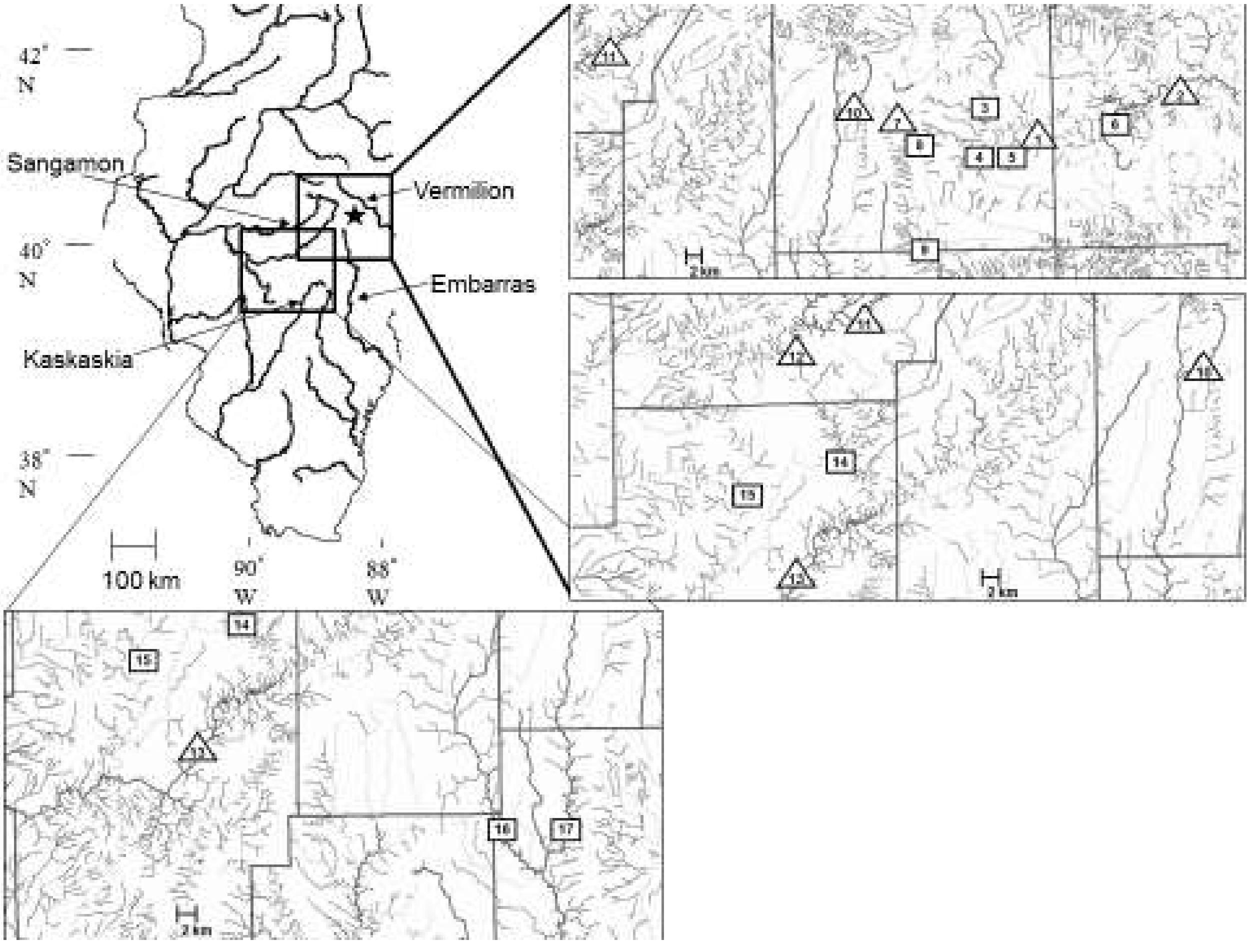
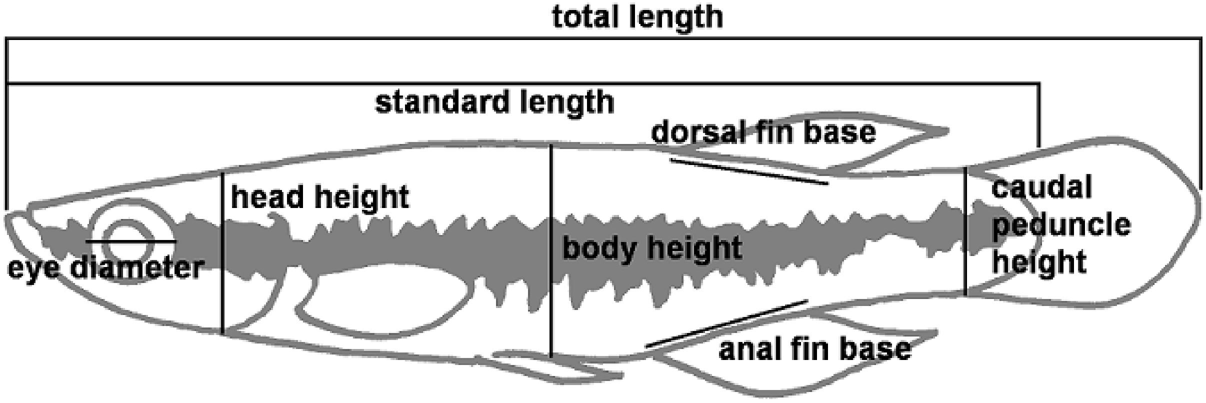


Figure 3.2. Principal Component 1 of fish from age class 1 as a function of sex. Error bars are ± 1 standard error.



Supplemental Figure 3.1. Map of the river system of Illinois with the four river drainages used in this study labeled. Enlarged sections show the counties in east-central Illinois and the sites used in this study (triangles are lakes, rectangles are streams). Two pairs of sites (Homer Lake/Homer Dam and Clear Lake/Long Lake) are grouped together because of close geographic proximity. Numbers refer to the "map number" in Table 1. The star indicates the location of the University of Illinois.



Supplemental Figure 3.2. Morphometric traits measured. Body width and head width are not shown.

Tables

Table 3.1. Habitat type and number of specimens collected at each site. Map number corresponds to the number on Supplemental Figure 1. For the lakes, the approximate year built is included in parentheses. For streams, the cumulative drainage area (CDA) is provided as a descriptor of stream size and local hydrology.

Map Number	Site	Habitat Type (Built)	CDA (km^2)	River Drainage	Total Specimens	Age				Sex	
						0	1	2	3	Male	Female
1	Homer Dam	Lake (1967)	-	Vermilion	10	9	1	0	0	4	6
1	Homer Lake	Lake (1967)	-	Vermilion	39	29	9	1	0	20	19
2	Clear Lake, Kickapoo State Park	Lake (1940s)	-	Vermilion	15	8	5	2	0	9	6
2	Long Lake, Kickapoo State Park	Lake (1940s)	-	Vermilion	21	5	15	1	0	13	8
3	Philips Tract	Stream	204	Vermilion	41	25	13	3	0	21	20
4	Horse Pasture	Stream	20	Vermilion	20	10	9	1	0	14	6
5	Salt Fork	Stream	619	Vermilion	38	20	17	1	0	22	16
6	Richter Site	Stream	1194	Vermilion	13	4	3	6	0	7	6
7	1st & Windsor	Lake (1980s)	-	Embarras	40	34	5	1	0	18	22
8	Curtis Creek	Stream	17	Embarras	13	11	2	0	0	8	5
9	Nanney Site	Stream	20	Embarras	75	28	39	5	3	50	25
10	Kaufman Park	Lake (1960s)	-	Kaskaskia	25	11	14	0	0	11	14
16	Nursing Creek	Stream	51	Kaskaskia	18	10	8	0	0	5	13
17	Antique Creek	Stream	939	Kaskaskia	30	23	7	0	0	18	12
11	Clinton Lake	Lake (1970s)	-	Sangamon	35	15	20	0	0	25	10
12	Weldon Springs State Park	Lake (1900s-1920s)	-	Sangamon	34	22	12	0	0	21	13
13	Lake Decatur	Lake (1920s)	-	Sangamon	11	3	8	0	0	8	3
14	Kickapoo Creek	Stream	194	Sangamon	42	28	14	0	0	18	24
15	Steven's Creek	Stream	39	Sangamon	52	36	16	0	0	28	24

Table 3.2. Results from principal component analysis on the size-regressed traits using the correlation matrix. Eigenvectors of each morphometric trait for the first four principle component axes are shown. Eigenvalues and the proportion of variation accounted for are listed below.

Variable	PC1	PC2	PC3	PC4
body width	-0.06	0.90	0.09	0.26
body height	0.40	-0.01	-0.07	-0.28
head width	0.33	0.42	-0.11	-0.36
head height	0.43	-0.01	-0.05	-0.18
anal fin base	0.40	-0.05	-0.14	0.34
dorsal fin base	0.36	-0.09	-0.31	0.69
caudal peduncle height	0.40	0.00	-0.11	-0.28
eye diameter	0.33	-0.07	0.92	0.15
eigenvalue	4.92	1.12	0.53	0.50
proportion variation	0.6146	0.1401	0.0668	0.0630

Table 3.3. F-values from analyses of variance on standard length of (A) age 0 and (B) age 1 individuals. Significant ($p<0.05$) factors are indicated **in bold**. Habitat type refers to lake or stream. Superscript refers to the term used in conjunction with the mean square error to generate the Satterthwaite approximation error degrees of freedom to calculate the F-value for that factor.

A: Age 0

Term #	Factor	DF	MS	F	P
1	habitat type (HT)^8	1	26.53	0.49	0.5039
2	drainage (D)^8	3	125.52	2.19	0.1673
3	**HT x D^8**	**3**	**400.71**	**6.90**	**0.0138**
4	sex^9	1	24.59	1.20	0.2975
5	sex x HT^9	1	19.21	0.94	0.3551
6	sex x D^9	3	10.10	0.50	0.6901
7	sex x HT x D^9	3	3.25	0.16	0.9179
8	population (HT x D)^9	8	56.96	2.84	0.0806
9	sex x population (HT x D)^9	8	20.06	0.84	0.5673
10	error	282	23.86		

Table 3.3 (cont.)
B: Age 1

Term #	Factor	DF	MS	F	P
1	**habitat type (HT)[8]**	**1**	**1285.07**	**7.86**	**0.0228**
2	drainage (D)[8]	3	358.99	2.21	0.1642
3	HT x D[8]	3	599.40	3.62	0.0642
4	sex[9]	1	1.14	0.04	0.8463
5	sex x HT[9]	1	36.26	1.25	0.2949
6	sex x D[9]	3	15.38	0.53	0.6735
7	sex x HT x D[9]	3	8.43	0.29	0.8325
8	**population (HT x D)[9]**	**8**	**169.33**	**5.74**	**0.0116**
9	sex x population (HT x D)[9]	8	29.48	1.56	0.1395
10	error	179	18.89		

Table 3.4. F-values from analyses of variance on PC1 of (A) age 0 and (B) age 1 individuals. Significant ($p<0.05$) factors are indicated **in bold**. Habitat type refers to lake or stream. Superscript refers to the term used in conjunction with the mean square error to generate the Satterthwaite approximation error degrees of freedom to calculate the F-value for that factor.

A: Age 0

Term #	Factor	DF	MS	F	P
1	habitat type (HT)^{8}	1	51.39	2.05	0.1895
2	drainage (D)^{8}	3	49.99	1.85	0.2170
3	HT x D^{8}	3	21.96	0.79	0.5307
4	sex^{9}	1	0.00	0.00	0.9729
5	sex x HT^{9}	1	0.83	0.44	0.5219
6	sex x D^{9}	3	3.58	1.89	0.2099
7	sex x HT x D^{9}	3	0.42	0.22	0.8784
8	**population (HT x D)^{9}**	**8**	**26.91**	**14.26**	**0.0005**
9	sex x population (HT x D)^{10}	8	1.89	1.14	0.3375
10	error	278	1.66		

Table 3.4 (cont.)
B: Age 1

Term #	Factor	DF	MS	F	P
1	habitat type (HT)[8]	1	0.34	0.03	0.8603
2	**drainage (D)[8]**	**3**	**88.95**	**8.56**	**0.0066**
3	HT x D[8]	3	6.52	0.62	0.6218
4	**sex[9]**	**1**	**52.61**	**7.69**	**0.0223**
5	sex x HT[9]	1	7.71	1.11	0.3227
6	sex x D[9]	3	4.21	0.60	0.6298
7	sex x HT x D[9]	3	9.88	1.41	0.3086
8	population (HT x D)[9]	8	10.71	1.50	0.2884
9	sex x population (HT x D)[10]	8	7.12	1.99	0.0505
10	error	179	3.58		

Table 3.5. F-values from analyses of variance on PC2 of (A) age 0 and (B) age 1 individuals. Significant ($p<0.05$) factors are indicated **in bold**. Habitat type refers to lake or stream. Superscript refers to the term used in conjunction with the mean square error to generate the Satterthwaite approximation error degrees of freedom to calculate the F-value for that factor.

