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Getting in shape: habitat-based morphological divergence for two sympatric fishes

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Freshwater fishes often show large amounts of body shape variation across divergent habitats and, in most cases, the observed differences have been attributed to the environmental pressures of living in lentic or lotic habitats. Previous studies have suggested a distinct set characters and morphological features for species occupying each habitat under the steady–unsteady swimming performance model. We tested this model and assessed body shape variation using geometric morphometrics for two widespread fishes, *Goodea atripinnis* (Goodeidae) and *Chirostoma jordani* (Atherinopsidae), inhabiting lentic and lotic habitats across the Mesa Central of Mexico. These species were previously shown to display little genetic variation across their respective ranges. Our body shape analyses reveal morphometric differentiation along the same axes for both species in each habitat. Both possess a deeper body shape in lentic habitats and a more streamlined body in lotic habitats, although the degree of divergence between habitats was less for *C. jordani*. Differences in the position of the mouth differed between habitats as well, with both species possessing a more superior mouth in lentic habitats. These recovered patterns are generally consistent with the steady–unsteady swimming model and highlight the significance of environmental forces in driving parallel body shape differences of organisms in divergent habitats. © 2014 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2015, **114**, 152–162.

ADDITIONAL KEYWORDS: atherinopsids – goodeids – morphometrics – phenotypic plasticity – swimming model.

INTRODUCTION

An important area of interest in evolutionary biology is the relationship between phenotypes and heterogeneous environmental gradients. At the population level, morphological trait divergence is the product of genetic differentiation and phenotypic plasticity via natural selection (Robinson & Wilson, 1994; Schluter, 2000; Franssen *et al*., 2013). Selection acts on phenotypes that promote population persistence and resource utilization (e.g. trophic characters, locomotor aptitudes, competition, and predation) leading to morphological divergence (Brönmark & Miner, 1992; Day, 2000; Hendry, Taylor & McPhail, 2002; Langerhans, 2008).

Phenotypically divergent populations in heterogeneous environments can arise via divergent selection on labile traits (Agrawal, 2001; Tobler *et al*., 2008). This is particularly true in the aquatic environment, which is highly variable both from spatial and temporal perspectives. The ability of a fish to move efficiently through water is highly dependent on its body shape, thereby limiting species to certain habitats or environmental gradients (Sfakiotakis, Lane & Davies, 1999; Triantafyllou, Triantafyllou & Yue, 2000; Müller & Van Leeuwen, 2006; Langerhans & Reznick, 2009). Phenotypic responses to flow velocity can be summed up as the interplay of trade-offs in steady and unsteady swimming. Steady swimming, the constant locomotion in a straight line (Langerhans, 2008), is necessary in high-flow environments because of an increase in hydrometric drag, which favours a streamlined body shape. Alternatively, low-flow environments correlate with unsteady swimming, where there are locomotion patterns with inconsistent changes in direction or velocity, often resulting in a

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deepened body shape (Brönmark & Miner, 1992; Hendry *et al*., 2002; Langerhans, 2008; Franssen, 2011). These traits in concordance with flow differences can result in divergent character selection of adaptive phenotypes (Langerhans, 2008).

As a result, Langerhans (2008) and, subsequently, Langerhans & Reznick (2009) proposed a steady–unsteady swimming performance model to predict the impacts of natural selection on morphology and locomotion abilities for fishes inhabiting different flow regimes. The prediction is based on the idea that morphology is strongly linked to swimming ability in fishes and this idea has been supported by many other studies examining the relationship between form and function (Webb, 1982, 1984; Sfakiotakis *et al*., 1999). The model predicts that fishes occupying habitats that require steady swimming (i.e. lotic habitats) should possess morphological features that enhance swimming performance in these habitats such as streamlined bodies, shallow/narrow caudal peduncle, and higher aspect-ratio caudal fins. The second portion of the model predicts that fish inhabiting low-flow environments (i.e. lentic habitats) should possess features that enhance unsteady swimming including deeper or larger caudal peduncles, smaller heads, and lower aspect-ratio caudal fins.

