Canadian Journal of Zoology
Revue canadienne de zoologie

**Morphological change and phenotypic plasticity in response
to water velocity
in three species of Centrarchidae**

Journal:	<i>Canadian Journal of Zoology</i>
Manuscript ID	cjz-2015-0096.R1
Manuscript Type:	Article
Date Submitted by the Author:	08-Sep-2015
Complete List of Authors:	Istead, Ashley; Trent University, Environmental & Resource Studies Program and Department of Biology Yavno, Stan; Trent University, Environmental & Life Sciences Graduate Program Fox, Michael; Trent University, Environmental and Research Studies Program
Keyword:	bluegill, common environment, G x E interaction, LOCOMOTION < Discipline, pumpkinseed, rock bass, reaction norms

SCHOLARONE™
Manuscripts

**Morphological change and phenotypic plasticity in response to water velocity
in three species of Centrarchidae**

Running Title: Morphology and plasticity in three centrarchids

A. E. ISTEAD¹, S. YAVNO², and M. G. FOX^{1,3}

¹Environmental and Resources Studies Program and Department of Biology, Trent University,
Peterborough, Ontario, K9J 7B8, CANADA

²Environmental and Life Sciences Graduate Program, Trent University, Peterborough
Ontario, K9J 7B8, CANADA

³Author to whom correspondence should be addressed at present address: Environmental and
Resources Studies Program and Department of Biology, Trent University, Peterborough,
Ontario, K9J 7B8, CANADA. Tel: +1 (705) 748-1011 x7873; email: mfox@trentu.ca

Abstract: Morphological responses to flowing water have been documented in many fishes, but rarely reported in species with gibbose body types, despite their use of riffles in rivers and streams. In this study, we compared morphological responses to water flow in three gibbose centrarchids; the bluegill *Lepomis macrochirus* (Rafinesque, 1819), pumpkinseed *Lepomis gibbosus* (L., 1758), and rock bass *Ambloplites rupestris* (Rafinesque, 1817). Flow-through plexiglass units, set to either a sustained flow or static conditions, were used to rear juveniles from each species for an 80-day period. All three species developed more streamlined body forms, longer and thinner caudal peduncles and longer pectoral fins when reared in sustained current. The pumpkinseed exhibited the highest level of phenotypic plasticity in response to water flow, followed closely by bluegill; rock bass was the least plastic species. Rock bass developed longer pre-dorsal and pre-pectoral lengths, which differ from the morphological changes observed in the bluegill and pumpkinseed. The findings not only suggest that some gibbose fishes are capable of exhibiting strong phenotypic responses to water flow, but that many morphological changes are species specific, even within the same taxonomic family. Lastly, all three species developed shorter dorsal fin base lengths when reared under flowing condition, which differs from some previously documented work on fusiform fishes. The results of this study provide a better understanding of how morphology in three closely related species varies in response to environmental conditions.

Key Words: bluegill; common environment; G x E interaction; locomotion; pumpkinseed; rock bass; reaction norms.

22 **Introduction**

23 Phenotypic plasticity, the ability for a genotype to express alternate phenotypes in
24 response to an environmental condition (West-Eberhard 1989; Agrawal 2001), facilitates
25 the modification of behavioural, life history, morphological and physiological traits
26 (Stearns 1989; Scheiner 1993; Hoverman and Relyea 2008). These changes can produce
27 phenotypes that are better matched to the environment (DeWitt, Sih and Wilson 1998),
28 increasing survival by allowing organisms to adjust to changing conditions (Pfennig et al.
29 2010) and thereby achieving higher fitness (Agrawal 2001; Parsons and Robinson 2006).
30 Morphological plasticity, which is well-documented in fishes, has revealed that
31 genetically similar individuals, reared under different environmental conditions, are
32 capable of exhibiting very distinct traits. Within a single species, morphological
33 differentiation is clearly visible among individuals that occupy different habitats
34 (Ehlinger and Wilson 1988; Imre et al. 2002), and fish can exhibit specialized
35 morphological traits based on their habitat (e.g. Ehlinger 1990; Gillespie and Fox 2003).

36 Water velocity is a crucial environmental factor that shapes the body form of
37 fishes because stronger hydrological forces may favor the evolution of more efficient
38 propulsive traits (Standen and Lauder 2005). Strong currents create significant drag
39 forces, favouring body forms that are more fusiform (i.e. narrower body depths, caudal
40 dimensions) which, in turn, improve swimming performance through a reduction in
41 hydrodynamic drag (Webb 1984a). Swimming against a current is energetically costly
42 because it requires sustained body movements in order to maintain a relative position
43 (Alcaraz and Urrutia 2008). If phenotypic plasticity allows the development of a more
44 hydrodynamic body form, the energy saved would be available for feeding, reproduction

and overall growth. Bearing in mind that phenotypic plasticity relates to specific traits and is not a general characteristic of the whole organism (Schlichting and Pigliucci 1998), phenotypic plasticity in one or a series of traits can be species-specific, and the degree to which phenotypic changes occur depends largely on the strength of the environmental gradient (Pakkasmaa and Piironen 2001; Fischer-Rousseau et al. 2010). However, several traits can respond in integrated fashion to a given environmental factor, especially when the species are closely related, and this kind of coordinated morphological response can be the result of environmental, functional and/or evolutionary integration (reviewed in Pugliucci 2003, Cooper et al. 2011 and Klingenberg 2014). Previous studies with fishes have demonstrated that environmental gradients can produce both shared and unique morphological responses in several species subjected to the same factors (Langerhans and DeWitt 2004; Langerhans 2008).

The effects of water velocity on fish morphology are frequently examined using taxa with streamlined body forms, such as Salmonidae (e.g. Pakkasmaa and Piironen 2001; Imre et al. 2002), Cyprinidae (e.g. Haas et al. 2010; Franssen et al. 2012), Poeciliidae (e.g. Zuniga-Vega et al. 2007; Leavy and Bonner 2009), and Atheriniformes (Leavy and Bonner 2009; Foster and Piller 2015). Salmonids in particular have fusiform bodies, and most of them use subcarangiform locomotion to propel themselves through the water column (Ojanguren and Brana 2003); many of these species also inhabit streams with moderate to high water velocities (Pakkasmaa and Piironen 2001). On the other hand, gibbose, laterally compressed fishes such as Centrarchidae spend the majority of their lives in lentic habitats (e.g. Osenberg et al. 1988) and they use median and paired fins for swimming (Wootton 1998; Higham 2007). Locomotory strategies, which are

inherently linked to body shape and morphological traits (Webb 1984a; Standen and Lauder 2005), are clearly distinct between centrarchids and salmonids. Therefore, morphological responses to water velocity may also differ between these two groups.

While the morphology of gibbose fishes may respond differently to water velocity exposure than that of more fusiform fishes, it does not necessarily mean that all gibbose fishes will respond to flowing water in the same way. For example, Riopel et al. (2008) found that morphological differentiation between two co-occurring, congeneric sunfishes was similar for some traits but not for others. Another study of intraspecific morphological divergence between littoral and pelagic residents of one of the sunfishes further demonstrated that the interaction of evolutionary history and contemporary habitat can have more effect on fish morphology than that of habitat alone (Weese et al. 2012).