A: Age 0

Term #	Factor	DF	MS	F	P
1	habitat type (HT)^{8}	1	4.54	0.62	0.4547
2	drainage (D)^{8}	3	2.44	0.31	0.8202
3	HT x D^{8}	3	10.94	1.35	0.3262
4	sex^{9}	1	1.99	2.96	0.1156
5	sex x HT^{9}	1	0.92	1.36	0.2723
6	sex x D^{9}	3	0.67	0.99	0.4436
7	sex x HT x D^{9}	3	1.04	1.55	0.2797
8	**population (HT x D)^{9}**	**8**	**7.90**	**11.72**	**0.0011**
9	sex x population (HT x D)^{9}	8	0.67	1.02	0.4229
10	error	278	0.67		

Table 3.5 (cont.)
B: Age 1

Term #	Factor	DF	MS	F	P
1	habitat type (HT)[8]	1	21.64	3.71	0.0898
2	drainage (D)[8]	3	4.94	0.85	0.5034
3	HT x D[8]	3	4.74	0.80	0.5258
4	sex[9]	1	0.09	0.13	0.7244
5	sex x HT[9]	1	2.55	3.92	0.0800
6	sex x D[9]	3	0.43	0.66	0.5973
7	sex x HT x D[9]	3	0.13	0.19	0.8983
8	**population (HT x D)[9]**	**8**	**6.04**	**9.31**	**0.0024**
9	sex x population (HT x D)[9]	8	0.65	0.93	0.4929
10	error	179	0.70		

Table 3.6. F-values from analyses of variance on PC3 of (A) age 0 and (B) age 1 individuals. Significant ($p<0.05$) factors are indicated **in bold**. Habitat type refers to lake or stream. Superscript refers to the term used in conjunction with the mean square error to generate the Satterthwaite approximation error degrees of freedom to calculate the F-value for that factor.

A: Age 0

Term #	Factor	DF	MS	F	P
1	**habitat type (HT)^{8}**	**1**	**15.93**	**15.03**	**0.0043**
2	drainage (D)^{8}	3	1.44	1.28	0.3450
3	HT x D^{8}	3	2.84	2.49	0.1355
4	sex^{9}	1	0.78	1.17	0.3066
5	sex x HT^{9}	1	0.52	0.76	0.4059
6	sex x D^{9}	3	0.85	1.19	0.3747
7	sex x HT x D^{9}	3	0.83	1.14	0.3903
8	population (HT x D)^{9}	8	1.12	1.57	0.2685
9	**sex x population (HT x D)^{9}**	**8**	**0.71**	**2.08**	**0.0374**
10	error	278	0.34		

Table 3.6 (cont.)
B: Age 1

Term #	Factor	DF	MS	F	P
1	**habitat type (HT)[8]**	**1**	**7.67**	**5.69**	**0.0435**
2	drainage (D)[8]	3	0.68	0.51	0.6888
3	HT x D[8]	3	1.30	0.96	0.4571
4	**sex[9]**	**1**	**2.28**	**5.32**	**0.0454**
5	sex x HT[9]	1	0.92	2.15	0.1784
6	sex x D[9]	3	0.72	1.67	0.2430
7	sex x HT x D[9]	3	1.48	3.45	0.0688
8	population (HT x D)[9]	8	1.39	3.22	0.0592
9	sex x population (HT x D)[9]	8	0.43	1.04	0.4072
10	error	179	0.41		

Table 3.7. F-values from analyses of variance on PC4 of (A) age 0 and (B) age 1 individuals. Significant ($p<0.05$) factors are indicated **in bold**. Habitat type refers to lake or stream. Superscript refers to the term used in conjunction with the mean square error to generate the Satterthwaite approximation error degrees of freedom to calculate the F-value for that factor.

A: Age 0

Term #	Factor	DF	MS	F	P
1	habitat type (HT)^{8}	1	1.50	0.74	0.4156
2	drainage (D)^{8}	3	3.79	1.73	0.2375
3	HT x D^{8}	3	2.05	0.92	0.4740
4	sex^{9}	1	0.66	1.49	0.2530
5	sex x HT^{9}	1	0.02	0.04	0.8399
6	sex x D^{9}	3	0.05	0.11	0.9497
7	sex x HT x D^{9}	3	0.14	0.28	0.8364
8	population (HT x D)^{9}	8	1.12	1.57	0.2685
9	**sex x population (HT x D)^{9}**	**8**	**2.18**	**4.62**	**0.0222**
10	error	278	0.47		

Table 3.7 (cont.)
B: Age 1

Term #	Factor	DF	MS	F	P
1	habitat type (HT)[8]	1	0.29	0.12	0.7422
2	drainage (D)[8]	3	3.32	1.35	0.3258
3	HT x D[8]	3	3.13	1.25	0.3551
4	**sex[9]**	**1**	**10.23**	**14.09**	**0.0047**
5	sex x HT[9]	1	1.30	1.76	0.2201
6	sex x D[9]	3	0.19	0.26	0.8518
7	sex x HT x D[9]	3	0.34	0.45	0.7216
8	population (HT x D)[9]	8	2.57	3.41	0.0511
9	sex x population (HT x D)[9]	8	0.75	1.82	0.0761
10	error	179	0.41		

Chapter 4: Fin size and shape in *Fundulus notatus*

Abstract

In fish, the anal and dorsal fins serve multiple functions, including those related to swimming, mate attraction, and reproduction. As such, they are likely to be targets of both natural and sexual selection. Many fish species occupy different habitats, yet how environmental differences influence these “multi-use” traits is largely unknown. Here, we examine the size and shape of the anal and dorsal fin in the blackstripe topminnow, *Fundulus notatus*, from lake and stream habitats across multiple ages and sexes, this allowing us to simultaneously examine the effects of habitat, sexual selection (as indicated by sexual dimorphism), and age on fin size and shape. Our results found no effect of habitat. Rather, the size and shape of anal and dorsal fins is a result of sexual dimorphism. Males have a longer and more pointed anal fin and longer, larger, and a more pointed dorsal fin than females and these appeared almost exclusively in the older age class. Additionally, the angle of these fins are tightly correlated among both males and females suggesting that these fins follow a similar growth trajectory as individuals become sexually mature. Together these results suggest that, despite the multiple roles of these fins, sexual selection is the predominant force determining fin size and shape differences in *F. notatus*.

Introduction

Many species possess traits that serve several functions. The colored dewlaps in some anoles, for instance, are involved in both species recognition and mate attraction (Ng *et al.* 2013). The size and shape of such traits often reflect the diverse tasks in which they are used. Tail feather size was originally shaped by natural selection acting on flying ability, although it was then increased through mate choice (Rowe *et al.* 2001). Similarly, tail shape in hummingbirds seems to be impacted by both natural and sexual selection (Bleiweiss 2009). Thus, studies exploring variation in such traits can offer rich insights into the functional and adaptive significance of multi-use traits.

The anal and dorsal fins are two traits in fish that may be particularly enlightening because they serve multiple functions that likely have strong fitness consequences. First, these fins are typically involved in fish movement and stability. Fish actively manipulate the size and shape of the dorsal and anal fins to aid in stabilization and maneuverability (Drucker and Lauder 2001, Webb 2006, Standen and Lauder 2007). Second, the dorsal and anal fins also frequently function in mate attraction and competitive displays. Males of many species often flare or flash their fins as part of their courtship activities or during male-male interactions (Foster 1967, Robinson *et al.* 2011). Finally, the fins play a role in reproductive success by assisting in fertilization. Some species contain modified fin rays that essentially act like hooks, allowing a male to cling to a female (Kottelat and Lim 1999). In other species, the two sexes will fold their anal fins in such a way as to create a "funnel" that is believed to help place the eggs on a substrate or ensure that sperm fertilize the eggs (Carranza and Winn 1954, Foster 1967).

Despite these multiple roles, surprisingly little is known about the variation that exists in these fins or how differences in environmental factors may affect them. Lake and stream habitats are ideal for pursuing such a question because fish in these environments likely face very different selective pressures due to differences in several ecological characteristics (Jackson *et al*. 2001). Streams have faster water velocities than lakes, which has strong implications for movements/maneuverability and stabilization. Many lakes- including those in this study- contain higher predator abundances because of stocking programs that introduce large piscivorous game fish into lakes (Larimore and Bayley 1996). This may impose strong selection on a fin size or shape that could enable fish to perform "fast start" responses (Tytell and Lauder 2008, Blake *et al*. 2009), which is often used to avoid predators (Webb 1984).

Here, we examine the size and shape of the anal and dorsal fin in the blackstripe topminnow, *Fundulus notatus*, from lake and stream habitats. We examined both males and females. The original species description of *F. notatus* indicates that males and females differ in anal and dorsal fin shape with males having pointed anal and dorsal fins and females having rounded fins. In another morphometric study, we measured and compared anal and dorsal fin base lengths (but not fin shape and size) and found that the base lengths differed between the sexes and age-classes, but did not differ as a function of habitat (Welsh *et al.* 2013).

Our study here allows us to examine the effects of habitat, sexual selection (as indicated by sexual dimorphism), and age on anal and dorsal fin size and shape. If aspects of the habitat (water velocity, predation, etc.) are an important source of selection on the size or shape of the anal and dorsal fins, then we would expect to see a significant difference between lake and stream populations. Similarly, if courtship/fertilization is an important source of selection on fin size or shape, we would expect to see a significant difference between the sexes. An interaction between sex and habitat would be consistent with different environments altering the direction of sexual selection. We also examined the effect of age to determine if these patterns differ as a function of development. Finally, we examined correlations between anal and dorsal fin traits to determine whether the two are tightly integrated or whether they vary independently of one another.

Methods

Study system and field collection

Blackstripe topminnows were collected from stream and man-made lake sites in east central Illinois. Lake Decatur, Weldon Springs State Park, and Homer Lake are reservoirs (that is, they were constructed by impounding adjacent streams/rivers and contain a dam that periodically releases water). The other lakes were pits created for human activity (drainage, mining, etc.) and then later filled in with water. While we do not know the exact year most of these were created, there were no lakes in central Illinois until the construction of the first reservoirs in the early 1900s (Smith 2002), and, thus, all of the sites were filled with water within the past 100 years (most likely within the past 50 years). To minimize the potential for gene flow between lake and stream populations to confound results, we tried to avoid sampling where the two habitat types met (i.e. below a dam or at the inlet/outlet of a lake). In total, twelve sites (six lakes and six streams) were sampled (Table 4.1). Topminnows were collected with seines and dip nets from late August until mid October in 2010.

Measurements

Upon returning from the field, fish were euthanized with an overdose of MS-222, preserved in formalin, and later transferred to 70% ethanol. After all sites were visited, individuals were measured for body size, aged, and sexed. Size was determined using standard length measured to the nearest tenth of a millimeter with Vernier calipers, aging was done to the nearest year using growth rings on their scales observed under light microscopy (DeVries and Frie 1996), and sex determination was done visually by examining lateral line patterning and black markings on the fins: males have more pronounced vertical black barring along their sides and more small black spots ("speckles") on their fins than females (Carranza and Winn 1954, Welsh in review).

Anal and dorsal fins were carefully removed from each specimen by cutting along the base of the fin as close to the body as possible using dissection scissors. Each fin was then photographed using a Zeiss AxioCam ICc1 camera connected to a dissecting microscope. Each photograph contained a grid of 1 mm x 1 mm lines in the background for calibration. From each photograph, size and shape measurements of both the anal and dorsal fins were then determined using Zeiss AxioVision (v. 4.8.2) or ImageJ (v. 1.44). Three separate traits were measured on each fin (Figure 4.1): (1) the length of the fin base, which was measured as a straight line from the front of the first (most anterior) fin ray to the back edge of the last (most posterior) ray, (2) the surface area of the fin, as measured by tracing around the entire fin using the "outline" feature, and (3) the angle between the longest fin ray and the adjacent ray on either side (anterior and posterior to the longest ray). The angle is used as a quantitative description of the "pointedness" of the fin (which has up until now only been qualitatively described as "pointed" or "rounded"- see Carranza and Winn 1954): a smaller (more acute) angle is more pointed than a larger (more obtuse) angle.

Statistical Analysis

Individuals were classified into age classes corresponding to the number of full years they completed (i.e. age 0 are those individuals less than 1 year of age, age 1 are individuals over 1 year of age but less than 2, etc.). Throughout all analyses, we only considered individuals in age classes 0 and 1 because only about half of the populations contained individuals beyond the age 2 year class. Also, all individuals less than about 32 mm were excluded from the analysis. Below this size it is difficult to distinguish immature males from females (Welsh in review) (see Table 4.1 for sample sizes).

Because these traits are influenced by the size of an individual, we first accounted for body size by regressing base length, surface area, and angle against standard length. We then analyzed the residuals separately in a generalized linear model. We modeled the effects of sex, habitat, and population nested within habitat, and all their interactions. Because fish from the same population may not be independent samples, we used population or the interaction with population as the error term to test effects. In other words, habitat was tested over population and the effects of sex and its interactions with the other effects were tested over sex*population(habitat). Rather than delete many populations from an analysis in order to include age as a factor, we chose to analyze the data separately for each age class. To be conservative, we only considered those populations that had at least 2 individuals of both sexes for a particular age; however, the results are robust enough that they are qualitatively the same even if analyses were expanded to populations containing at least 1 individual of both sexes for a particular age. This caused us to delete 4 populations (Lake Decatur, Long Lake, Kaufman Park, and Nursing Creek) from the anal and dorsal fin age class 0 analyses, 1 population (Philip's Tract) from the anal fin age class 1 analysis, and 3 populations (Long Lake, Lake Decatur, and Philip's Tract) from the dorsal fin age class 1 analysis.