Previous studies comparing body shapes of lentic and lotic fish populations have found substantial differences in shape between fishes in these habitats (Walker, 1997; Hendry *et al*., 2002; McGuigan *et al*., 2003; Langerhans, 2008; Krabbenhoft, Collyer & Quattro, 2009; Schaefer, Duvernell & Kreiser, 2011; Webster *et al*., 2011; Franssen *et al*., 2013). Additional biotic and abiotic components of habitats play a role in differing body shapes and have been shown to impact fitness and functional success, such as resource and foraging requirements, predator avoidance, and character displacement as a result of competition (Brönmark & Miner, 1992; Robinson & Wilson, 1994; Adams & Huntingford, 2004; Svanbäck *et al*., 2008). Recently, morphological shape divergence has been shown in anthropogenically altered habitats of freshwater fish (Haas, Blum & Heins, 2010; Franssen, 2011; Franssen *et al*., 2013). Therefore, understanding how populations adapt to different habitats may provide an insight into the consequences of anthropogenic stream modifications and the evolutionary process.

Central Mexico is relatively depauperate from an ichthyological perspective (Miller, Minckley & Norris, 2005). The region is dominated by two distantly-related fish groups, the New World Silversides (Atherinopsidae) and the Splitfins (Goodeidae), which diversified in the region. Silversides (genus *Chirostoma*) diversified within the last 0.52 Myr and occur in both lentic and lotic habitats but reach their greatest diversity in the Central Mexican Lakes (Barbour, 1973; Bloom *et al*., 2013). The Mesa Silverside, *Chirostoma jordani* (Atherinopsidae), is one of the most widely distributed species in Central Mexico, occurring in the Ríos Lerma, Grande de Santiago, Panuco, Cazones, Tecolutla, and Ameca, and the isolated populations in the Rio Mezquital and Laguna Santiaguilla basins, as well as numerous inland lakes including (but not limited to) Lakes Chapala, Cuitzeo, and Patzcuaro, and the endorheic Valle de México (Fig. 1) (Barbour, 1973; Miller *et al*., 2005). The Splitfins (Goodeidae) are comprised of two subfamilies, EmPetrichthynae and Goodeinae; of those, Goodeinae is more diverse, containing approximately 42 species that have diversified since the middle Miocene (Doadrio & Domínguez-Domínguez, 2004). Many species are restricted to a particular drainage basin or spring habitat but at least one species, the Tiro or Blackfinned Goodea (*Goodea atripinnis*), is widespread throughout the region occurring in the Ríos Lerma-Grande de Santiago, Ameca, Balsas, Armeria, the endorheic Lago de Magdelana basin, and inland lakes on the Mesa Central (Miller *et al*., 2005).

Chirostoma jordani and *G. atripinnis* are generalists in terms of their habitat occupancy because both species occur in lentic and lotic habitats (Miller *et al*., 2005). Furthermore, previous studies have indicated that both species display limited genetic variation across their respective ranges (Doadrio & Domínguez-Domínguez, 2004; Bloom *et al*., 2009; K. R. Piller, unpubl. data). This situation offers the unique opportunity to investigate body shape differences of two sympatric species with limited genetic structure across a habitat gradient and to test hypotheses with regard to divergent selection, which can drive micro-evolutionary change within species. First, we hypothesize that there will be differences in body shape between lentic and lotic habitats for populations of *C. jordani* (Atherinopsidae) and *G. atripinnis* (Goodeidae) as a result of divergent selection pressures of these habitats. Second, we the test the steady–unsteady swimming hypothesis of Langerhans (2008) and hypothesize that populations inhabiting lotic environments will be more fusiform and streamlined in overall body shape relative to populations occupying lentic waters, thereby optimizing the locomotion abilities of populations inhabiting these divergent environments.