Centrarchidae (sunfish and bass) are endemic to eastern North America, where they primarily inhabit the calm, shallow waters of lakes and streams (Scott and Crossman 1973). This family includes several species that are found in post-glacial waterbodies, and their abundance has, in part, facilitated numerous eco-morphological studies (e.g. Ehlinger and Wilson 1988; Robinson et al. 1993, 2000; Gillespie and Fox 2003). Most centrarchids possess body shapes that are gibbose and laterally compressed; their morphology includes anal spines, median fins with both hard and soft rays, and thoracic pelvic fins (Nelson 2006). The taxonomic group is also quite plastic, with at least one experimental comparison determining that phenotypic plasticity was four times more powerful than genetic factors at influencing divergence in body morphology in pumpkinseed *Lepomis gibbosus* (L., 1758) reared in littoral and pelagic enclosures (Robinson and Wilson 1996). Brinsmead and Fox (2002) found that both rock bass

Ambloplites rupestris (Rafinesque, 1817) and pumpkinseed had generally deeper bodies in lacustrine habitats than conspecifics caught in nearby lotic habitats, while Yavno and Fox (2013) used an experimental comparison to demonstrate that pumpkinseed reared in flowing water developed caudal peduncles that were narrower, and pectoral fins that were more posteriorly positioned than individuals that were reared under static conditions. Together, these studies indicate that centrarchids can respond, morphologically, to changes in water velocity and habitat type.

To better understand how gibbose fishes respond to changes in water velocity, we examined differences in the morphology of three centrarchid species that vary in their phylogenetic and morphological similarity to one-another. Comparisons that include multiple species are better at revealing general patterns, and greatly improve our understanding of how biotic and abiotic factors can influence the phenotypes of organisms with similar body plans (Wimberger 1992), or specific taxonomic groups (e.g. Werner and Hall 1976; Riopel et al. 2008). However, few studies have used experimental conditions to simultaneously compare phenotypic changes in gibbose species from the same family (Werner and Hall 1977; Yavno et al. 2014). From hydrodynamic theory, we predicted that species exposed to sustained water flow will develop a more streamlined body and exhibit an anterior shift in fin positions because selection will favor phenotypes that reduce drag and optimize the angle of attack in fast-moving water (Webb 1984a,b; Ehlinger and Wilson 1988; Swain and Holtby 1989). Moreover, fish experiencing sustained current are likely to develop a shallower caudal peduncle, making the body more fusiform (Webb 1984a; Lauder and Drucker 2004; Blake 2004), as well as longer

median/paired fins, which increase stability when holding position in moving water (Webb 1984a,b; Swain and Holtby 1989).

To test whether plasticity in body morphology is consistent with hydrodynamic theory in gibbose fishes, we reared three co-occurring centrarchid species in the presence and absence of water flow, and compared their morphological responses. We also used this experiment to test the predictions that (1) the morphological response to habitat would be similar in the three gibbose species, and that (2) the similarity of the response would be related to their evolutionary proximity.

Materials and methods

Species selection and sampling

Pumpkinseed, bluegill *L. macrochirus* (Rafinesque, 1819) and rock bass were selected as the three study species based on their abundance in waterways surrounding the Laurentian Great Lakes, previous empirical evidence of morphological divergence in response to a variable water regime (e.g. Brinsmead and Fox 2002), and differences among the species in the timing of evolutionary divergence. Being of the same genus, the pumpkinseed and the bluegill are more closely related to each other than to the rock bass; mitochondrial DNA evidence suggests that the divergence of *Ambloplites* from *Lepomis* occurred approximately 28 million years ago (see Bolnick et al. 2008). While all three species possess a deep body shape, *Lepomis* are less gibbose than rock bass during early ontogeny (Scott and Crossman, 1973).

Young-of-year (YOY) for each species were collected along the Otonabee River (Ontario, Canada; 44°22' N, 78°17' W) using a 9 m bag seine (mesh size = 3 mm). YOY

were collected when individuals were approximately 25 mm SL (Table 1), which is comparable to the size of centrarchids used in previous plasticity comparisons (e.g. Robinson and Wilson 1996; Parsons and Robinson 2006; Yavno et al. 2014). Fish were transported to a holding facility and kept in 56-76 L aquaria for 48 h prior to the commencement of the study; aquaria were equipped with a carbon filter, aerated, and all fish were fed frozen brine shrimp *ad libitum* once daily.

Rearing protocol

YOY were reared in 12 plexiglass channels (0.9 m long x 0.15 m wide x 0.25 high), under one of two treatments (flow and static). The water velocity in flow treatments was set to 4.0 cm/sec (calibrated using a SonTek FlowTracker[®]) to provide a velocity of ≥ 1.5 total body lengths/sec, and adjusted halfway through the experiment to compensate for somatic growth, ensuring fish continue to experience a flow of ≥ 1.5 total body lengths/sec. This velocity is the minimum at which median/paired fins begin to undulate during locomotion in centrarchids (Standen and Lauder 2005). Static units were supplied with a minimum continuous flow to ensure a complete turnover in 24h. Wire mesh was placed in each channel as a means of confining the fish to the centre of the channel (i.e. the area where they would experience the most consistent flow). Channels were supplied with fresh Otonabee River water using hoses fastened to the front, and flow rates were controlled with mechanical adapters.

Experimental rearing was staggered because the spawning period differs for each of the three species (see Scott and Crossman 1973), and individuals of the same size were not simultaneously available. Rearing of YOY rock bass began in early July 2013,

whereas YOY bluegill and pumpkinseed were reared in mid-August. In both cases, rearing lasted for 80 days, which is a sufficient period of time for morphological differences between populations to develop (see Robinson and Wilson 1996; Yavno and Fox 2013). Because the two *Lepomis* species could not be easily differentiated from one-another at this early stage of ontogeny, the species were reared together and identification was conducted at the end of the study; this also eliminated excessive handling stress at the start, and likely reduced the initial number of mortalities.

At the beginning of the study, YOY were removed from the aquaria and placed randomly into a channel assigned to their taxon (i.e. rock bass or *Lepomis*) until individuals from each group were evenly distributed. Again, because pumpkinseed and bluegill are not easily distinguishable from one-another early in their first year of life, a larger number of *Lepomis* were collected to ensure a sufficient sample size of both species. Weight was estimated from SL using length-weight regressions taken from Murphy et al. (1991), and revealed that while all YOY had similar SL, rock bass were 1.5 times heavier than YOY *Lepomis* (Table 1). Rock bass were reared at 22 fish per unit (2 static, 2 flow treatments; $n = 88$ fish), whereas *Lepomis* were held at 38 fish per unit (4 static, 4 flow treatments; $n = 304$ fish); the difference resulting in roughly equivalent biomass loading. Flow visualization experiments indicate that general fish locomotion produces wake turbulence to the posterior of the body (e.g. Müller et al. 1997), however a search of the literature did not reveal any experimental data on the morphological effects of wake turbulence on proximate individuals. Bluegill, pumpkinseed and rock bass do not exhibit social swimming behaviour at any life stage (Brown and Colgan 1985), and no schooling behaviour was evident in any species during the rearing period. Given that fish

were not swimming in close proximity to one another, differences in rearing densities likely had a negligible effect on the velocity that individual fish experienced within each channel.

While pumpkinseed exhibit morphological divergence when reared in sympatry with bluegill, phenotypic changes due to competition are attributed to pumpkinseed shifting their foraging strategies and consuming alternate prey items (e.g. Yavno et al. 2014). Bluegill can induce decreased somatic growth in other centrarchid species, but the effect is independent of bluegill density, and the change in growth is also linked to resource availability (Marchetti 1999). Moreover, agonistic behaviours become less prevalent in juvenile centrarchids as rearing density increases (e.g. Fleming and Johansen 1984). During the trials, all fish were fed *ad libitum*, once daily using only frozen brine shrimp; agonistic interactions between individuals were not observed during any portion of the experiments, and all fish were observed consuming prey items during the feeding period so any effects of competition on morphological change were likely limited. Water flow was terminated during feeding to prevent brine shrimp from being carried away with the current, and ensuring the availability of brine shrimp for all fish.