We also considered the extent to which traits were correlated with one another. We measured Pearson correlation coefficients amongst all the individuals we had measured for both the raw trait values and the size-corrected traits. We then compared correlations among size-corrected traits for each age and sex. Because of tight correlations between angles for both fins (see Results below), we also calculated the coefficient of variation (CV) for the raw dorsal and anal fin angles to see if these correlations have resulted in low variability. CV is the ratio of the standard deviation to the mean and describes the dispersion (variability) of a trait. CV was calculated separately for each sex and age class. All analyses were performed in SAS V.2 (Cary, NC).

Results

Effects of Sex, Habitat, and Population on Individual Traits

Habitat had no effect on any aspect of anal or dorsal fin size or shape. Instead, differences were a result of sexual dimorphism, primarily among the older age class (i.e. age class 1) (Tables 4.2-4.7). Specifically, males had a longer anal fin base length and smaller (more acute) anal fin angle than females. Similarly, for the dorsal fin, males had a longer base length, larger surface area, and smaller angle (Figure 4.2). Differences in the angle were the only trait that appeared in the earlier age class and the pattern was the same as for age class 1 (i.e. males had a smaller angle than females).

Correlations Among Traits

There were strong correlations among raw trait values that were mediated by size. Table 4.8 shows the raw correlations among all individuals. Anal fin base length, anal fin surface area, dorsal fin base length, and dorsal fin area were positively correlated with size. In contrast, neither anal fin angle nor dorsal fin angle were tightly correlated with size, but they were tightly correlated with one another.

After controlling for standard length, functional within-trait relationships remained. For example, dorsal fin surface area was generally positively correlated with dorsal fin base length. More interesting was the relationship that emerged between dorsal and anal fin angles. Even after controlling for standard length, dorsal fin and anal fin angle remained tightly correlated with one another (Table 4.9, Figure 4.3). The tight correlation emerged both among age 1 males and females suggesting that the relationship arises due to variation among individuals within each sex as opposed to arising simply due to variation among the sexes (Figure 4.4, age Class 1 - males: $R = 0.677$, p = 0.0001, $n = 27$; females: $R = 0.709$, p = 0.0003, $n = 21$). For age class 0, the relationship between dorsal and anal fin angle was positive for females but not statistically significant ($R = 0.201$, p = 0.510, $n = 13$). For males, the relationship differed between habitats. In streams, anal fin angle and dorsal fin angle tended to be positively correlated ($R = 0.385$, p = 0.272, $n = 10$). However, there was a negative correlation between dorsal angle and anal angle for age 0 males from the lake habitat (Figure 4.4a, $R = -0.974$, $p = 0.0010$, $n = 6$).

Coefficient of Variation

Males had a higher variability (i.e. greater coefficient of variation) in both anal and dorsal fin angle (Table 4.10). Interestingly, the amount of variation differed between the sexes across the two age classes- male variation was greater in the older age class whereas female variation decreased with age. This pattern was consistent for both fins.

Discussion

The size and shape of anal and dorsal fins of the blackstripe topminnow is a result of sexual dimorphism. Differences in size and shape appeared almost exclusively in the older age class (age class 1). Males have a longer and more pointed anal fin and longer, larger, and a more pointed dorsal fin than females. Differences between the sexes in both of these fins are known from other species, and are often hypothesized to exist because of either male-male competition or to attract mates and enhance reproductive success (Oliveira and Almada 1995, Kottelat and Lim 1999, Hankison *et al*. 2006, McGrath and Hilton 2011). In *F. notatus*, these differences are believed to be due to their sex-specific uses in courtship and spawning (Carranza and Winn 1954, Foster 1967).

The shape of the dorsal and anal fins (i.e. the angle) is tightly correlated, particularly among age 1 individuals. This is present among both males and females suggesting that it is genuine and not simply due to large discrepancies between males and females. This suggests that anal and dorsal fins follow a similar growth trajectory as individuals become sexually mature, which likely reflects the strong developmental integration of endo- and exoskeletal patterning that exists in fishes (Mabee *et al*. 2002). There were no other consistently strong relationships between elements of different fins. These two fins are correlated in triggerfish as well, although the aspects of shape in triggerfish are primarily due to the length and height of the fins and not the angle (Dornburg *et al*. 2011). Whether tight correlations are a general principle of fish fins is unknown, as very few studies have explicitly examined for correlations between anal and dorsal fins.

Despite the strong correlations between anal and dorsal fin angles in *F. notatus*, these traits still exhibit substantial variation, as evidenced by the coefficients of variation. Males had higher amounts of variation than females and their variation increased with age (whereas it decreased with age for females). Coefficients of variation have been used to infer the potential for the traits to respond to selection in the future (Houle 1992). The correlation between dorsal and anal fin angle could conceivably result from either (a) correlated selection favoring pointed dorsal and anal fins or (b) a common genetic basis such as pleiotropy. Selection analyses and breeding studies are required to resolve these options.

Surprisingly, habitat did not strongly affect the size and shape of either fin. There was no difference in either fins' size or shape between lakes and streams. Blackstripe topminnows may respond behaviorally such that they are not faced with high water velocities. Other species are known to alter their activity in response to changes in flow, often by seeking out refugia (reviewed in Liao 2007). In the field, topminnows are almost never seen in the main stem of streams and rivers but, rather, are primarily observed in shallow pools and side coves (personal observations). This microhabitat preference may also help them avoid the predatory fish, as these larger fish tend to be found in deeper water. Therefore, while lakes and streams differ in many potential selective pressures, *F. notatus* may have overcome these differences through behavioral responses. Alternatively, differences between habitats may show up on “real-time” analyses (i.e. using high speed video recordings of live fish). Several species of fish have found that they are actively able to alter the shape of their fins while swimming (Lauder and Drucker 2004) and, thus, it may be that effects of habitat would appear in biomechanical studies of living individuals. This would be an interesting area to pursue for future studies, but would not explain the lack of a difference found in the size of the fins.

References

Blake R. W., J. Li, and K. H. S. Chan. 2009. Swimming in four goldfish *Carassium auratus* morphotypes: understanding functional design and performance employing artificially selected forms. *Journal of Fish Biology* **75:** 591-617.

Bleiweiss R. 2009. The tail end of hummingbird evolution: parallel flight system development in living and ancient birds. *Biological Journal of the Linnean Society* **97:** 467-493.

Carranza J. and H. E. Winn. 1954. Reproductive behavior of the blackstripe topminnow, *Fundulus notatus*. *Copeia* **1954:** 273-278.

DeVries D. R. and R. Frie. 1996. Growth and age estimation. In: Murphy BR, Willis DW, eds. *Fisheries Technique*. Bethesda, MD: American Fisheries Society.

Dornburg, A., B. Sidlauskas, F. Santini, L. Sorenson, T. J. Near, and M. E. Alfaro. 2011.

The influence of an innovative locomotor strategy on the phenotypic diversification of triggerfish (Family: Balistidae). *Evolution* **65:** 1912-1926.

Drucker E. G. and G. V. Lauder. 2001. Locomotor function of the dorsal fin in teleost fishes: experimental analysis of wake forces in sunfish. *Journal of Experimental Biology* **204:** 2943-2958.

Foster N. R. 1967. Comparative studies on the biology of killifishes (Pisces, Cyprinodontidae). PhD Dissertation, Cornell University.

Hankinson S.J., M. J. Childress, J. J. Schmitter-Soto, and M. B. Ptacek. 2006. Morphological divergence within and between Mexican sailfin mollies, *Poecilia velifera* and *Poecilia petenensis*. *Journal of Fish Biology* **68:** 1610-1630.

Houle D. 1992. Comparing evolvability and variability of quantitative traits. *Genetics* **130:** 195-204.

Jackson D. A., P. R. Peres-Neto, and J. D. Olden. 2001. What controls who is where in freshwater fish communities - the roles of biotic, abiotic, and spatial factors. *Canadian Journal of Fisheries and Aquatic Sciences* **58:** 157-170.

Kottelat M. and K. K. P. Lim. 1999. Mating behavior of *Zenarchopterus gilli* and *Zenarchopterus buffonis* and function of the modified dorsal and anal fin rays in some species of *Zenarchopterus* (Teleostei: Hemiramphidae). *Copeia* **1999:** 1097-1101.

Larimore R. W. and P. B. Bayley. 1996. The fishes of Champaign County, Illinois, during a century of alterations of a prairie ecosystem. *Illinois Natural History Survey Bulletin* **35:** 53-183.

Lauder G. V. and E. G. Drucker. 2004. Morphology and experimental hydrodynamics of fish fin control surfaces. *IEEE Journal of Oceanic Engineering* **29:** 556-571.

Liao J. C. 2007. A review of fish swimming mechanics and behaviour in altered flows. *Philosophical Transactions of the Royal Society B- Biological* **362:** 1973-1993.

Mabee P. M., P. L. Crotwell, N. C. Bird, and A. C. Burke. 2002. Evolution of median fin modules in the axial skeleton of fishes. *Journal of Experimental Zoology* **294:** 77-90.

McGrath P. E. and E. J. Hilton. 2012. Sexual dimorphism in longnose gar *Lepisosteus osseus*. *Journal of Fish Biology* **80:** 335-345.

Ng, J., E. L. Landeen, R. M. Logsdon, and R. E. Glor. 2013. Correlation between Anolis lizard dewlap phenotype and environmental variation indicates adaptive divergence of a signal important to sexual selection and species recognition. *Evolution* **67:** 573-582.

Oliveira R. F. and V.C. Almada. 1995. Sexual dimorphism and allometry of external morphology in *Oreochromis mossambicus*. *Journal of Fish Biology* **46:** 1055-1064.

Robinson D. M., M. S. Tudor, and M. R. Morris. 2011. Female preference and the evolution of exaggerated male ornament: the shape of the preference function matters. *Animal Behaviour* **81:** 1015-1021.

Rowe, L. V., M. R. Evans, and K. L. Buchanan. 2001. The function and evolution of the tail streamer in hirundines. *Behavioral Ecology* **12:** 157-163.

Smith P. W. 2002. *The Fishes of Illinois*. Illinois: University of Illinois Press.

Standen E. M. and G. V. Lauder. 2007. Hydrodynamic function of dorsal and anal fins in brook trout (*Salvelinus fontinalis*). *The Journal of Experimental Biology* **210:** 325-339.

Tytell E. D. and G. V. Lauder. 2008. Hydrodynamics of the escape response in bluegill sunfish, *Lepomis macrochirus*. *Journal of Experimental Biology* **211:** 2259-3369.

Webb P. W. 1984. Body form, locomotion and foraging in aquatic vertebrates. *American Zoologist* **24:** 107–120.

Webb P. W. 2006. Stability and maneuverability. In: *Fish Biomechanics* (ed. Shadwick, R. E. and Lauder, G. V.), Amsterdam: Elsevier. pp. 281-332

Welsh D. P., M. Zhou, S. M. Mussmann, L. G. Fields, C. L. Thomas, S. P. Pearish, S. L. Kilburn, J. L. Parker, L. R. Stein, J. A. Bartlett, C. R. Bertram, T. J. Bland, K. L. Laskowski, B. C. Mommer, X. Zhuang, R. C. Fuller. 2013. The effects of age, sex, and habitat on body size and shape of the blackstripe topminnow, *Fundulus notatus* (Cyprinodontiformes: Fundulidae) (Rafinesque 1820). *Biological Journal of the Linnean Society* **108:** 784-789.

Figures

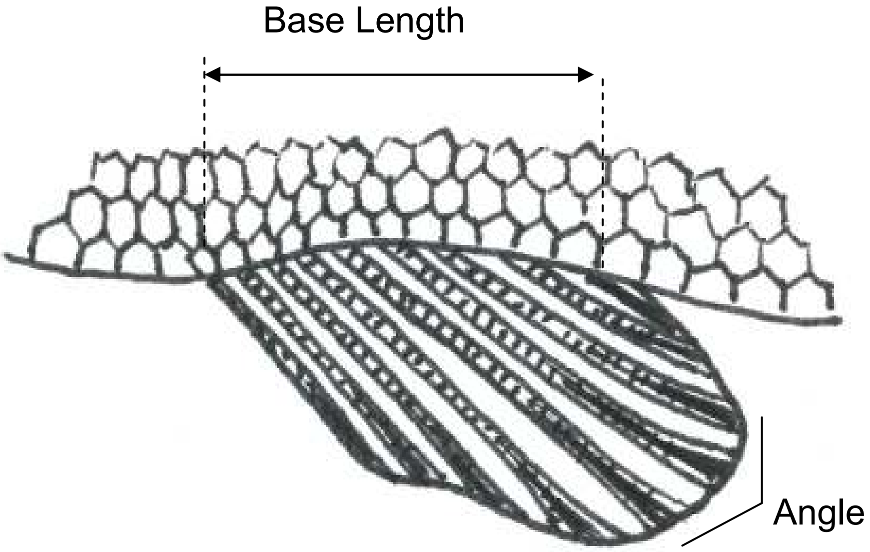


Figure 4.1. Traits measured on each fin. Surface area not shown.