MATERIAL AND METHODS

Geometric morphometrics

We examined the shape variation for 178 individuals of *C. jordani* and 189 individuals of *G. atripinnis* from

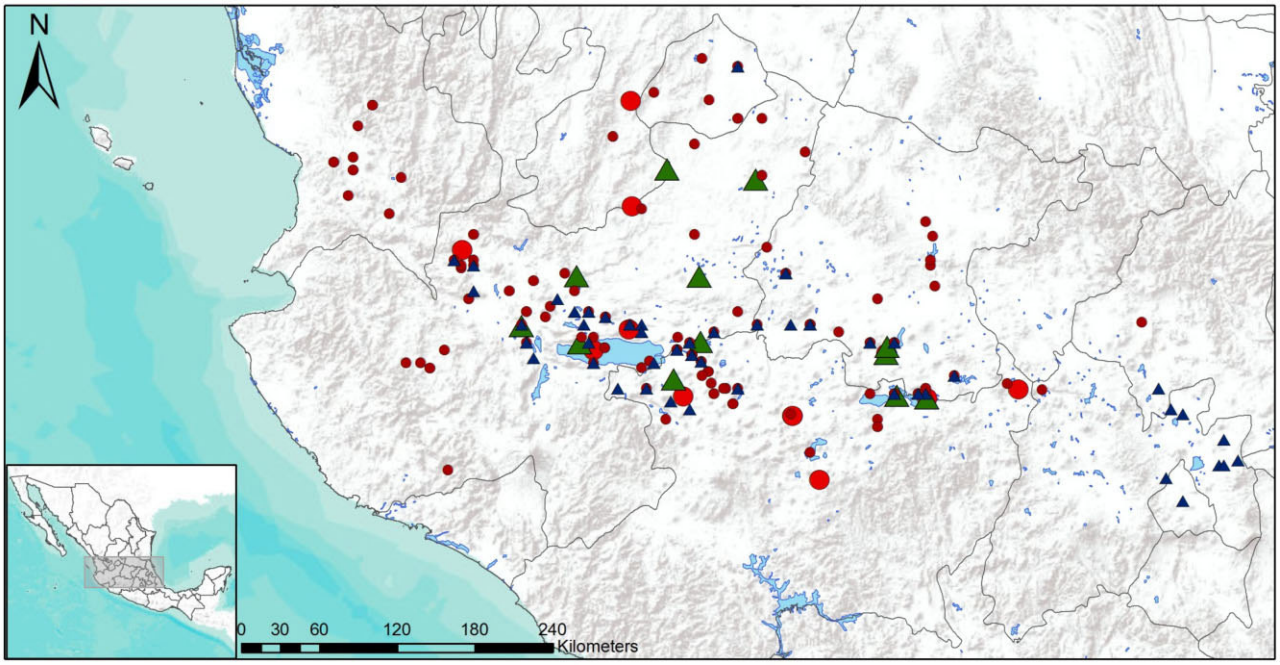

Figure 1. Map of the distribution of *Goodea atripinnis* (circles) and *Chirostoma jordani* (triangles). Small symbols correspond to vouchered museum records (http://www.fishnet2.org; June 2014). Large symbols correspond to the location of museum specimens used in the analysis.