At the end of each experiment, fish were removed from the units and euthanized using a lethal dose of MS-222. At this point, *Lepomis* were identified as either bluegill or pumpkinseed based from their lateral bar markings and spots (see Brown and Colgan 1981).

Morphometric analysis

Morphological differences were assessed from photographs taken of the lateral and dorsal aspects (see Yavno et al. 2013; Yavno and Fox 2013; Yavno et al. 2014). Photographs were taken under standardized conditions (e.g. height, incandescent lighting, perspective, specimen orientation and alignment) using a Fujifilm FinePix L30 camera mounted to a secured base. Fins were extended to their maximum natural position, and all photographs included a scientific ruler that permitted a standardization of scale among morphometric measurements across all digital photographs. Measurements were quantified using ImageJ 1.46 software (National Institute of Health Image; <http://rsb.info.nih.gov/ij/>) to assess divergence in 12 morphological characters commonly associated with locomotion in numerous fish taxa (Webb 1984*a, b*; Blake 2004; Yavno et al. 2013) (Fig. 1).

Statistical analysis

Because the data did not meet the assumption of normality (Kolmogorov-Smirnov test; $P < 0.05$) or homogeneity of error variance, all morphometric measurements were first $\log_{10}$ transformed prior to statistical analysis. Following the approach of Fischer-Rousseau et al. (2010), replicate effects were examined by comparing differences in multivariate body shape among treatments. A measure of overall body shape was derived using a Principal Component Analysis (PCA) that was performed on the 17 morphological measures that comprise a traditional truss-based centroid (Fig 1.) (Robinson et al. 2000). The 17 truss measurements form a quadrilateral network across the body, and include 11 of the 12 functional measures. The addition of 6 ‘non-functional’ measures adds a level of redundancy when assessing size and shape, and

reduces the influences of erroneous measures on any final shape/size score (Strauss and Bookstein 1982). While the redundant measures may also respond to water flow and differ by species, they were excluded from the main analyses; final comparisons only included traits that are homologous across most fish species, and have clear functional roles for locomotion (e.g. Brinsmead and Fox 2002; Yavno et al. 2013). Differences in shape between treatment replicates were tested using the canonical scores for each of the twelve axes. Because no significant differences were detected between replicates in any of the three species (MANOVA; $P > 0.05$ on each axis for all three species), replicates were pooled for subsequent statistical analyses.

To account for allometric relationships between linear trait measurements and body size, size-free residuals were calculated by regressing the log-transformed measurements for each trait against the canonical scores from the first axis of the aforementioned PCA (88.3% of the total variation). PCA that are conducted on trait measures that span several major body regions in fishes can reveal substantial shape variation between groups (Claverie and Wainwright 2014), and the canonical scores from the primary axis can serve as a better representation of body size than one trait measure along a single body region (e.g. standard length). In the present study, size correction was confirmed by regressing the size-free residuals for each trait against a traditional truss-based centroid (Strauss and Bookstein 1982) (Pearson correlation; $P > 0.05$ in all cases). This first set of statistical analyses were conducted using Statistica 6.1 (Statsoft Inc.)

Following the method used by Hoverman and Relyea (2008), a factorial Multivariate Analysis of Variance (MANOVA) was conducted to examine the effects of water velocity on the morphology of the three species. To compare the level of

phenotypic plasticity exhibited by each species, this method was expanded by separating size-free residuals by species and performing one full factorial MANOVA independently for each species. All MANOVAs were performed in SPSS 22 (IBM Inc.), and because of unequal sample sizes, MANOVAs were performed using bootstrap replicates ($n = 500$) to increase the robustness of each analysis. Univariate tests (Analysis of Variance) were only conducted if significant multivariate effects were detected in the primary MANOVA (performed with all three species), and Student-Neuman-Keuls (SNK) analyses were used as a post-hoc comparison where univariate tests were significant. Probabilities were adjusted to $\alpha \leq 0.0042$ using Bonferroni corrections to guarantee an experiment-wide probability of $P < 0.05$. Effect sizes (multivariate eta-squared, η^2) were provided to indicate the relative influence of each factor on morphological change.

The size-free residuals, calculated by regressing 12 raw trait measures against the first canonical axis of the 17 trait PCA (described above), were used in a second PCA to visually identify patterns of differentiation by species and treatment in multivariate space. Interspecific differences in plasticity were assessed using the size-free residuals of each morphometric trait. The means of these residuals were first determined by species and treatment, and the absolute value of difference in these means between static and flow treatments were calculated by species and used as an indicator of the magnitude of phenotypic response to flow. These differences were then assessed in a two-way ANOVA (independent variables were species and trait) to determine whether there were significant differences in plasticity and if so, whether the differences were related to the taxonomic similarity of the species. Effect sizes (partial eta-squared, η^2) were provided to indicate the relative influence of each factor on morphological change.

Results

Morphological trait differentiation

Species, water velocity and their interaction significantly affected morphological trait differentiation (Table 2A). Ten of the twelve morphological traits showed significant species-level effects; maximum pectoral fin length and body depth were the only traits to not differ among the three species (Table 2B). SNK analyses revealed that rock bass exhibited pre-pelvic lengths that were significantly shorter (Fig. 2L), and pre-pectoral lengths (Fig. 2K) that were significantly longer than those of bluegill and pumpkinseed. Bluegill and pumpkinseed also had shorter anal and dorsal fin base lengths (Fig. 2G, H) than those of rock bass. Body width differed among all three species (Fig. 2B), with pumpkinseed exhibiting the widest bodies and rock bass exhibiting the narrowest.

Six of the 12 traits were significantly influenced by water velocity (Table 2B). In all three species, body width shortened (Fig. 2B) and pectoral fins lengthened (Fig. 2I) when fish were reared under a sustained current. Water velocity induced all three species to develop shallower caudal peduncles in the posterior dimension (Fig. 2D). Species by water velocity interactions influenced four of the six traits. A sustained current induced lengthening along both the dorsal and ventral side of the caudal peduncle (Fig. 2E, F), but the degree of change was greater in bluegill and pumpkinseed than in rock bass (i.e. species by water velocity interaction). Pre-dorsal length was reduced in bluegill and pumpkinseed held under a sustained current, whereas it increased in rock bass given the same treatment (Fig. 2J). Anterior caudal peduncle depth decreased in all three species

held in current, but the level of change was lower in rock bass than in both *Lepomis* species. A sustained current also induced narrower body depths in all three species (Fig. 2A), but the difference was not significant.

In the PCA of size-free residuals, the first and second axis together explained 46% of the total variation between species and treatment groups. Because there was clear evidence of species and water velocity separation along the first two axes, and each of the remaining axes contributed less than 10% additional variation, only axes one and two were used for visualizing patterns. The first axis showed a strong positive correlation with caudal peduncle depth and moderate negative correlations with caudal peduncle and pectoral fin lengths, whereas the second axis showed a strong positive correlation with pre-pectoral length, pre-dorsal length and anal fin base length, and a moderate negative correlation with body width (Table 3). The PCA scatterplot (Fig. 3) indicated a strong morphological similarity between the pumpkinseed and bluegill, and the two congeners exhibited a similar morphological response to being reared in flowing water. The more distantly related rock bass showed a directional response to flowing water that was similar to that exhibited by the two congeners, but a less marked morphological shift to the change in rearing environment.