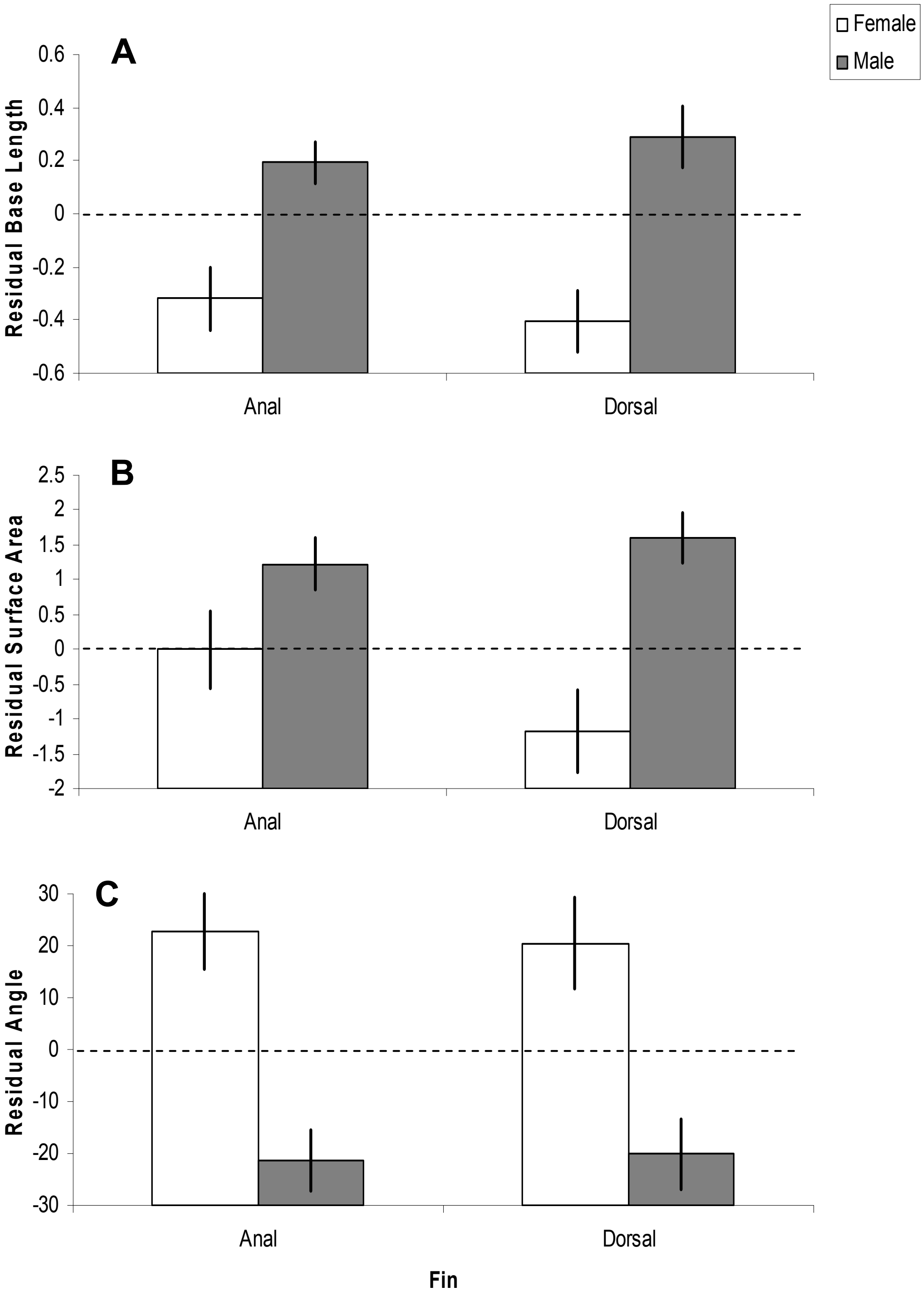


Figure 4.2. Sexual dimorphism of (A) residual base length, (B) residual surface area, and (C) residual angle of the anal and dorsal fins in age class 1. Error bars are ± 1 standard error. Please note the change in y-axis scale. All comparisons are significant except for the residual surface area of the anal fin.

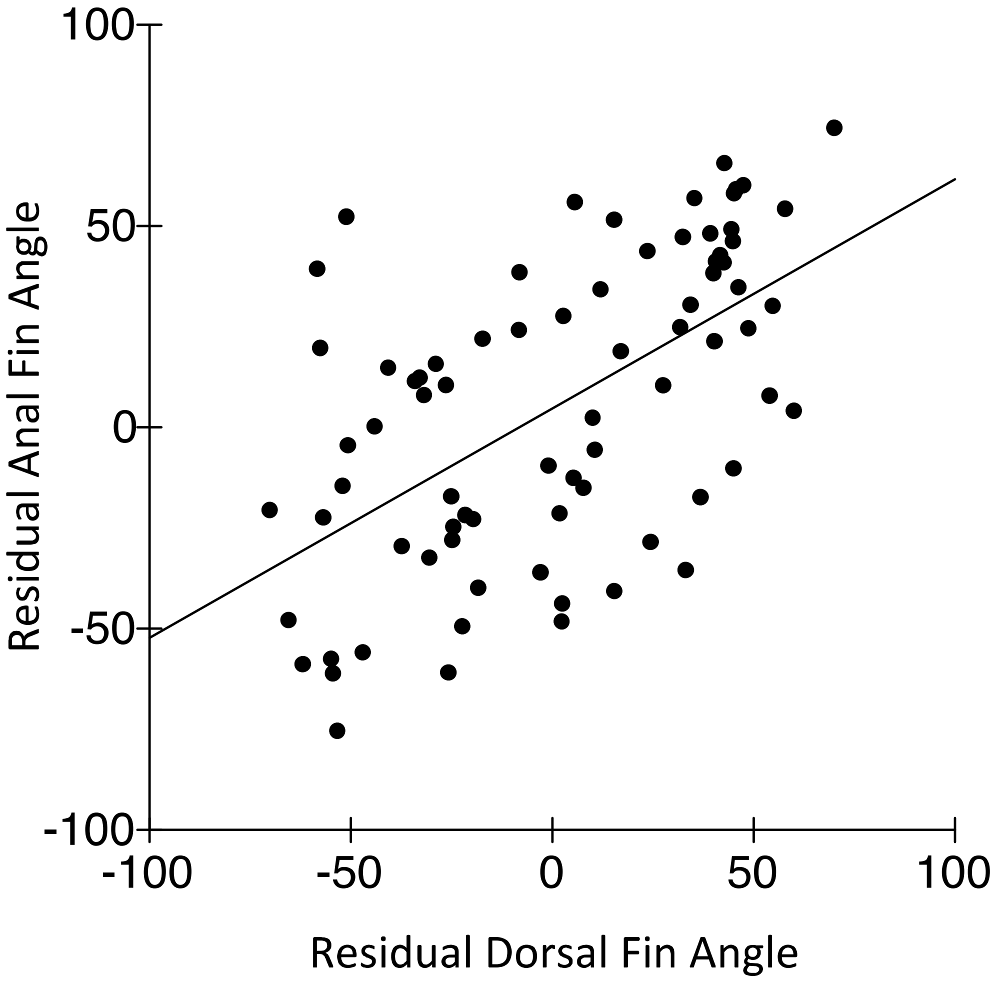


Figure 4.3. The relationship between residual dorsal fin angle and residual anal fin angle.

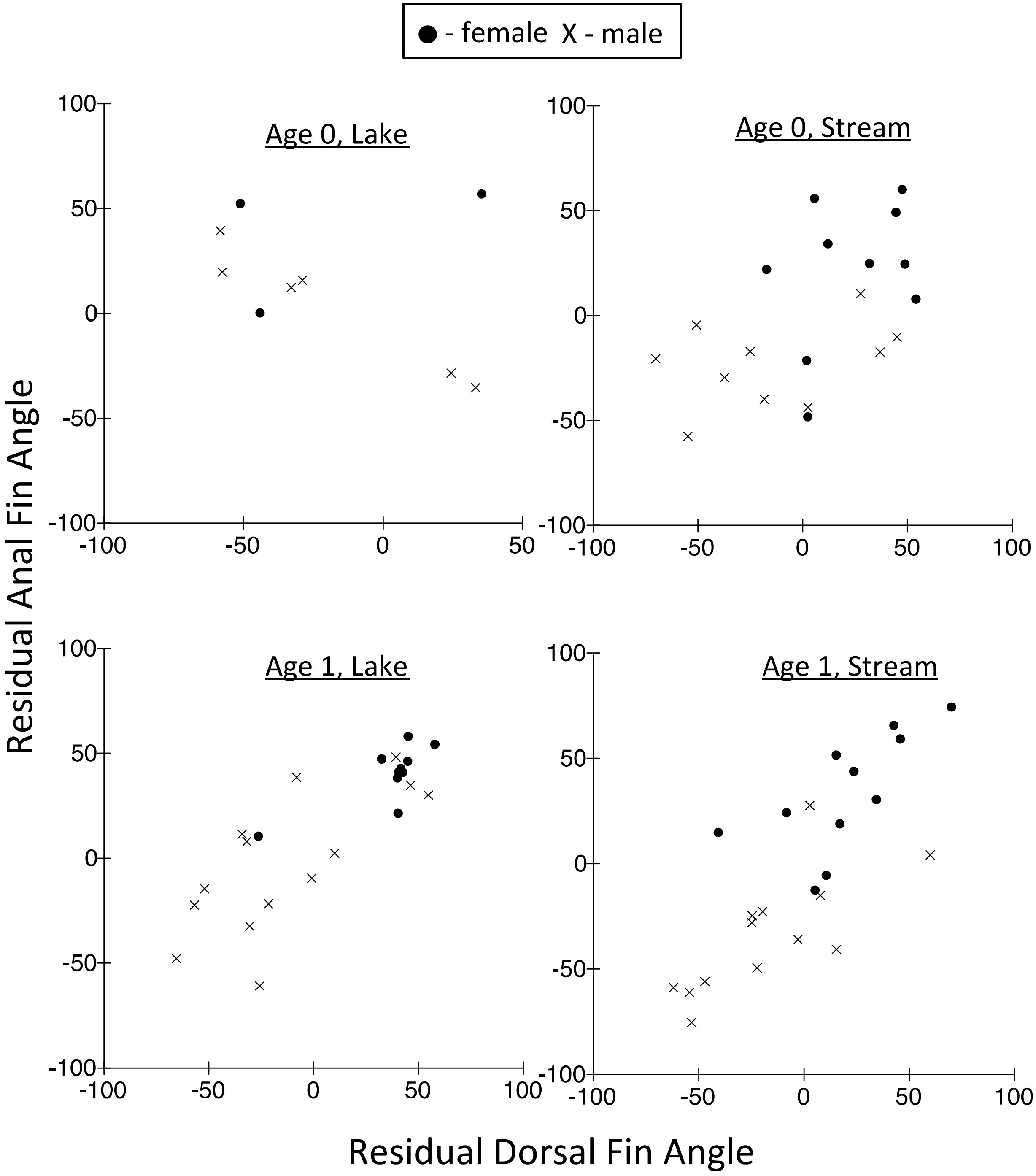


Figure 4.4. The relationship between residual dorsal fin angle and residual anal fin angle for each combination of age and habitat.

Tables

Table 4.1. Number of specimens collected at each site that was of the appropriate size for use in the analyses. Note that for some specimens, not every measurement of the fin was possible because of damage to the fins and, thus, sample sizes for a population for a particular analysis may be lower than the numbers shown here.

Site	Habitat Type	Total Specimens	Age Class		Sex	
			0	1	Male	Female
Homer Lake	Lake	15	7	8	8	7
Clear Lake, Kickapoo State Park	Lake	15	8	5	9	6
Long Lake, Kickapoo State Park	Lake	12	1	11	9	3
Philip’s Tract	Stream	9	6	3	6	3
Salt Fork	Stream	13	9	4	6	7
Kaufman Park	Lake	13	0	13	5	8
Nursing Creek	Stream	9	1	8	5	4
Antique Creek	Stream	15	11	4	10	5
Weldon Springs State Park	Lake	27	15	12	18	9
Lake Decatur	Lake	11	0	8	5	3
Kickapoo Creek	Stream	19	8	11	8	11
Steven’s Creek	Stream	20	7	13	9	11

Table 4.2. Analyses of variance on residual anal fin base length of (A) age 0 and (B) age 1 individuals. Significant ($p<0.05$) factors are shown in **bold**. Superscript refers to the term used in conjunction with the mean square error to generate the Satterthwaite approximation error degrees of freedom to calculate the F-value for that factor.