both lotic (i.e. rivers, streams, creeks) and lentic (i.e. lakes, reservoirs) habitats from central Mexico using museum specimens (see Supportiing information, Tables S1, S2). The specimens used in the present study were collected during two general time periods: 1960s and 2000s. We tested for age-related effects and found no significant differences in body shape and so all specimens were combined in subsequent analyses. Based on definitions by Wetzell (2001), a lotic system is defined as a body of water with unidirectional water movements along a slope in response to gravity. In the case of the present study, this includes rivers, streams, and creeks. A lentic body of water is defined as a system with still or calm water, although there may be water movement by mechanisms other than gravity. These types of bodies of water include lakes, ponds, presas (reservoirs), and spring pools. The body shape of *C. jordani* and *G. atripinnis* was quantified using a geometric morphometric approach. The left lateral side of all specimens was photographed using a Nikon SLR digital camera. In the family of Goodeidae, the adult range is from 50 mm standard length (Webb *et al.*, 2004) and specimens smaller than 50 mm were considered as juveniles. For *C. jordani*, Olvera-Blanco *et al.* (2009) reported that both males and females reach maturity by one year of age; this corresponds to approximately 50 mm standard length. Any juveniles were excluded from the study to reduce any possible biases due to ontogenetic effects. Additionally, any damaged or warped specimens were removed from all analyses. TPSDIG2 (Rohlf, 2005) was used to digitize twelve homologous landmarks (Fig. 2). Standard length was measured with calipers to the nearest 0.1 mm for each specimen. Procrustes superimposition was used to remove position, orientation, and size biases for each species separately, and was carried out using MORPHOJ 1.05f (Klingenberg, 2011). Each species aligned data will be referred to as the 'shape data'.

Multivariate analysis

To correct for possible allometric shape variation within species, a pooled within habitat allometric regression between the shape data and log centroid size was performed in MORPHOJ 1.05f (Klingenberg, 2011; Sidlauskas, Mol & Vari, 2011). Canonical variate analysis (CVA) was run in MORPHOJ 1.05f using the residuals from allometric regression to control for any allometric shape variation. CVA was used find the shape features that best distinguish between the two habitat types. To reduce data dimensionality, a principal component analysis (PCA) was run using the residuals of the allometric regression without further pooling (Sidlauskas *et al.*, 2011). Each species was analyzed separately. Separate nonparametric multivariate analysis of variance (NP-MANOVA) for each species were used to test for significant

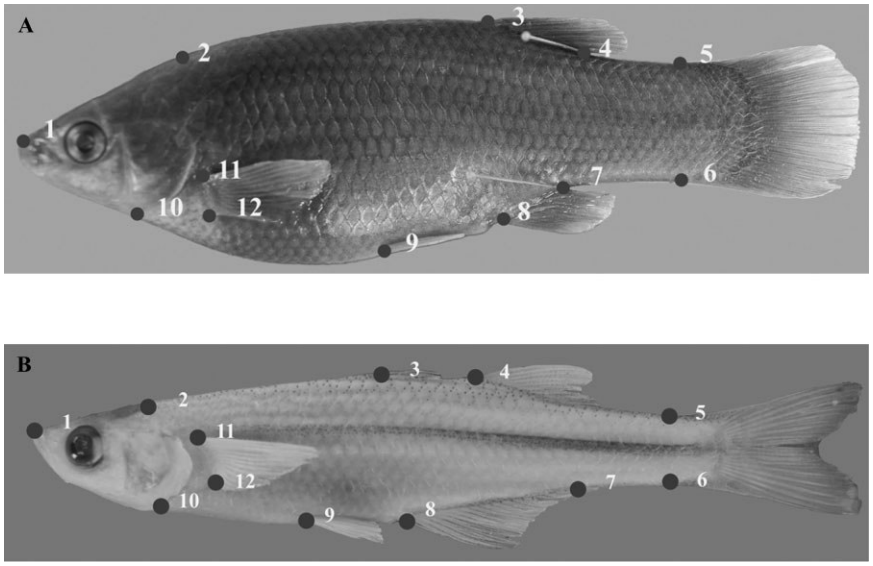

Figure 2. A, geometric landmarks for *Goodea atripinnis* (1) anterior tip of the snout, (2) posterior aspect of the neurocranium, (3) anterior origin of the dorsal fin, (4) posterior insertion of the dorsal fin or spiny fin dorsal fin, (5) dorsal insertion of the caudal fin, (6) ventral insertion of the caudal fin, (7) posterior insertion of the anal fin, (8) anterior insertion of the anal fin, (9) origin of pelvic fin, (10) the insertion of the operculum on the profile, (11) upper insertion of the pectoral fin, (12) lower insertion of the pectoral fin. B, geometric landmarks for *Chirostoma jordani*, (1) tip of snout, (2) anterior border of epiphyseal bar at midline dorsal neurocranium, (3) origin of first dorsal fin, (4) insertion of second dorsal fin, (5) dorsal base of caudal fin, (6) ventral base of caudal fin, (7) insertion of anal fin, (8) origin of anal fin, (9) origin of pelvic fin, (10) intersection of gill opening and ventral body margin, (11) origin of pectoral fin, (12) insertion of pectoral fin.