Comparison of phenotypic plasticity

The magnitude of phenotypic response to flow differed significantly among species (two way ANOVA, species factor: $F_{2,35} = 5.70$, $P = 0.01$). The greatest plasticity was exhibited by the pumpkinseed, and the least by the rock bass, and while the plastic response of the bluegill was closer to the pumpkinseed than to that of the rock bass, there

were no significant differences between that of the bluegill and either of the other species (Fig. 4). This order of greater to lower plasticity (from pumpkinseed to rock bass) is also evident in the PCA plot (Fig. 3) in that the Euclidian distances between the centroids of treatment groups were greatest in pumpkinseed (Fig. 3; denoted by arrows), and least in rock bass.

Discussion

Morphological divergence between centrarchids held under flow and static water conditions were evident in all three species. Morphological differentiation in bluegill and pumpkinseed was very strongly influenced by water velocity, whereas the magnitude of morphological change in response to water flow was lower in rock bass. It was also evident that some morphological changes, such as those related to body shape, responded in integrated fashion across the three species, whereas others, such as those related to the position of fins on the body, differed between the two lepomids and the rock bass. Replicated patterns of morphological divergence are commonly found in many taxa of fishes inhabiting benthic and pelagic zones of the same lake (Cooper et al. 2010 and examples within), although these patterns do not extend to every morphological trait (see Robinson and Parsons 2002). The similarity between the two more closely related lepomids and the differences with the more distantly related rock bass occurs in both the nature of the morphological response to current and the degree of plasticity in this response, possibly due to a phylogenetic constraint in the ability of the latter to respond to current (see Pugliucci 2003). This linkage of phylogeny and phenotypic response to

differences in habitat may not be the norm in fishes. In a comparative study of morphological differences four species inhabiting streams and reservoirs, Franssen et al. (2015) found that the response of head shape to reservoirs was more similar between the bluegill and one cyprinid species than between the two cyprinid species examined.

The prediction that fish exposed to flow would develop a more streamlined body was generally supported. In all three species, fish held under a sustained current developed body forms that were narrower and shallower than the body form of fish that were held under static conditions. The morphological changes we observed were consistent with both theoretical predictions of how external traits might change in response to increases in water velocity (Blake 2004), and previous observations made on wild populations (e.g. Dynes et al. 1999; Brinsmead and Fox 2002; Bhagat et al. 2006), including observations where impoundments turned fluvial environments into reservoirs (Haas et al. 2010, but see Franssen et al. 2012). During sustained swimming, streamlined body forms reduce energy lost to hydrodynamic drag, which is a resisting force generated when fish move through the water (Sfakiotakis et al. 1999; Langerhans 2008). Drag is inherently linked to both the size and position of physical structures along the body, and streamlined body forms are predicted in fish that engage in steady swimming (Webb 1984a; McGuigan et al. 2003; Blake 2004). Interestingly, Pakkasmaa and Piironen (2001) investigated streamlining in two fusiform species, and found that while brown trout *Salmo trutta* (Linnaeus, 1758) became more streamlined in faster water velocities, juvenile Atlantic salmon *Salmo salar* (Linnaeus, 1758) became deeper bodied. McGuigan et al. (2003) also saw no evidence of shallower bodies in rainbow fish (*Melanotaenia eachamensis* (Allen & Cross, 1982), *M. duboulayi* (Castelnau, 1878) that inhabit lotic

environments. Taxon-specific responses to flow may reflect fine-scale adaptations to microhabitats within streams, or alternatively, evolutionary constraints to morphological plasticity (Robinson and Parsons 2002).

A sustained current also induced longer, shallower caudal peduncles in all three species. The posterior region of the body plays a significant role during locomotion, and the shape of the caudal peduncle is crucial for sustained swimming. Caudal peduncles can undergo a change termed ‘narrow necking,’ which reduces the anterior depth of the caudal region; this change can lead to a considerable reduction in drag, while simultaneously allowing fish to generate large amplitude displacements for better locomotory performance (Webb 1984*a, b*; Blake 2004). Indeed, we observed a narrowing along the anterior caudal peduncle in all three centrarchids. The similar response of all three species in traits related to drag reduction is suggestive of functional, and possibly, evolutionary morphological integration in centrarchids, although further testing is needed with older individuals and more centrarchid species to confirm these explanations.

In our study, significant species by water velocity interactions were evident in the development of median and paired fin position. Under a sustained water velocity, pre-dorsal and pre-pectoral fin lengths shortened in bluegill and pumpkinseed, but lengthened in rock bass. The position of external traits relative to centre of mass can affect balance and stability within a current (Drucker and Lauder 2005; Standen and Lauder 2005; Tytell and Lauder 2008), and some empirical work on fusiform species suggests that anteriorly positioned fins are more likely to develop under flowing conditions (Swain and Holtby 1989; McGuigan et al. 2003).

Some studies have suggested that centrarchids from fluvial and/or lotic habitats possess more anteriorly positioned fins than those occupying lacustrine habitats (e.g. Bhagat et al. 2006), which is consistent with hydrodynamic theory (Webb 1984b). In contrast, Brinsmead and Fox (2002) found that the position of pectoral fins did not differ between pumpkinseed from lotic habitats and those from vegetated lacustrine sites. Anteriorly oriented fins assist in manoeuvring through complex habitats (e.g. submerged macrophytes), which suggests that the streams they sampled may have contained areas of dense vegetation. Bluegill and pumpkinseed spend their early life stages in vegetated areas (Osenberg 1988), while YOY rock bass are more pelagic (Scott and Crossman 1973). Because pelagic habitats are further from shore, and they encompass areas of open water, fish that swim through unstructured environments may benefit from posteriorly oriented fins. Regardless of water velocity, pectoral fins in rock bass are already more posteriorly oriented than those in *Lepomis*, suggesting that the position of this trait has been acquired by the genotype because it has beneficial fitness consequences (*sensu* Yavno and Fox 2013). An increase in water flow induced even further rearward positioning, and the same trend was visible with respect to the position of the dorsal fins. While it is difficult to ascertain why water velocity induced even further rearward positioning of median and paired fins in rock bass, the changes in pectoral fin position between the three species may be reflective of an interaction between the contemporary physical environment and the evolutionary history of the species (Weese et al. 2012). Further study is needed to determine how this interaction affects fitness in each species, and whether this change is manifested in other rock bass populations.

Pectoral fin length was greater in all three centrarchids when reared under a sustained current, but statistically significant morphological divergence between flow and static treatments was only detected in *Lepomis* species. Naspleda et al. (2012) found that pumpkinseed collected from streams in Spain possessed longer paired fins than those collected in lakes, which is consistent with the results of Pakkasmaa and Piironen (2001), in which higher water velocities induced longer pectoral fins in brown trout. Leavy and Bonner (2009) found longer pectoral fins in several taxa of fishes are a characteristic of fishes with greater swimming speed that inhabit fluvial environments. As water velocity increases, fish tend to experience higher drag forces, which are oftentimes accompanied by increasing instability (Langerhans 2008). Longer paired fins could be an adaptive morphological response that enables fish to counteract opposing forces through the use of appendages (Webb 1984a, b; Brinsmead and Fox 2002). Pectoral fins serve as an important mechanism for self-correction (Silva et al. 2012) and overriding torque forces that are generated along other areas of the body (Lauder and Drucker 2004). Thus, longer paired fins in centrarchids are likely an adaptive response for re-establishing stability during steady locomotion. Pectoral fins are used for propulsion in centrarchids; larger fins displace larger volumes of water, which helps to generate more thrust (Drucker and Lauder 2001; Lauder et al. 2002; Lauder and Drucker 2004; Tytell and Lauder 2008).