A) Age 0

Term #	Factor	df	MS	F	P
1	habitat type (HT)^4	1	0.240	0.10	0.7659
2	sex^5	1	1.427	2.96	0.1553
3	sex x HT^5	1	0.872	1.82	0.2473
4	population (HT)^5	5	2.273	4.66	0.0584
5	sex x population (HT)^6	5	0.488	0.84	0.5289
6	error	49	0.582		

B) Age 1

Term #	Factor	df	MS	F	P
1	habitat type (HT)^4	1	0.008	0.03	0.8667
2	**sex^5**	**1**	**5.078**	**18.40**	**0.0010**
3	sex x HT^5	1	0.0001	0.00	0.9875
4	population (HT)^5	8	0.260	1.03	0.4821
5	sex x population (HT)^6	8	0.252	0.55	0.8134
6	error	53	0.454		

Table 4.3. Analyses of variance on residual anal fin surface area of (A) age 0 and (B) age 1 individuals. Significant ($p<0.05$) factors are shown in **bold**. Superscript refers to the term used in conjunction with the mean square error to generate the Satterthwaite approximation error degrees of freedom to calculate the F-value for that factor.

A) Age 0

Term #	Factor	df	MS	F	P
1	habitat type (HT)^4	1	12.169	0.28	0.6169
2	sex^5	1	1.183	0.05	0.8300
3	sex x HT^5	1	26.899	1.15	0.3350
4	population (HT)^5	5	39.483	1.81	0.2660
5	**sex x population (HT)^6**	**5**	**21.855**	**4.89**	**0.0011**
6	error	48	4.469		

B) Age 1

Term #	Factor	df	MS	F	P
1	habitat type (HT)^4	1	38.935	3.16	0.1078
2	sex^5	1	13.687	1.26	0.2890
3	sex x HT^5	1	2.089	0.19	0.6707
4	population (HT)^5	8	12.819	1.15	0.4257
5	sex x population (HT)^6	8	11.181	1.34	0.2397
6	error	67	8.347		

Table 4.4. Analyses of variance on residual anal fin angle of (A) age 0 and (B) age 1 individuals. Significant ($p<0.05$) factors are shown in **bold**. Superscript refers to the term used in conjunction with the mean square error to generate the Satterthwaite approximation error degrees of freedom to calculate the F-value for that factor.

A) Age 0

Term #	Factor	df	MS	F	P
1	habitat type (HT)^4	1	222.965	0.63	0.5012
2	**sex**^{5}	**1**	**16435**	**48.59**	**0.0049**
3	sex x HT^5	1	1678.425	5.16	0.1122
4	population (HT)^5	5	453.035	1.34	0.4238
5	sex x population (HT)^6	4	365.326	0.32	0.8619
6	error	33	1138.343		

B) Age 1

Term #	Factor	df	MS	F	P
1	habitat type (HT)^4	1	1160.993	3.41	0.0899
2	**sex**^{5}	**1**	**50236**	**59.43**	**<0.0001**
3	sex x HT^5	1	1135.940	1.34	0.2752
4	population (HT)^5	8	300.377	0.36	0.9175
5	sex x population (HT)^6	8	844.531	0.99	0.4571
6	error	50	855.326		

Table 4.5. Analyses of variance on residual dorsal fin base length of (A) age 0 and (B) age 1 individuals. Significant ($p<0.05$) factors are shown in **bold**. Superscript refers to the term used in conjunction with the mean square error to generate the Satterthwaite approximation error degrees of freedom to calculate the F-value for that factor.

A) Age 0

Term #	Factor	df	MS	F	P
1	habitat type (HT)^4	1	0.005	0.01	0.9316
2	sex^5	1	0.328	0.31	0.6041
3	sex x HT^5	1	1.127	1.06	0.3614
4	population (HT)^5	5	0.624	0.62	0.6945
5	sex x population (HT)^6	5	1.009	1.49	0.2122
6	error	44	0.689		

B) Age 1

Term #	Factor	df	MS	F	P
1	habitat type (HT)^4	1	0.024	0.04	0.8415
2	**sex^5**	**1**	**6.327**	**11.14**	**0.0135**
3	sex x HT^5	1	0.128	0.22	0.6511
4	population (HT)^5	6	0.548	0.95	0.5230
5	sex x population (HT)^6	6	0.575	1.27	0.2889
6	error	53	0.454		

Table 4.6. Analyses of variance on residual dorsal fin surface area of (A) age 0 and (B) age 1 individuals. Significant ($p<0.05$) factors are shown in **bold**. Superscript refers to the term used in conjunction with the mean square error to generate the Satterthwaite approximation error degrees of freedom to calculate the F-value for that factor.

A) Age 0

Term #	Factor	df	MS	F	P
1	habitat type (HT)^4	1	20.148	0.89	0.3904
2	sex^5	1	7.649	0.55	0.4953
3	sex x HT^5	1	13.790	0.96	0.3763
4	population (HT)^5	5	20.128	1.54	0.3224
5	**sex x population (HT)^6**	**5**	**13.030**	**3.16**	**0.0164**
6	error	41	4.122		

B) Age 1

Term #	Factor	df	MS	F	P
1	habitat type (HT)^4	1	8.241	0.55	0.4853
2	**sex^5**	**1**	**132.326**	**28.34**	**0.0010**
3	sex x HT^5	1	2.439	0.53	0.4911
4	population (HT)^5	6	15.244	3.40	0.0812
5	sex x population (HT)^6	6	4.490	0.58	0.7429
6	error	55	7.711		

Table 4.7. Analyses of variance on residual dorsal fin angle of (A) age 0 and (B) age 1 individuals. Significant ($p<0.05$) factors are shown in **bold**. Superscript refers to the term used in conjunction with the mean square error to generate the Satterthwaite approximation error degrees of freedom to calculate the F-value for that factor.

A) Age 0

Term #	Factor	df	MS	F	P
1	habitat type (HT)^{4}	1	3612.96	5.54	0.0769
2	**sex**^{5}	**1**	**6603.643**	**12.62**	**0.0226**
3	sex x HT^{5}	1	1335.220	2.59	0.1847
4	population (HT)^{5}	5	680.908	1.24	0.4108
5	sex x population (HT)^{6}	5	550.813	0.47	0.7932
6	error	20	1169.013		

B) Age 1

Term #	Factor	df	MS	F	P
1	habitat type (HT)^{4}	1	308.511	1.18	0.3137
2	**sex**^{5}	**1**	**16427**	**12.53**	**0.0149**
3	sex x HT^{5}	1	54.665	0.04	0.8458
4	population (HT)^{5}	5	231.504	0.17	0.9608
5	sex x population (HT)^{6}	5	1324.597	1.35	0.2693
6	error	33	983.331		

Table 4.8. Overall correlations between standard length (SL), anal fin angle, anal fin base length, anal fin surface area, dorsal fin angle, dorsal fin base length, and dorsal fin surface area. Pearson's correlation coefficients, p-values, and sample sizes are listed. P-values less than 0.0001 are listed in **bold**.

	SL	Anal Angle	Anal Base Length	Anal Surface Area	Dorsal Angle	Dorsal Base Length	Dorsal Surface Area
SL	1	-0.11716 (0.2064) 118	**0.76248** **(<.0001)** **158**	**0.8849** **(<.0001)** **153**	-0.21991 (0.0313) 96	**0.67616** **(<.0001)** **146**	**0.74029** **(<.0001)** **141**
Anal Angle		1	-0.2089 (0.0238) 117	-0.1447 (0.1196) 117	**0.58535** **(<.0001)** 77	-0.12391 (0.1971) 110	-0.14826 (-0.1275) 107
Anal Base Length			1	**0.76708** **(<.0001)** 153	-0.23431 (0.0223) 95	**0.64758** **(<.0001)** 144	**0.64283** **(<.0001)** 139
Anal Surface Area				1	-0.22833 (0.026) 95	**0.64272** **(<.0001)** 141	**0.74697** **(<.0001)** 136
Dorsal Angle					1	-0.30247 (0.003) 94	-0.30984 (0.0022) 95
Dorsal Base length						1	**0.81093** **(<.0001)** **139**
Dorsal Surface Area							1

Table 4.9. Correlations among size corrected anal fin angle, anal fin base length, anal fin surface area, dorsal fin angle, dorsal fin base length, and dorsal fin surface area across all individuals. Pearson's correlation coefficients, (p-values), and sample sizes are listed. P-values less than 0.0001 are listed in bold.

	anal angle residual	anal base length residual	anal surface area residual	dorsal angle residual	dorsal base length residual	dorsal surface area residual
anal angle residual	1	-0.19119 (0.0389) 117	-0.09579 (0.3042) 117	**0.58195** **(<.0001)** 77	-0.11357 (0.2375) 110	-0.13336 (0.1709) 107
anal base length residual		1	0.2671 (0.0008) 153	-0.10674 (0.3032) 95	0.27102 (0.001) 144	0.18161 (0.0324) 139
anal surface area residual			1	-0.08093 (0.4356) 95	0.13211 (0.1184) 141	0.31404 (0.0002) 136
dorsal angle residual				1	-0.22254 (0.0311) 94	-0.22043 (0.0318) 95
dorsal base length residual					1	**0.62514** **(<.0001)** 139
dorsal surface area residual						1

Table 4.10. Coefficients of variation (CV) of the angle of each fin for both sexes across the two age classes.

Fin	Sex	Age	CV
Anal	Female	0	24.98
		1	17.17
	Male	0	33.85
		1	40.00
Dorsal	Female	0	24.89
		1	16.73
	Male	0	33.19
		1	38.91

Chapter 5: Genetics and water velocity on size and shape of *Fundulus notatus*

Abstract

Phenotypic differences between populations of the same species occupying different habitats are common and could reflect phenotypic plasticity and/or genetic differentiation between different populations. Body size and shape are common phenotypic differences in fish that vary among habitats. Here, we explored whether body size and in the blackstripe topminnow, *Fundulus notatus*, is a result of genetic differentiation and/or phenotypic plasticity. We created families from lake and stream parents and reared offspring under both moving water (lotic) and still water (lentic) conditions to explore the role of genetics and water velocity on size and shape. We found that both genetics and sex-specific responses to water velocity influence body size. Dorsal fin length showed a complex interaction between genetics and water velocity that was independent of sex. Both genetics and phenotypic plasticity result in pattern where stream fish (regardless of rearing environment) and lake fish raised in the lotic environment have a longer dorsal fin base than lake fish raised in the lentic environment. The caudal fin aspect ratio was the only aspect of body shape that showed a difference among the sexes. Interestingly, lake fish exhibit greater phenotypic plasticity than stream fish. These patterns offer new sights into the processes driving phenotypic differences in fish.

Introduction

Phenotypic differences between populations of the same species occupying different habitats are common. These differences could reflect phenotypic plasticity and/or genetic differentiation. Plasticity, which is the ability of an organism to express different phenotypes depending on the environment it is in, is a widespread occurrence in nature (Travis 1994). Sex-specific differences in phenotypic plasticity are also known in several taxa (Stillwell and Davidowitz 2010). However, not all traits may show phenotypic plasticity, as the ability to be plastic may have fitness related costs (Relyea 2002). As such, differences that exist can be due simply to genetic divergence between different populations.

Fish body size and shape are good traits to focus on when examining phenotypic plasticity and genetic differences. Many species grow faster under moving water (Nicoletto 1996, Azuma *et al*. 2002, Grünbaum *et al*. 2007), suggesting plasticity. However, differences in body size and shape due because of genetic differences have also been found between stream and lake habitats in several species of fish (Skúlason *et al*. 1996, Thompson *et al*. 1997, Albert *et al*. 2008). Body size and shape in fish may reflect adaptation to the environment. Both size and shape have strong effects on an individual's maneuverability in many species and, because of this, are believed to be targets for selection (Walker 1997, Fraser *et al*. 2011).

Changes in flow regime impact a fish's body size and shape (Webb 1984, Pakkasmaa and Piironen 2001, Langerhans 2008, Sagnes and Statzner 2009). Many studies have documented differences in body size and/or shape between lakes and streams (Hendry *et al*. 2002, McGuigan *et al*. 2003, Neat *et al*. 2003, Collin and Fumagalli 2011, Welsh *et al*. 2013). As mentioned previously, water velocity is known to affect fish body size through impacts on growth, although the results seem to be inconsistent- some fish reared under moving water growing faster (and attained larger body size) than those raised in still water (Davison 1997, Grünbaum *et al*. 2007) whereas other studies have shown no effect (or a negative effect) of stream speed on growth rates (Bhagat *et al*. 2006, Páez *et al*. 2008, Fischer-Rousseau *et al*. 2010). With regard to body shape, individuals in high water velocities are predicted to be narrower and shallower ("more streamlined") when compared to those in low velocities (Langerhans 2008). Again, however, other species either show no difference in shape or the opposite pattern (Pakkasmaa and Piironen 2001, McGuigan *et al*. 2003, Neves and Monteiro 2003, Krabbenhoft *et al*. 2009). The caudal fin is predicted to vary based on flow regime, with a larger aspect ratio (larger ratio of height to surface area) in high flow environments because it aids in thrust while minimizing drag, which is beneficial for prolonged periods of swimming (Langerhans 2008) and research in other fish species have found evidence that water velocity induces changes that are a result of phenotypic plasticity (Imre *et al.* 2002). The dorsal and anal fins may also vary, as biomechanical studies have found them to be utilized during swimming for stabilization and maneuverability (Lauder and Drucker 2004, Standen and Lauder 2007), and water velocity alters the development of median fin development in newly hatched salmonids (Cloutier *et al*. 2010).