differences in the distribution of habitat groups (lentic and lotic) for all populations in morphospace because the assumptions of multivariate normal were not met. NP-MANOVA is an equivalent design to an ANOVA, allowing the testing of multiple factors and interactions, but allows for relaxed assumptions by relying on a permutation procedure (Anderson, 2001). The NP-MANOVA model included the PC axes that accounted for > 80% of the variation as the dependent variables with habitat type (to test the lentic versus lotic effect) as the fixed effects. Goodeids are sexually dimorphic; therefore, the effect of sex was tested for as well. The interaction between the sex and habitat factors was found to be significant and so a separate analysis for both sexes was implemented. The NP-MANOVA was carried out in R software using the vegan package (R Development Core Team, 2011; Oksanen *et al*., 2013). To examine the shape changes between specimens found in lentic and lotic habitats for each species, thin spline plates were created from the residuals of the allometric regression for each habitat type (lentic versus lotic).

RESULTS

The PCA of the allometric regression residuals summarized 81.2% of the variation for *C. jordani* in the first seven PC axes and 83.3% of the variation in the first six PC axes for *G. atripinnis.* Using NP-MANOVA, morphological divergence was detected for the habitat variable for *C. jordani* (Table 1). When testing for morphological divergence, all variables had a significant effect on body shape variation for *G. atripinnis* (Table 1). A significant interaction between sex and habitat type was found for *G. atripinnis*, although the results from both sex match the pooled analysis (see Supporting information, Figs S1, S2; Table S3).

Because only two habitat types were examined, only one CV axis could be extracted from the CVA. The CVA plots show distinct habitat groups for both species with only a few individuals overlapping between groups for each species (Figs 3, 4). Dorsal fin position, pectoral fin position, anal fin, pelvic fin position, mouth position, and caudal peduncle length were characterized as the most important shape variables for distinguishing the lotic and lentic specimens for *C. jordani* (Table 2). In *G. atripinnis*, the shape features that best explain the difference between habitat types were caudal peduncle length, anal fin, head size, mouth position, and dorsal fin position (Table 3).

Specimens of *C. jordani* from lentic habitats had a more superior mouth, a reduced caudal peduncle, elongate anal fin, and a deeper body shape (Fig. 5A).

Table 1. Results of the nonparametric multivariate analysis of variance for the species-specific body shape divergence in lentic and lotic habitats using the PC axes that capture > 80% of the variation for each species. Both sexes were pooled for *G. atripinnis*. Effect terms are ranked by the relative variance explained in each model

Species	Model	*F* value	r^2	*P* value
Chirostoma jordani	Habitat	12.316	0.066	< 0.001
Goodea atripinnis	Habitat	69.187	0.268	< 0.001
	Sex	8.105	0.031	< 0.001
	Habitat × Sex	6.135	0.024	< 0.001