While the dorsal fin is one of the least understood morphological traits in teleosts, recent evidence suggests that it plays a critical role in the locomotory and propulsive ability of sunfish (Drucker and Lauder 2001; Standen and Lauder 2005). Dorsal fins are typically shorter along the base when fish inhabit pelagic or lotic environments. Dynes et al. (1999) observed that pelagic brook charr *Salvelinus fontinalis* (Mitchill, 1814) possess

shorter dorsal fin bases than their littoral counterparts, and Fischer-Rousseau et al. (2010) found that increasing water velocity led to the development of shorter dorsal fin base lengths in rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792). The latter study also found that the height of the fin decreases nearly proportionally with length, which reduces the surface area of the fin, and in turn decreases the effects of drag (*sensu* Sagnes et al. 2000). Our results are consistent with the aforementioned studies, as all three species responded to sustained current by developing shorter dorsal fin base lengths, however we cannot confirm that the overall surface area of the fin decreases in response to increasing water velocity. In other fusiform taxa, such as Atlantic and coho salmon *Oncorhynchus kisutch* (Walbaum, 1792) and blacktail shiner (*Cyprinelle venosta* Girard, 1856), dorsal fins seem to lengthen along the base when those species are exposed to a current (Swain and Holtby 1989; Pakkasmaa and Piironen 2001; Haas et al. 2010).

While the effects of water velocity on fish morphology have been well documented in fusiform fishes, the present study demonstrates that gibbose species also exhibit clear phenotypic responses to water flow. Our results suggest that water velocity may play an important role in shaping morphological development in gibbose species, but that certain morphological responses are not shared, even in related species.

Morphological changes occurred in the same direction for bluegill and pumpkinseed, and both of these congeners exhibited greater levels of phenotypic plasticity than did the more distantly-related rock bass in response to current. These results are consistent with those of the nested variation morphology study of Riopel et al. (2008), and support their hypothesis that variation in lepidomid body form is the result of both deterministic selection and evolutionary contingency.

Non-random mortality of different phenotypes could have potentially affected the results of our study by shifting the morphological traits of the survivors in a different direction for a particular species or treatment. While this cannot be completely ruled out, it is unlikely because the rate of mortality was similar between treatments for both species groups (*Lepomis*: 22% static; 17% flow; rock bass: 36% static; 34% flow; Table 1). The higher mortality rate of rock bass could be the result of stronger selection pressure on particular phenotypes, but this is not suggested by the lower degree of morphological separation by treatment, relative to that of *Lepomis* (Fig. 3).

Finally, while some of our findings were not consistent with those described in more fusiform fishes, they generally matched theoretical predictions for drag reduction and the generation of thrust. In general, the three centrarchids we studied developed more streamlined body forms and larger, more anteriorly positioned pectoral fins in response to water flow; changes that theoretically enhance locomotion under lotic conditions. Future studies should assess which locomotory traits directly affect fitness in sunfish, and then incorporate those traits into experimental analyses of phenotypic plasticity. Their inclusion would help to clarify which phenotypic strategies are adaptive responses to environmental perturbations.

Acknowledgements

We wish to thank A. Rooke for field and laboratory assistance, and S. Bocking, G. Burness, E. Nol and B. Robinson for their comments and suggestions during and after the study. Funding was provided by the Natural Sciences and Engineering Research Council of Canada to MGF, and by the Ontario Ministry of Training, Colleges and Universities in

478 the form of an Ontario Graduate Scholarship to SY. The authors wish to thank two
479 anonymous reviewers for their helpful comments and suggestions on an earlier version of
480 this manuscript.

481

Draft

References

- Agrawal, A.A. 2001. Phenotypic plasticity in the interactions and evolution of species. *Science*, 294: 321-326. doi:10.1126/science.1060701.
- Alcaraz, G., and Urrutia, V. 2008. Growth in response to sustained swimming in young montezumae swordtails, *Xiphophorus montezumae*. *Mar. Freshw. Res.* 41: 65–72. doi: 10.1080/10236240801896447.
- Bhagat, Y., Fox, M.G., and Ferreira, M. 2006. Morphological differentiation in introduced pumpkinseed *Lepomis gibbosus* (L.) occupying different habitat zones in Portuguese reservoirs. *J. Fish Biol.* 69: 79–94. doi: 10.1111/j.1095-8649.2006.01267.x.
- Blake, R.W. 2004. Fish functional design and swimming performance. *J. Fish Biol.* 65: 1193–1222. doi: 10.1111/j.0022-1112.2004.00568.x
- Bolnick, D.I., Turelli, M., López-Fernández, H., Wainwright P.C., and Near, T.J. 2008. Accelerated mitochondrial evolution and “Darwin’s Corollary”: asymmetric viability of reciprocal F1 hybrids in centrarchid fishes. *Genetics*, 178: 1037–1048.
- Brinsmead, J., and Fox, M.G. 2002. Morphological variation between lake and stream dwelling rock bass and pumpkinseed populations. *J. Fish Biol.* 61: 1619–1638. doi: 10.1111/j.1095-8649.2002.tb02502.x.
- Brown, J.A., and Colgan, P.W. 1981. The use of lateral-body bar markings in identification of young-of-year sunfish (*Lepomis*) in Lake Opinicon, Ontario. *Can. J. Zool.* 59: 1852–1855.
- Brown, J.A., and Colgan, P.W. 1985. The ontogeny of social behaviour in four species of centrarchid fish. *Behaviour*, 92: 254–276.

- 505 Claverie, T., and Wainwright, P.C. 2014. A morphospace for reef fishes: elongation is the
506 dominant axis of body shape evolution. PLoS ONE, 9: e112732. doi:
507 10.1371/journal.pone.0112732.
- 508 Cooper, W.J., Wernle, K.M., and Albertson, R.C. 2011. Functional and genetic
509 integration in the skulls of Lake Malawi cichlids. *Evol. Biol.* 38: 316-334. doi:
510 10.1007/s11692-011-9124-9.
- 511 DeWitt, T.J., Sih, A., and Wilson, D.S. 1998. Costs and limits of phenotypic
512 plasticity. *Trends Ecol. Evol.* 13: 77–81. doi: 10.1016/S0169-5347(97)01274-3.
- 513 Douglas, M.E., and Mathews, W.J. 1992. Does morphology predict ecology? Hypothesis
514 testing within a freshwater stream fish assemblage. *Oikos*, 65: 213–224. doi:
515 10.2307/3545012.
- 516 Drucker, E.G., and Lauder, G.V. 2001. Locomotor function of the dorsal fin in teleost
517 fishes: experimental analysis of wake forces in sunfish. *J. Exp. Biol.* 204: 2943–
518 2958. doi: 10.1242/jeb.01922.
- 519 Drucker, E.G., and Lauder, G.V. 2005. Locomotor function of the dorsal fin in rainbow
520 trout: kinematic patterns and hydrodynamic forces. *J. Exp. Biol.* 208: 4479–4494.
521 doi: 10.1242/jeb.01922.
- 522 Dynes, J., Bernatchez, L., Magnan, P., and Rodríguez, M.A. 1999. Genetic and
523 morphological variation between two forms of lacustrine brook charr. *J. Fish Biol.*
524 54: 955–972. doi: 10.1111/j.1095-8649.1999.tb00850.x.
- 525 Ehlinger, T.J., and Wilson, D.S. 1988. Complex foraging polymorphism in bluegill
526 sunfish. *Proc. Nat. Acad. Sci. U.S.A.* 85: 1878–1882. doi: 10.1073/pnas.85.6.1878.