In the blackstripe topminnow, *Fundulus notatus*, fish differ in body size (and to a lesser extent shape) between lakes and streams (Welsh *et al.* 2013). In particular, individuals from streams have a larger body size. This pattern was substantiated in a comparative study examining the Fundulidae family (Chapter 2). Differences in body shape are mainly due to sexual dimorphism, but stream fish were found to have a longer dorsal fin base than lake fish. Such across population patterns are highly intriguing for evolutionary ecologists because they raise two questions. The first question is what ultimate (i.e. selective) forces gave rise to these patterns. Lakes and streams differ in a number of attributes including water velocity, predation, and prey types that might generate selection on body size/shape. The second question that arises is whether population differences are due to genetic differentiation, phenotypic plasticity, or a combination of the two? This study addresses the latter question. Specifically, the goals of this experiment are to determine the extent to which variation in body size and shape in the blackstripe topminnow is affected by genetics and/or water velocity and whether these patterns are consistent across the sexes.

Methods

Experimental Design

Wild caught individuals from one lake (Long Lake in Kickapoo State Park) and one stream (a tributary of the Salt Fork of the Vermilion River) were collected in May and June of 2012. Ten lake and ten stream families were then created by placing one male and one female from the same location into a 20 L tank containing a sponge filter and 4-6 yarn "mops" for spawning substrate. Two or three mops were attached to PVC pipe to let it sink to the bottom of the tank and the others were attached to small Styrofoam balls to act as floating substrate. To encourage egg production, fish were fed ad libitum 1-2 times per day with a mixture of Tetramin flake food and frozen brine shrimp (*Artemia* spp). Each tank was checked 1-2 times per day for eggs. The eggs were then placed into a small, labeled plastic container with water. Pairs were kept together until about 20 eggs were obtained or no new eggs were produced for five successive days.

Once eggs hatched, offspring (fry) were kept in the plastic container until the yolk was fully absorbed and they started to feed exogenously (usually 7-10 days after hatching). Fry were fed live brine shrimp nauplii ad libitum once per day. Once the fry were feeding exogenously, ten of each family were haphazardly collected and assigned to one of two water velocity treatments: lentic (still water) or lotic (moving water). The lentic treatment was created by setting up 10 L aquaria with a sponge filter but otherwise no water flow. The lotic treatment was created by setting up 10 L aquaria with a sponge filter and a Maxi-Jet 600 power head to generate water flow. This power head was chosen because it has an output of 160 gallons per hour (605 liters per hour), resulting in the strongest current of about 0.4 cm/s (as measured by a Hontzsch HFA flow meter), which is similar to the highest water velocity experienced by topminnows in the streams in the area. The power head contained a pre-filter sponge over the intake to reduce fry mortality due to the suction of the power head.

To prevent overly stressing the fry, the flow was gradually introduced into the tank by connecting the power head to a timer. The introduction consisted of 5 days of no flow (to ensure that they were feeding in their tanks and no mortality occurred from the transfer into the tank) followed by 3 week periods of increasing duration of flow (1 hour, 2 hours, 4 hours, 6 hours, 12 hours). This was designed to mimic the natural exposure that this species likely faces, as eggs and fry in both lakes and streams are typically found in shallow, side pools/coves that are fairly stagnant (Welsh personal observation, Foster 1969) and even adults are not typically found in the parts of a stream with the strongest velocity (Welsh personal observation, Carranza and Winn 1954, Foster 1969). Fry were fed a diet that changed as they grew, starting with live brine shrimp nauplii and then transitioning to frozen Daphnia and, eventually, frozen brine shrimp and flake food. In order to try to minimize size differences between tanks and treatments simply due to differences in amount of food, food was given in set amounts of 1.5 mL of the food per fish in the tank once per day. To assess how many fish were in the tank (i.e. account for any mortality), a quick "head count" was done during every feeding and a more thorough consensus was performed every 1-2 months.

Offspring were maintained in the lab under the rearing conditions for 7 months. This duration was chosen because this was the time it took them to reach about 32 mm in size (standard length), which is the smallest size at which sexually dimorphic morphometric traits typically appear (mainly the pattern on the black stripe on the side and on the fins- see Carranza and Winn 1954, Welsh in review). Once offspring reached 7 months of age, they were removed from their tank, sexed, and their right side photographed on a 1 mm by 1 mm grid background using a 12.1 megapixel Nikon Coolpix L110.

Body Size and Shape

Geometric morphometric techniques were used to determine the body size and shape of individuals based off of the digital photographs (reviewed in Rohlf and Marcus 1993, Zelditch *et al*. 2012). These techniques allow for comparisons of size and shape independent of each other based on anatomical landmarks defined by Cartesian coordinates. Using the TPS software series (http://life.bio.sunysb.edu/morph), we placed a series of 30 landmarks (10 homologous Type I, 20 "sliding" semi-landmarks) (Figure 5.1) on each photograph using tpsDig (v2.14). Type I landmarks are placed on distinctive, homologous features of the fish (i.e. center of the eye, anterior base of dorsal fin, etc.) whereas semi-landmarks are used to cover large regions of the body where there are no distinctive features (curves, large areas without homologous features, etc.) (Bookstein 1997, Mitteroecker and Gunz 2009). The "unbend" module was used in tpsUtil to compensate for bending of specimens.

A Generalized Procrustes analysis (GPA) on the "unbent" landmarks was performed in tpsRelw (v1.49) to remove variation in landmark configurations that is due to differences in factors unrelated to shape, such as size, position, and orientation (Rohlf and Slice 1990). This produced measures of size (centroid size) and shape (partial warp scores and uniform components) that are independent of each other. Thin plate splines in tpsRelw were used to visualize the shape changes that occurred along each principal component axis.

Centroid size was used as the measure of body size. A principal components analysis using the partial warp scores and uniform components in a covariance matrix was used as a measure of body shape. We only considered the first three principal components because individually they accounted for at least 10% of the variation and together they explained over 65% of the variation.

We also measured caudal fin aspect ratio. This was done to test the general prediction by Langerhans (2008) that fish in high flow environments should have a higher aspect ratio. Aspect ration was measured from the digital pictures using ImageJ (v.1.44p) (using the 1 mm grid as calibration). We calculated caudal fin aspect ratio as h^2/s, where "h" is the height of the fin and "s" is the surface area (Pauly 1989). Height was measured by a straight vertical line from one edge of the caudal fin to the other edge, and surface area was calculated by tracing around the entire fin (excluding the caudal peduncle) with the "polygon selection" tool.

Analyses

To explore whether body size and shape is affected by genetics and/or water velocity, we used an analysis of variance (ANOVA) that considers the main effects of parental habitat (i.e. genetic differences based on where the parents were from- lake or stream), rearing habitat (i.e. effects due to water velocity- lentic or lotic), and sex (male or female), as well as all interactions between these main effects. Because individuals within a particular tank cannot be considered independent samples, we conducted all analyses using the tank means. Separate ANOVAs were conducted for body shape (PC1, PC2, PC3), body size (centroid size) and caudal fin aspect ratio. Each analysis first began with a consideration of sex by including it, and all interactions with sex, using the mean for each sex in each tank. If sex or any interactions were strongly non-significant ($p>0.3$), we removed those effects and re-ran the analysis using the mean for each tank with the sexes combined. Because of uneven mortality between different treatments and different families, we also tested whether there was an effect of density on body size, shape, or caudal fin. We did this by including the number of fish in the tank at the end of the experiment as a term in the model. As above, this term was removed if non-significant. The final model for each analysis above (body size, body shape, and caudal fin) was first run on every family. However, because of uneven mortality, we then compared this analysis to one using just those families that had surviving offspring in both treatments (lentic and lotic). All analyses were conducted using SAS (v. 9.3).

Results

Body Size

Parental habitat and the interaction between sex and rearing habitat had effects on body size (Table 5.1). Males were much smaller than females when raised under lotic conditions, but trended toward being larger under lentic conditions (Figure 5.2). Regardless of sex, fish from stream parents were smaller than fish from lake parents. There was also a trend toward fish from lake parents to be larger when raised under lotic conditions than lentic conditions ($F_{1,27} = 3.61$, $p = 0.0680$) (Figure 5.3). There was no effect of density on body size ($F_{1,21} = 0.00$, $p = 0.9627$). These results were qualitatively the same when analyzing just those families with offspring in both rearing habitats.

Body Shape and Caudal Fin Aspect Ratio

PC1 was related to eye position and dorsal fin length. In particular, a negative PC1 value corresponded to a more centrally located eye and shorter dorsal fin whereas a positive PC1 value corresponded to a more ventrally placed eye and a longer dorsal fin (Figure 5.4). PC1 did not differ between sexes, but, rather, varied as a function of the interaction between parental habitat and rearing habitat (Table 5.1). Stream offspring had high values for PC1 regardless of whether they were raised in lentic or lotic treatments. Lake offspring were similar to stream offspring when raised under the lotic treatment (i.e. in flowing water), but differed when raised in the lentic treatment (i.e. still water). Specifically, lake offspring had a negative PC1 value (i.e. a centrally located eye and a shorter dorsal fin) when raised in the lentic treatment (Figure 5.4). PC1 was the only component of shape that showed any significant effects of parental habitat, rearing habitat, or sex (or their interactions) (results not shown). However, there these effects disappeared when considering just those families with offspring under both treatments (all $p > 0.3$), although this could be due to low sample size ($n = 14$ tanks). There was no effect of density on PC1 ($F_{1,21} = 2.31$, $p = 0.1437$).

There was pronounced sexual dimorphism in the caudal fin aspect ratio, where males had a larger aspect ratio than females (Figure 5.5). There was no effect of density on aspect ratio ($F_{1,21} = 0.20$, $p = 0.6620$). As with body shape, this result was qualitatively the same when we only considered those families with offspring in both rearing habitats.

Discussion

The goals of this experiment were to determine the extent to which genetics and/or water velocity influence body size and shape in the blackstripe topminnow and whether these patterns are consistent across the sexes. We found that both genetics and sex-specific responses to water velocity influence body size. Dorsal fin length showed a complex interaction between genetics and water velocity that was independent of sex. The caudal fin aspect ratio was the only aspect of body shape that showed a difference among the sexes. Despite only using families from one lake and one stream, the results were generally similar to those described for this family (chapter 2) and for this species (chapter 3). We discuss the findings of this study in more detail below.

Genetic Effects and Phenotypic Plasticity

In nature, one-year old *F. notatus* are larger in streams than in lakes and have longer dorsal fin bases (chapter 3). A phylogenetic analysis across the family indicated a similar pattern where fish are larger in lotic habitats than they are in lentic habitats (chapter 2). The phylogenetic study also duplicated the earlier finding for size in *F. notatus* where animals from lotic habitats were larger than those lentic habitats. Here, we sought to determine whether these patterns were likely attributable to phenotypic plasticity and/or genetic differentiation.

For size, there is an interaction between parental habitat (i.e. genetics) and rearing habitat (i.e. phenotypic plasticity) (Figure 5.3). The interaction is attributable to the fact that lake offspring differ in size between rearing habitats but stream offspring do not. Considering only the direction of plasticity in the lake fish, our results are consistent with previous studies in that fish in flowing water are larger. However, the lack of plasticity in the stream fish results in a pattern where the genetic signature contradicts previous findings because stream fish are, on average, smaller than lake fish. This result is not simply due to any idiosyncrasies from the fish used as parents, as the parents from the stream were, indeed, larger than those from the lake ($F_{1,24} = 5.25$, $p = 0.0310$).

A somewhat similar pattern can be seen for shape (PC1). Here, PC1 corresponds to the length of the dorsal fin base length. In chapter 3, we showed a slight effect where stream fish had slightly longer dorsal fin bases than did lake fish. Figure 5.4 shows the effects of parental environment and rearing environment on PC1. Again, lake offspring were plastic in their development whereas stream fish were not. Plasticity in lake fish again produces this same pattern, because fish raised in the lotic environment had a larger dorsal fin base than did fish raised in the lentic environment (which matches the patterns documented in chapter 3). However, unlike with size, the genetic signature for PC1 is also consistent with previous results because stream fish, on average, had greater values for PC1.

The motivating question for this chapter is whether genetics and/or environment produces the phenotypic patterns seen in size and shape across population in *F. notatus*. We can answer this somewhat for size. Simple genetic differentiation among populations does not cause stream fish to be larger than lake fish. Does phenotypic plasticity cause the pattern? This question is more difficult to assess. If both populations had showed enhanced growth in lotic treatments, then this would unequivocally implicate phenotypic plasticity. However, the heterogeneous response to rearing environment prevents such a conclusion. Other factors that might result in the across population pattern include possible differences in food availability (assuming there is more food in streams which equates to bigger fish), differences in growing season (water temperatures rise sooner in streams than in lakes), or differences in size-selective predation where large individuals are more likely to be preyed upon in lakes. Phenotypic plasticity is not always present (Pigliucci 1997) and, even when it is, there can be population-level differences in plasticity (Donohue *et al*. 2000, Lind and Johansson 2007).