- 527 Ehlinger, T.J. 1990. Habitat choice and phenotype-limited feeding efficiency in bluegill:
528 individual differences and trophic polymorphism. *Ecology*, 71: 886–896. doi:
529 10.2307/1937360.
- 530 Fischer-Rousseau, L., Chu, K.P., and Cloutier, R. 2010. Developmental plasticity in fish
531 exposed to a water velocity gradient: a complex response. *J. Exp. Zool. B* 314: 67–
532 85. doi: 10.1002/jez.b.21311.
- 533 Fleming, A., and Johansen, P.H. 1984. Density and agonistic behaviour in young-of-the-
534 year largemouth bass (*Micropterus salmoides*). *Can. J. Zool.* 62: 1454–1455. doi:
535 10.1139/z84-209.
- 536 Foster, K., Bower, L., and Piller, K. 2015. Getting in shape: habitat-based morphological
537 divergence for two sympatric fishes. *Biol. J. Linn. Soc.* 114: 152-162.
- 538 Franssen, N.R., Jarris, J., Clark, S.R., Schaefer, J.F., and Stewart, L.K. 2012. Shared and
539 unique morphological responses of stream fishes to anthropogenic habitat alteration.
540 *Proc. R. Soc. Lon. B Biol. Sci.* 280: 20122715. doi: 10.1098/rspb.2012.2715.
- 541 Gillespie, G.J., and Fox, M.G. 2003. Morphological and life-history differentiation
542 between littoral and pelagic forms of pumpkinseed. *J. Fish Biol.* 62: 1099-1115. doi:
543 10.1046/j.1095-8649.2003.00100.x.
- 544 Haas, T.C., Blum, M.J., and Heins, D.C. 2010. Morphological responses of a stream fish
545 to water impoundment. *Biol. Lett.* 6: 803-806. doi:10.1098/rsbl.2010.0401.
- 546 Higham, T.E. 2007. Feeding, fins and braking manoeuvres: locomotion during prey
547 capture in centrarchid fishes. *J. Exp. Biol.* 210: 107–117. doi: 10.1242/jeb.02634.
- 548 Hoverman, J.T., and Relyea, R.A. 2008. Temporal environmental variation and
549 phenotypic plasticity: a mechanism underlying priority effects. *Oikos*, 117: 23-32.

- 550 Imre, I., McLaughlin, R.L., and Noakes, D.L.G. 2002. Phenotypic plasticity in brook
551 charr: changes in caudal fin induced by water flow. *J. Fish Biol.* 61: 1171–1181. doi:
552 10.1111/j.1095-8649.2002.tb02463.x.
- 553 Klingenberg, C.P. 2014. Studying morphological integration and modularity at multiple
554 levels: concepts and analysis. *Trans. R. Soc. Lon. B Biol. Sci.* 369: 20130249. doi:
555 10.1098/rstb.2013.0249.
- 556 Langerhans, R.B. 2008. Predictability of phenotypic differentiation across flow regimes
557 in fishes. *Integr. Comp. Biol.* 48: 750–768. doi: 10.1093/icb/icn092.
- 558 Langerhans, R.B., and DeWitt, T. 2004. Shared and unique features of evolutionary
559 diversification. *Am. Nat.* 164: 335–349.
- 560 Lauder, G.V., and Drucker, E.G. 2004. Morphology and experimental hydrodynamics of
561 fish fin control surface. *J. Ocean Eng.* 29: 556–571. doi: 10.1109/JOE.2004.833219.
- 562 Lauder, G.V., Nauen, J.C., and Drucker, E.G. 2002. Experimental hydrodynamics and
563 evolution: function of median fins in ray-finned fishes. *Integr. Comp. Biol.* 42:
564 1009–1017. doi: 10.1093/icb/42.5.1009.
- 565 Leavy, T.R., and Bonner, T.H. 2009. Relationships among swimming ability, current
566 velocity association, and morphology for freshwater lotic fishes. *N. Am. J. Fish.*
567 *Manage.* 29: 72–83. doi: 10.1577/M07-040.1.
- 568 Marchetti, M.P. 1999. An experimental study of competition between the native
569 Sacramento perch (*Archoplites interruptus*) and introduced bluegill (*Lepomis*
570 *macrochirus*). *Biol. Invasions*, 1: 55–65.

- 571 McGuigan, K., Franklin, C.E., Moritz, G., and Blows, M.W. 2003. Adaptation of
572 Rainbow fish to lake and stream habitats. *Evolution*, 57: 104–118.
573 doi: 10.1111/j.0014-3820.2003.tb00219.x.
- 574 Müller, U.K., Van Den Heuvel, B.L.E., Stamhuis, E.J., and Videler, J.J. 1997. Fish foot
575 prints: morphology and energetics of the wake behind a continuously swimming
576 mullet (*Chelon labrosus* Risso). *J. Exp. Biol.* 200: 2893–2906.
- 577 Murphy, B.R., Willis, D.W., and Springer, T.A. 1991. The relative weight index in
578 fisheries management: status and needs. *Fisheries*, 16: 30–38. doi: 10.1577/1548-
579 8446(1991)016<0030:TRWIIF>2.0.CO;2.
- 580 Nasplada, J., Vila-Gispert, A., Fox, M.G., Zamora, L., and Ruiz-Navarro, A. 2012.
581 Morphological variation between non-native lake and stream-dwelling pumpkinseed
582 *Lepomis gibbosus* in the Iberian Peninsula. *J. Fish. Biol.* 81: 1915–1935.
- 583 Nelson, J.S. 2006. *Fishes of the World*. 4th ed. John Wiley and Sons, Inc., Hoboken.
- 584 Nilsson, C., Reidy, C.A., Dynesius, M., and Revenga, C. 2005. Fragmentation and flow
585 regulation of the world's large river systems. *Science*, 308: 405–408.
- 586 Ojanguren, A.F., and Brana, F. 2003. Effects of size and morphology on swimming
587 performance in juvenile brown trout (*Salmo trutta* L.) *Ecol. Freshw. Fish*, 12: 241-
588 246. doi: 10.1046/j.1600-0633.2003.00016.x.
- 589 Osenberg, C.W., Werner, E.E., Mittelbach, G.G., and Hall, D.J. 1988. Growth patterns in
590 bluegill (*Lepomis macrochirus*) and pumpkinseed (*L. gibbosus*) sunfish:
591 environmental variation and the importance of ontogenetic niche shifts. *Can. J. Fish.*
592 *Aquat. Sci.* 45:17–26. doi: 10.1139/f88-003.

- 593 Pakkasmaa, S., and Piironen, J. 2001. Water velocity shapes juvenile salmonids. *Evol.*
594 *Ecol.* 14: 721–730.
- 595 Parsons, K.J., and Robinson, B.W. 2006. Replicated evolution of integrated plastic
596 responses during early adaptive divergence. *Evolution*, 60: 801–813. doi:
597 10.1554/05-213.1.
- 598 Pfennig, D.W., Wund, M.A., Snell–Rood, E.C., Cruickshank, T., Schlichting, C.D., and
599 Moczek, A.P. 2010. Phenotypic plasticity’s impacts on diversification and
600 speciation. *Trends Ecol. Evol.* 25: 459–467.
- 601 Pugliucci, M. 2003. Phenotypic integration, studying the ecology and evolution of
602 complex phenotypes. *Ecol. Lett.* 6: 265–272.
- 603 Riopel, C., Robinson, B.W., and Parsons, K.J. 2008. Analyzing nested variation in the
604 body form of Lepomid sunfishes. *Environ. Biol. Fishes*, 82: 409–420. doi:
605 10.1007/s10641-007-9303-9.
- 606 Robinson, B.W., and Parsons, K.J. 2002. Changing times, spaces, and faces: tests and
607 implications of adaptive morphological plasticity in the fishes of northern postglacial
608 lakes. *Can J. Fish. Aquat. Sci.* 59: 1819–1833.
- 609 Robinson, B.W., and Wilson, D.S. 1996. Genetic variation and phenotypic plasticity in a
610 trophically polymorphic population of pumpkinseed sunfish (*Lepomis gibbosus*).
611 *Evol. Ecol.* 10: 631–652. doi: 10.1007/BF01237711.
- 612 Robinson, B.W., Wilson, D.S., and Margosian, A.S. 2000. A pluralistic analysis of
613 character release in pumpkinseed sunfish (*Lepomis gibbosus*). *Ecology*, 81: 2799–
614 2812. doi: 10.2307/177342.

- 615 Robinson, B.W., Wilson, D.S., Margosian, A.S., and Lotito, P.T. 1993. Ecological and
616 morphological differentiation of pumpkinseed sunfish in lakes without bluegill
617 sunfish. *Evol. Ecol.* 7: 451–464. doi: 10.1007/BF01237641.
- 618 Sagnes, P., Champagne, J.Y., and Morel, R. 2000. Shifts in drag and swimming potential
619 during grayling ontogenesis: relations with habitat use. *J. Fish Biol.* 57: 52–68. doi:
620 10.1111/j.1095-8649.2000.tb00775.x.
- 621 Schaefer, J., and Ryan, A. 2006. Developmental plasticity in the thermal tolerance of
622 zebrafish *Danio rerio*. *J. Fish Biol.* 69: 722–734. doi: 10.1111/j.1095-
623 8649.2006.01145.x.
- 624 Scheiner, S.M. 1993. Genetics and evolution of phenotypic plasticity. *Annu. Rev. Ecol.*
625 *Syst.* 24: 35–68. doi: 10.1146/annurev.es.24.110193.000343.
- 626 Schlichting, C.D., and Pigliucci, A. 1998. Phenotypic evolution: a reaction norm
627 perspective. Sinauer Associates, Sunderland, MA.
- 628 Scott, W.B., and Crossman, E.J. 1973. Freshwater fishes of Canada. Fisheries Research
629 Board of Canada, Ottawa.
- 630 Sfakiotakis, M., Lane, D.M., and Davies, J.B. 1999. Review of fish swimming modes for
631 aquatic locomotion. *J. Ocean Eng.* 24: 237–252. doi: 10.1109/48.757275.
- 632 Silva, A.T., Katopodis, C., Jantos, J.M., Ferreira, M.T., and Pinheiro, A.N. 2012.
633 Cyprinid swimming behaviour in response to turbulent flow. *Ecol. Eng.* 44: 314–
634 328. doi: 10.1016/j.ecoleng.2012.04.015.
- 635 Simon, K.D., Bakar, Y., Temple, S.E., and Mazlan, A.G. 2010. Morphometric and
636 meristic variation in two congeneric archer fishes *Toxotes chatareus* (Hamilton

- 1822) and *Toxotes jaculatrix* (Pallas 1767) inhabiting Malaysian coastal waters. J. Zhejiang Univ. Sci. B 11: 871–879.
- Standen, E.M., and Lauder, G.V. 2005. Dorsal and anal fin function in bluegill sunfish, *Lepomis macrochirus*: three-dimensional kinematics during propulsion and maneuvering. J. Exp. Biol. 208: 2753–2763. doi: 10.1242/jeb.01706.
- Stearns, S.C. 1989. The Evolutionary significance of phenotypic plasticity. BioScience, 39: 436–445. doi: 10.2307/1311135.
- Strauss, R.E., and Bond, C.E. 1990. Taxonomic methods: morphology. In Methods for fish biology. Edited by C.B. Schreck and P.B. Moyle. American Fisheries Society, Bethesda. pp. 109–140.
- Strauss, R.E., and Bookstein, F.L. 1982. The truss: body form reconstructions in morphometrics. Syst. Zool. 31: 113–135. doi: 10.2307/2413032.
- Swain, D.P., and Holtby, L.B. 1989. Differences in morphology and behavior between juvenile coho salmon (*Oncorhynchus kisutch*) rearing in lake and in its tributary stream. Can. J. Fish. Aquat. Sci. 46: 1406–1414. doi: 10.1139/f89-180.
- Tytell, E.D., and Lauder, G.V. 2008. Hydrodynamics of the escape response in bluegill sunfish, *Lepomis macrochirus*. J. Exp. Biol. 211: 3359–3369. doi: 10.1242/jeb.020917.
- Webb, P.W. 1984a. Form and function in fish swimming. Sci. Am. 25: 72–82. doi: 10.1038/scientificamerican0784-72.
- Webb, P.W. 1984b. Body form, locomotion and foraging in aquatic vertebrates. Am. Zool. 24: 107–120. doi: 10.1093/icb/24.1.107.

- 659 Weese, D.J., Ferguson, M.M., and Robinson, B.W. 2012. Contemporary and historical
660 evolutionary processes interact to shape patterns of within-lake phenotypic
661 divergences in polyphenic pumpkinseed sunfish, *Lepomis gibbosus*. *Ecol. Evol.* 2:
662 574–592. doi: 10.1002/ece3.72.
- 663 Werner, E.E., and Hall, D.J. 1976. Niche shifts in sunfishes: experimental evidence and
664 significance. *Science*, 191: 404–406. doi: 10.1126/science.1246626.
- 665 Werner, E.E., and Hall, D.J. 1977. Competition and habitat shift in two sunfishes
666 (Centrarchidae). *Ecology*, 58: 869–876. doi: 10.2307/1936222.
- 667 West-Eberhard, M.J. 1989. Phenotypic plasticity and the origins of diversity. *Annu. Rev.*
668 *Ecol. Syst.* 20: 249–278. doi: 10.1146/annurev.es.20.110189.001341.
- 669 Wimberger, P.H. 1992. Plasticity of fish body shape. The effects of diet, development,
670 family and age in two species of *Geophagus* (Pisces: Cichlidae). *Biol. J. Linn. Soc.*
671 45: 197–218. doi: 10.1111/j.1095-8312.1992.tb00640.x.
- 672 Wootton, R.J. 1998. Body form and locomotion. *In* *Ecology of Teleost Fishes* 2nd ed.
673 *Edited by* R.J. Wootton. Kluwer Academic Publishers, Dordrecht. pp. 13–18. doi:
674 10.1007/978-94-009-0829-1.
- 675 Yavno, S., and Fox, M.G. 2013. Morphological change and phenotypic plasticity in
676 native and non-native pumpkinseed sunfish in response to sustained water velocities.
677 *J. Evol. Biol.* 26: 2383–2395. doi: 10.1111/jeb.12230.
- 678 Yavno, S., Fox, M.G., Vila-Gispert, A., and Bhagat, Y. 2013. Morphological differences
679 between native and non-native pumpkinseed in traits associated with locomotion.
680 *Environ. Biol. Fishes*, 96: 507–518. doi: 10.1007/s10641-012-0053-y.