While we may be unable to resolve the question of genetics versus plasticity for body size, we are able to answer this question for body shape. Both genetics and phenotypic plasticity result in pattern where stream fish (regardless of rearing environment) and lake fish raised in the lotic environment have a longer dorsal fin base than lake fish raised in the lentic environment. Hence, both genetics and environment contribute to these subtle changes in body shape documented between lakes and streams.

An intriguing question raised by this work is why the lake fish are more plastic with respect to water velocity than are the stream fish. It has been suggested that different levels of plasticity in different populations are due to differences in either genetic drift or selection on plasticity and not because of the costs of plasticity, itself (Steiner and Van Buskirk 2008, Lind and Johansson 2009). We currently have no information about the level of gene flow between populations or of any genetic differences between populations, so we cannot make inferences whether genetic drift explains the difference in plasticity. If the plasticity difference is due to selection due to environmental differences, one possible abiotic factor is water flow. Lakes have little in the way of moving water whereas streams vary both spatially and temporally in water flow. Spatially, water flow varies where flow is greatest in the middle of the stream compared to the sides where nooks and crannies along the stream bank provide areas of low flow. Flow also varies temporally with rainfall. Illinois streams frequently flood in the spring, but have reduced water flow in late summer/early fall. In the summer of 2012, many streams stopped flowing altogether. However, given this variation, one would hypothesize that stream fish would be more plastic in their growth than lake fish.

Growth may be canalized for stream fish. Wimberger (1994) suggested that polymorphisms (in morphology and other features) are less likely to occur in streams than lakes because they have less temporal stability. Canalization in streams assumes that responding plastically to every alteration in water flow is actually bad and, instead, fish develop in such a way as to buffer themselves from this environmental variation. While intriguing, this hypothesis does not explain why lake fish are plastic. It is also unknown whether this finding can be generalized to all lake populations in this species. *F. notatus* is found in many lakes and streams throughout central Illinois (Welsh *et al*. 2013), so this is a great system to explore the "repeatability" of such patterns. The lake population may be also an ideal system for future research related to the costs of phenotypic plasticity- which, as Lind and Johansson (2009) have noted, has been very difficult to find in natural populations- as they have suggested that efforts devoted to identifying costs should focus on highly plastic populations.

Effects Due to Sex

In comparison to the other chapters, this study found relatively mild differences between males and females. In chapters 2 and 3, body shape differences were often dominated by effects of sex. However, here, the only aspect of the body shape that showed sexual dimorphism was the caudal fin aspect ratio. Males had a larger caudal fin aspect ratio than females. Interpreting these results in the light of the previous chapters is more challenging, as the previous chapters did not investigate caudal fin aspect ratio. Why sex-specific differences should exist in the caudal fin aspect ratio is unclear. A large caudal fin aspect ratio is believed to be important for maintaining long periods of steady swimming while a smaller ratio is more important for short bursts and quick maneuvers (Webb 1984). Foster (1967) does report that males seem to engage in more swimming overall, both in terms of steady swimming and maneuvers. Hence, an adaptive explanation for this pattern is that the extra swimming demand in males may favor the emergence of sexual dimorphism in caudal fin aspect ratio.

The effect of sex on size found here is fairly consistent with the patterns described in the previous chapters. Chapter 2 found a subtle pattern across the family where females were larger than males provided that there were differences between the sexes, although *F. notatus* did not show this effect in either the comparative study (chapter 2) or the field survey (chapter 3). Here, we found that females were larger than males when raised in lotic treatments, but there were no such difference for the lentic treatment. Sex-specific differences in body size due to phenotypic plasticity is known in a small number of other species, including insects (Stillwell and Davidowitz 2010, Stillwell *et al*. 2010) and turtles (Fonseca 2010). A larger size due to water velocity has been attributed to several factors in other fish species, including enhanced food conversion efficiency, changes in aerobic metabolism, and reduced agonistic behavior (Davison 1997). All of these factors could differ between the sexes in *F. notatus*, resulting in the interaction between water velocity and sex. In fact, males are known to engage in more agonistic interactions than females (Carranza and Winn 1954, Foster 1967). Why this pattern is not observed in streams in nature is unclear, but may be related to their behavior. Both sexes of the blackstripe topminnow are only very rarely observed in the strongest parts of a stream, so it may be that females simply do not experience strong water velocities consistently enough to generate the differences observed in the lab (where they experienced more consistency in water velocity). Studies on microhabitat use between sexes within a stream would be needed to explore this possibility further.

References

Albert A. Y. K., S. Sawaya, T. H. Vines, A. K. Knecht, C. T. Miller, B. R. Summers, S.

Balabhadra, D. M. Kingsley, and D. Schluter. 2008. The genetics of adaptive shape shift in stickleback: pleiotropy and effect size. *Evolution* **62:** 76-85.

Bhagat Y., M. G. Fox, and M. T. Ferreira. 2006. Morphological differentiation in introduced pumpkinseed *Lepomis gibbosus* (L.) occupying different habitat zones in Portuguese reservoirs. *Journal of Fish Biology* **69 (**Suppl. C)**:** 79–94.

Bookstein F. L. 1997. Landmark methods for forms without landmarks: morphometrics of group differences in outline shape. *Medical Image Analysis* **1:** 225-243.

Carranza J. and H. E. Winn. 1954. Reproductive behavior of the blackstripe topminnow, *Fundulus notatus*. *Copeia* **1954:** 273–278.

Cloutier R., A. Caron, T. Grünbaum, and N. R. Le François. 2010. Effects of water velocity on the timing of skeletogenesis in the Artcic Charr, *Salvelinus alpinus* (Salmoniformes: Teleostei): an empirical case of developmental plasticity. *International Journal of Zoology* **2010:** 1-15.

Collin H. and L. Fumagalli. 2011. Evidence for morphological and adaptive genetic divergence between lake and stream habitats in European minnows (*Phoxinus phoxinus*, Cyprinidae). *Molecular Ecology* **20:** 4490–4502.

Collyer, M. L., C. A. Stockwell, D. C. Adams, and M. H. Reiser. 2007. Phenotypic plasticity and contemporary evolution in introduced populations: evidence from translocated populations of white sands pupfish (*Cyrpinodon tularosa*). *Ecological Research* **22:** 902-910.

Davison W. 1997. The effects of exercise training on teleost fish, a review of recent literature. *Comparative Biochemistry and Physiology A: Molecular and Integrative Physiology* **117A:** 67–75.

Donohue K., D. Messiqua, E. H. Pyle, M. S. Heschel, and J. Schmitt. 2000. Evidence of adaptive divergence in plasticity: density- and site-dependent selection on shade-avoidance responses in *Impatiens capenses*. *Evolution* **54:** 1956-1968.

Fischer-Rousseau L., K. P. Chu, and R. Cloutier. 2010. Developmental plasticity in fish exposed to a water velocity gradient: a complex response. *Journal of Experimental Zoology B–Molecular and Developmental Evolution* **314B:** 67–85.

Fonseca C. P. C. 2010. Phenotypic plasticity, sexual dimorphism, and Rensch's rule in turtles. PhD Dissertation, Iowa State University.

Foster N. R. 1967. Comparative studies on the biology of killifishes (Pisces, Cyprinodontidae). PhD Dissertation, Cornell University.

Fraser D. J., L. K. Weir, L. Bernatchez, M. M. Hansen, and E. B. Taylor. 2011. Extent and scale of local adaptation in salmonid fishes: review and meta-analysis. *Heredity* **106:** 404-420.

Grünbaum T., R. Cloutier, P. M. Mabee, and N. R. Le Francois. 2007. Early developmental plasticity and integrative responses in arctic charr (*Salvelinus alpinus*): effects of water velocity on body size and shape. *Journal of Experimental Zoology* **308B:** 396–408.

Helland, I. P., L. A. Vøllestad, J. Freyhof, and T. Mehner. 2009. Morphological differences between two ecologically similar sympatric fishes. *Journal of Fish Biology* **75:** 2756-2767.

Hendry A. P., E. B. Taylor, and J. D. McPhail. 2002**.** Adaptive divergence and the balance between selection and gene flow: lake and stream stickleback in the Misty system. *Evolution* **56:** 1199–1216.

Howick G. L. and W. J. O'Brien. 1983. Piscivorous feeding behavior of largemouth bass: an experimental analysis. *Transactions of the American Fisheries Society* **112:** 508-516.

Imre I. R. L. McLaughlin, and D. L. G. Noakes. 2002. Phenotypic plasticity in brook charr: changes in caudal fin induced by water flow. *Journal of Fish Biology* **61:** 1171-1181.

Krabbenhoft T. J., M. L. Collyer, and J. M. Quattro. 2009. Differing evolutionary patterns underlie convergence on elongate morphology in endemic fishes of Lake Waccamaw, North Carolina. *Biological Journal of the Linnean Society* **98:** 636-645.

Langerhans R. B. 2008. Predictability of phenotypic differentiation across flow regimes in fishes. *Integrative and Comparative Biology* **48:** 750–768.

Larimore R. W. and P. B. Bayley. 1996. The fishes of Champaign County, Illinois, during a century of alterations of a prairie ecosystem. *Illinois Natural History Survey Bulletin* **35:** 53–183.

Lauder G. V. and E. G. Drucker. 2004. Morphology and experimental hydrodynamics of fish fin control surfaces. *IEEE Journal of Oceanic Engineering* **29:** 556-571.

Lind M. I. and F. Johansson. 2007. The degree of phenotypic plasticity is correlated with the spatial environmental heterogeneity experienced by island population of *Rana temporaria*. *Journal of Evolutionary Biology* **20:** 1288-1297.

Lind M. I. and F. Johansson. 2009. Costs and limits of phenotypic plasticity in island populations of the common frog *Rana temporaria* under divergent selection pressures. *Evolution* **63:** 1508-1518.

McGuigan K, C. E. Franklin, C. Moritz, and M. W. Blows. 2003. Adaptation of rainbow fish to lake and stream habitats. *Evolution* **57:** 104-118.

Mitteroecker P. and P. Gunz. 2009. Advances in geometric morphometrics. *Evolutionary Biology* **36:** 235-247.

Neat F. C., W. Lengkeek, E. P. Westerbeek, B. Laarhoven, J. J. Videler. 2003. Behavioural and morphological differences between lake and river populations of *Salaria fluviatilis*. *Journal of Fish Biology* **63:** 374-387.

Neves F. M. and L. R. Monteiro. 2003. Body shape and size divergence among populations of Poecilia vivipara in coastal lagoons of south-eastern Brazil. *Journal of Fish Biology* **63:** 928-941.

Páez D. J., R. Hedger, L. Bernatchez, and J. J. Dodson. 2008. The morphological plastic response to water current velocity varies with age and sexual state in juvenile Atlantic salmon, *Salmo salar*. *Freshwater Biology* **53:** 1544-1554.

Pakkasmaa S. and J. Piironen. 2001. Water velocity shapes juvenile salmonids. *Evolutionary Ecology* **14:** 721-730.

Pauly D. 1989. A simple index of metabolic level in fishes. *Fishbyte, Newsletter of the Network of Tropical Fisheries Scientists* **7:** 22.

Pigliucci M. 1997. Ontogenetic phenotypic plasticity during the reproductive phase in *Arabidopsis thaliana* (Brassicaceae). *American Journal of Botany* **84:** 887-895.

Relyea R. A. 2002. Costs of phenotypic plasticity. *The American Naturalist* **159:** 272-282.

Rohlf, F. J. and L. F. Marcus. 1993. A revolution in morphometrics. *Trends in Ecology and Evolution* **8:** 129-132.

Rohlf, F. J. and D. E. Slice. 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Zoology* **39:** 40-59.

Sagnes P. and B. Statzner. 2009. Hydronamic abilities of riverine fish: a functional link between morphology and velocity use. *Aquatic Living Resources* **22:** 79-91.

Skúlason S., S. S. Snorrason, D. L. G. Noakes, and M. M. Ferguson. 1996. Genetic basis of life history variations among sympatric morphs of Arctic char, *Salvelinus alpinus*. *Canadian Journal of Fisheries and Aquatic Sciences* **53:** 1807-1813.

Standen E. M. and G. V. Lauder. 2007. Hydrodynamic function of dorsal and anal fins in brook trout (*Salvelinus fontinalis*). *The Journal of Experimental Biology* **210:** 325-339.

Stillwell R. C. and G. Davidowitz. 2010. Sex differences in phenotypic plasticity of a mechanism that controls body size: implications for sexual size dimorphism. *Proceedings of the Royal Society B* **277:** 3819-3826.

Stillwell R. C., W. U. Blanckenhorn, T. Teder, G. Davidowitz, and C. W. Fox. 2010. Sex differences in phenotypic plasticity affect variation in sexual size dimorphism in insects: from physiology to evolution. *Annual Review of Entomology* **55:** 227-245.

Thompson C. E., E. B. Taylor, J. D. McPhail. 1997. Parallel evolution of lake-stream pairs of threespine sticklebacks (Gasterosteus) inferred from mitochondrial dna variation. *Evolution* **51:** 1955-1965.

Travis J. 1994. Evaluating the adaptive role of morphological plasticity. In: Wainwright P. C. and S. M. Reilly, eds. *Ecological Morphology*. Chicago: University of Chicago Press.

Van Buskirk J. and U. K. Steiner. 2009. The fitness costs of developmental canalization and plasticity. *Journal of Evolutionary Biology* **22:** 852-860.