- 681 Yavno, S., Rooke, A.C., and Fox, M.G. 2014. Morphological change and phenotypic
682 plasticity in native and non-native pumpkinseed sunfish in response to
683 competition. *Naturwissenschaften*, 101: 479–49. doi: 10.1007/s00114-014-1177-
684 z.
- 685 Zúñiga-Vega, J., Reznick, D.N., and Johnson, J.B. 2007. Habitat predicts reproductive
686 superfetation and body shape in the livebearing fish, *Poeciliopsis turrubarensis*.
687 *Oikos*, 116: 995-1005. doi: 10.1111/j.2007.0030-1299.15763.x.

Draft

Table 1. Initial (*i*) and final (*f*) number of fish (*N*), standard length (SL), and weight (W) for bluegill (*Lepomis macrochirus* Rafinesque, 1819), pumpkinseed (*Lepomis gibbosus* (L., 1758)), and rock bass (*Ambloplites rupestris* Rafinesque, 1817) collected from the field and used in the rearing experiment.

Treatment	Sampling period	N_i	N_f	SL_i^*	SL_f^*	W_i^*	W_f^*
<i>A. rupestris</i>	13 June 2012 – 11 July 2012	88	57	17.9 - 29.7 (24.68)		0.09 - 0.41 (0.25)	
Flow		44	29		29.4 - 44.9 (38.1)		1.64
Static		44	28		27.3 - 43.7 (37.3)		1.55
<i>Lepomis</i>	1 August 2012 - 19 August 2012	304	244	18.6 - 28.0 (24.03)		0.07 - 0.27 (0.17)	
Flow		152	126				
<i>L. gibbosus</i>			22		25.1 - 36.1 (30.3)		0.70
<i>L. macrochirus</i>			104		20.8 - 36.7 (28.2)		0.55
Static		152	118				
<i>L. gibbosus</i>			18		23.7 - 36.2 (30.4)		0.71
<i>L. macrochirus</i>			100		21.6 - 37.1 (28.6)		0.60

* Values for SL and W are expressed in mm and g, respectively, and represent range (minimum – maximum) with mean in parentheses. Weights were estimated using length-weight relationships developed by Murphy, Willis and Springer (1991), and W_f is represented as only the mean.

Table 2. A) Results of a MANOVA (Wilks’ λ with F approximations) on the effects of water velocity treatments on the morphology of three species of Centrarchidae. B) Univariate tests (P -values) of main effects (Species, Water Velocity) and their interaction for twelve morphological traits that are considered functionally significant for locomotion (*, $P \leq 0.0042$). Effect sizes are represented by a multivariate or partial eta-squared (η^2) for the multivariate and univariate results, respectively.

A) Multivariate		Wilks’ λ	d.f.	F	P	η^2
Species		0.409	24,538	12.62	< 0.0001*	0.360
Water velocity		0.301	12,269	52.06	< 0.0001*	0.699
Species x Water velocity		0.645	24,538	5.49	< 0.0001*	0.084
B) Univariate						
Morphological trait	Species	η^2	Water velocity	η^2	Species x Water velocity	η^2
Body depth	0.13	0.013	0.005	0.026	0.24	0.009
Body width	< 0.0001*	0.117	< 0.0001*	0.048	0.49	0.004
Anterior caudal peduncle depth	< 0.0001*	0.025	< 0.0001*	0.308	< 0.0001*	0.079
Posterior caudal peduncle depth	< 0.0001*	0.042	< 0.0001*	0.311	0.06	0.011
Dorsal caudal peduncle length	< 0.0001*	0.065	< 0.0001*	0.247	< 0.0001*	0.065
Ventral caudal peduncle length	< 0.0001*	0.087	< 0.0001*	0.270	< 0.0001*	0.032
Anal fin base length	< 0.0001*	0.321	0.82	0.000	0.69	0.002
Dorsal fin base length	< 0.0001*	0.150	0.02	0.016	0.71	0.002
Maximum pectoral fin length	0.02	0.021	< 0.0001*	0.118	0.08	0.014
Pre-dorsal length	< 0.0001*	0.264	0.04	0.011	0.0001*	0.045
Pre-pectoral length	< 0.0001*	0.304	0.84	0.000	0.25	0.007
Pre-pelvic length	< 0.0001*	0.112	0.73	0.000	0.29	0.008

Table 3. Correlations between the 12 morphological traits and the two major axes in Principal Component Analysis, conducted on size-corrected residuals using varimax rotation.

Morphological Traits	Axis 1	Axis 2
Body depth	0.204	0.016
Pre-dorsal length	0.253	0.759
Pre-pectoral length	0.119	0.878
Pre-pelvic length	− 0.015	− 0.199
Dorsal fin base length	− 0.140	− 0.032
Anal fin base length	0.095	0.748
Dorsal caudal peduncle length	− 0.687	− 0.379
Ventral caudal peduncle length	− 0.692	− 0.357
Anterior caudal peduncle depth	0.847	− 0.242
Posterior caudal peduncle depth	0.721	0.237
Maximum pectoral length	− 0.615	− 0.179
Body width	0.187	− 0.592
Cumulative % of total variance explained	22.9	45.6

Figure captions

Fig. 1. Twelve morphological traits, considered functionally significant for locomotion (dorsal view: body width in the plane between the origin of each pectoral fin; lateral view: (1-2) pre-dorsal length, (1-3) pre-pectoral length, (1-4) pre-pelvic length, (2-4) body depth, (2-6) dorsal fin base length, (3-0) maximum pectoral fin length, (5-7) anal fin base length, (6-7) anterior caudal peduncle depth, (6-8) dorsal caudal peduncle length, (7-9) ventral caudal peduncle length, and (8-9) posterior caudal peduncle depth). In addition to the 12 morphological traits, (2-7) anterior dorsal to posterior anal fin, (4-5) anterior pelvis to anterior dorsal fin, (4-6) anterior pelvic to posterior dorsal fin, and (7-8) caudal peduncle truss were in both the principal components analysis and to calculate the traditional centroids

Fig. 2. Relative morphological changes to water velocity for the 12 morphological traits (A, body depth; B, body width; C, anterior caudal peduncle depth; D, Posterior caudal peduncle depth; E, dorsal caudal peduncle length; F, ventral caudal peduncle length; G, anal fin base length; H, dorsal fin base length; I, maximum pectoral fin length; J, pre-dorsal length; K, pre-pectoral length; L, pre-pelvic length) for each centrarchid species (RB, ■ and - - -; BG, ● and —; PS, ▲ and —). Log-transformed morphological measurements were regressed against PCA1 scores to remove the effects of body size, and all morphological changes are expressed as residuals. Lowercase letters represent Student-Neuman-Keuls pairwise comparisons across both treatments; treatments with shared letters are not significantly different ($P > 0.05$)

Fig. 3. Scatterplot of scores from Principal Component Analysis (with varimax rotation), performed on size-corrected residuals for 12 morphological traits, with 50% ellipsoids about the centroid of each group for static (black) and flowing (grey) water treatments. Arrows denote the shift of the centroid between static and flowing treatments for each centrarchid species (RB, ■ and - - -; BG, ● and — -; PS, ▲ and —).

Fig. 4. Phenotypic response of three centrarchid species to flow, as determined by the size-corrected difference between static and flow treatments in the dimension of 12 morphological measures. See Methods section for calculation details.

Draft

Draft

Fig. 1

Fig. 2

Fig. 3

Fig. 4