Walker J. A. 1997. Ecological morphology of lacustrine three-spine stickleback *Gasterosteus aculeatus* L. (Gasterosteridae) body shape. *Biological Journal of the Linnean Society* **61:** 3-50.

Webb P. W. 1984. Form and function in fish swimming. *Scientific American* **251:** 58–68.

Welsh D. P., M. Zhou, S. M. Mussmann, L. G. Fields, C. L. Thomas, S. P. Pearish, S. L. Kilburn, J. L. Parker, L. R. Stein, J. A. Bartlett, C. R. Bertram, T. J. Bland, K. L. Laskowski, B. C. Mommer, X. Zhuang, R. C. Fuller. 2013. The effects of age, sex, and habitat on body size and shape of the blackstripe topminnow, *Fundulus notatus* (Cyprinodontiformes: Fundulidae) (Rafinesque 1820). *Biological Journal of the Linnean Society* **108:** 784-789.

West-Eberhard M. J. 2003. *Developmental plasticity and evolution*. Oxford University Press, New York.

Wimberger P. H. 1994. Trophic polymorphisms, plasticity, and speciation in vertebrates. In: Stouder D. J., K. L. Fresh, and R. J. Feller, eds. *Theory and Application in Fish Feeding Ecology*. Columbia: University of South Carolina Press.

Zelditch, M. L., D. L. Swiderski, and H. D. Sheets. 2012. *Geometric morphometrics for Biologists: A Primer*. 2nd edition. Academic Press, Massachusetts.

Figures

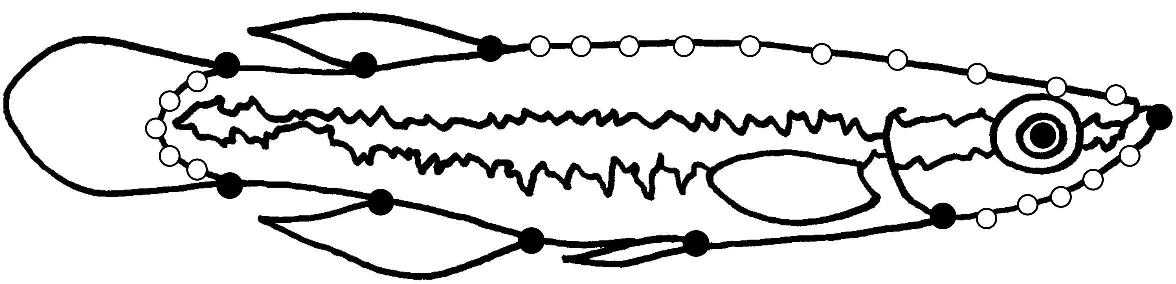


Figure 5.1. Landmarks used for geometric morphometric analysis. Black dots refer to homologous Type I landmarks and white dots denote semi-landmarks.

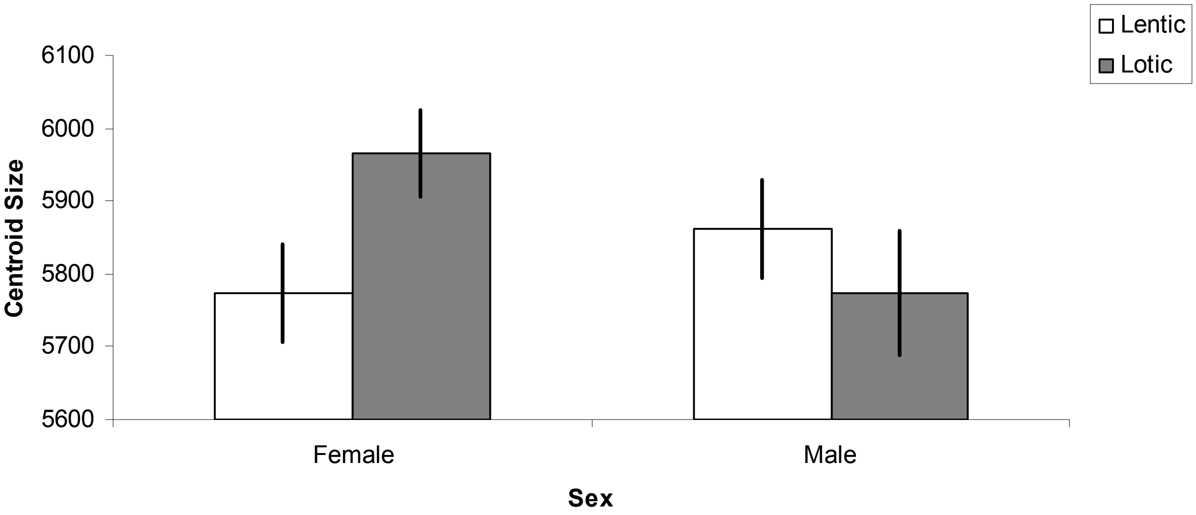


Figure 5.2. Body size as a function of the interaction between sex and <u>rearing habitat</u>. Error bars are ± 1 standard error.

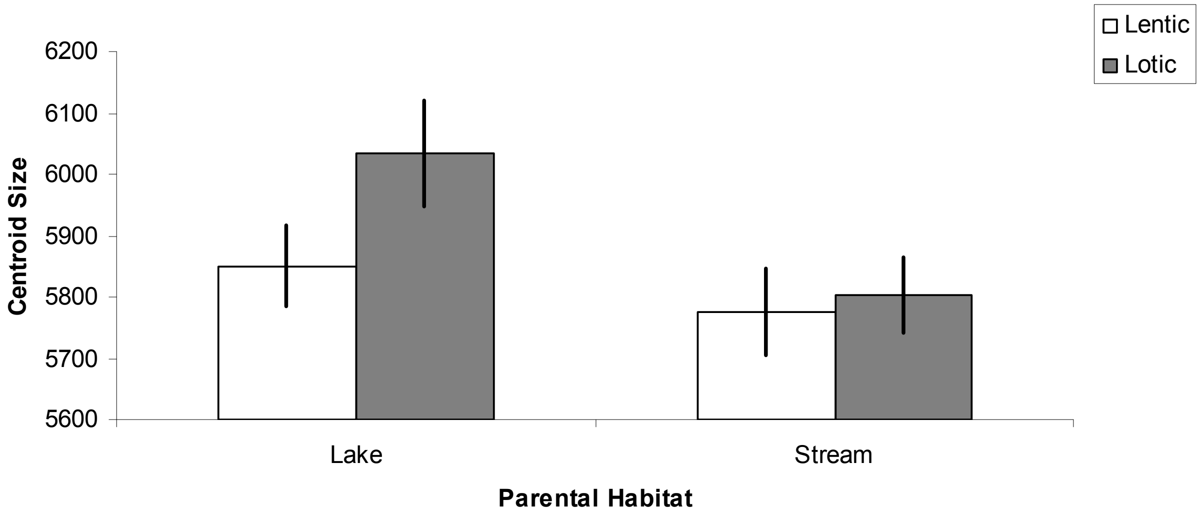


Figure 5.3. Body size as a function of parental habitat and rearing habitat. Error bars are ± 1 standard error.

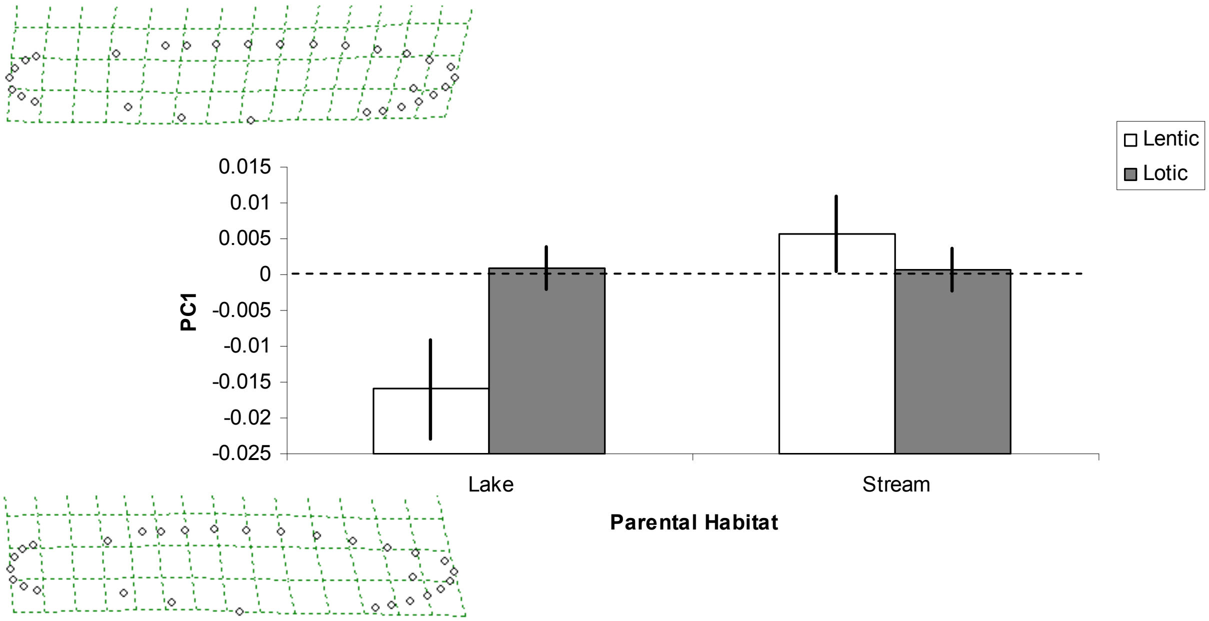


Figure 5.4. PC1 as a function of the interaction between parental habitat and rearing habitat. Error bars denote 1 standard error. Thin plate splines are shown to illustrate the shape changes along PC1.

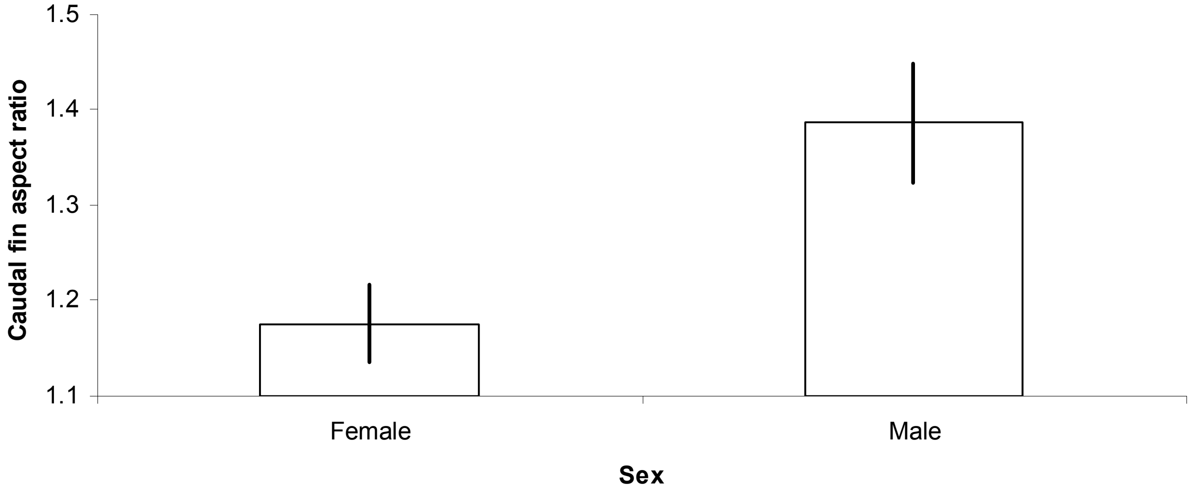


Figure 5.5. Caudal fin aspect ratio as a function of sex. Error bars are ± 1 standard error.

Tables

Table 5.1. F-values from analyses of variance on (A) Centroid Size, (B) PC1, and (C) Caudal Fin Aspect Ratio. For PC1, sex was removed from the analysis because it was strongly non-significant ($p>0.3$). For all, any interactions that were strongly non-significant ($p>0.3$) were removed. Significant ($p<0.05$) factors are indicated in **bold**.

A) Centroid Size

Factor	DF	MS	F	P
parental habitat	**1**	**246434.1**	**7.26**	**0.0120**
rearing habitat	1	105240.8	3.10	0.0896
parental habitat x rearing habitat	1	122710	3.61	0.0680
sex	1	83726.6	2.47	0.1280
sex x rearing habitat	**1**	**235070**	**6.92**	**0.0139**
error	27	33952.1		

B) PC1

Factor	DF	MS	F	P
parental habitat	1	0.00068	4.16	0.0535
rearing habitat	1	0.00021	1.29	0.2682
parental habitat x rearing habitat	**1**	**0.00072**	**4.43**	**0.0469**
error	22	0.00016		

Table 5.1 (cont.)
C) Caudal Fin Aspect Ratio

Factor	DF	MS	F	P
parental habitat	1	0.031	0.88	0.3600
rearing habitat	1	0.013	0.37	0.5478
parental habitat x rearing habitat	1	0.048	1.39	0.2531
sex	**1**	**0.310**	**8.93**	**0.0076**
sex x parental habitat	1	0.105	3.01	0.0988
error	19	0.035		