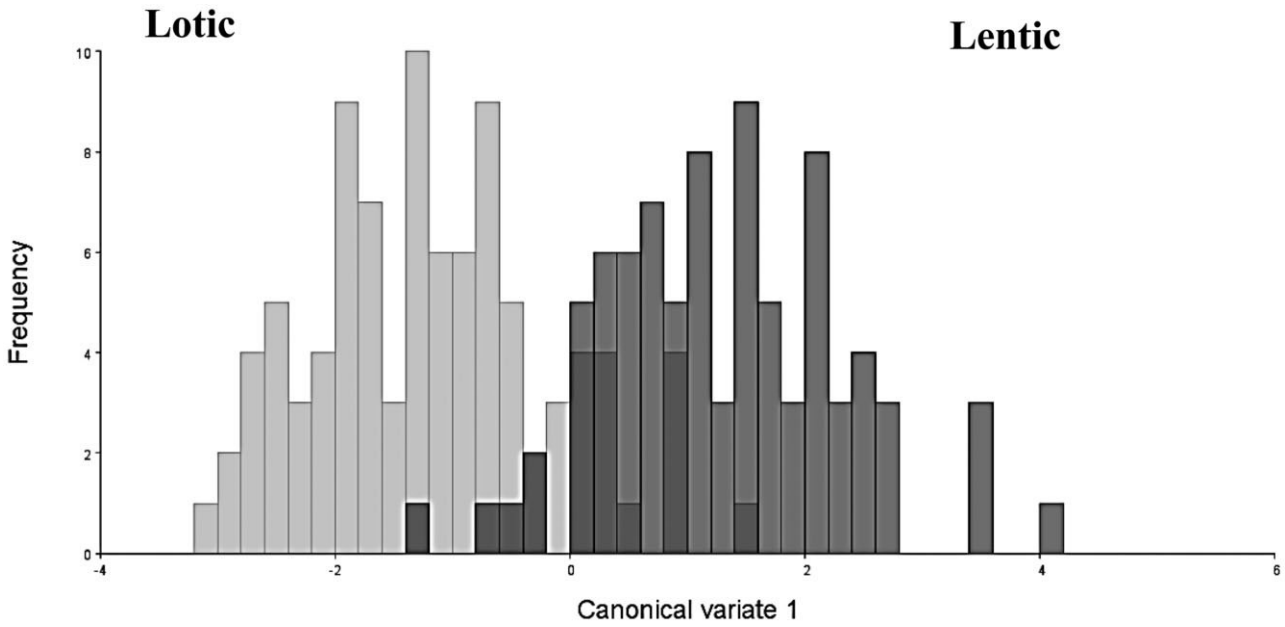

Figure 3. A plot of the canonical variate (CV) analysis results for *Chirostoma jordani*, with the CV scores of specimens on the *x*-axis and the frequency of the individuals on *y*-axis.

Specimens from lotic habitats tended to have a more inferior mouth, longer caudal peduncle, shortened anal fin, and compressed body shape (Fig. 5B). Individuals of *G. atripinnis* from the lentic sites were characterized by a deeper body, shortened head, a more superior mouth, anteriorly positioned anal and dorsal fin, and wider, elongated caudal peduncle, whereas individuals collected from lotic sites had shallower body, an elongated head, a more inferior mouth, posteriorly positioned anal and dorsal fin, and a short, narrow caudal peduncle (Fig. 6).

DISCUSSION

Habitat-associated morphological divergence is common in fishes (Walker, 1997; Hendry *et al*., 2002; McGuigan *et al*., 2003; Langerhans, 2008; Tobler *et al*., 2008; Krabbenhoft *et al*., 2009; Schaefer *et al*., 2011; Webster *et al*., 2011). The results of the present study demonstrate morphological divergence for two distantly-related species in two contrasting habitats and support the steady–unsteady swimming performance model of Langerhans (2008). Both *G. atripinnis* and *C. jordani* independently show a morphological shift towards a fusiform body shape in lotic systems, demonstrating similar phenotypic responses to similar environmental gradients and flow regimes, despite the lack of intraspecific genetic variation across their respective ranges (Doadrio & Domínguez-Domínguez, 2004; Bloom *et al*., 2009; K. R. Piller, unpubl. data

The two study species exhibit possible adaptive responses to divergent habitats, including mouth position, dorsal fin position, anal fin position, and caudal peduncle (length and width). Divergent selection pressure is considered to be a major driving force behind intraspecific polymorphism (Svanbäck *et al*., 2008). At least two mechanisms can explain morphological divergence among populations: (1) phenotypic plasticity, the existence of a range of phenotypes

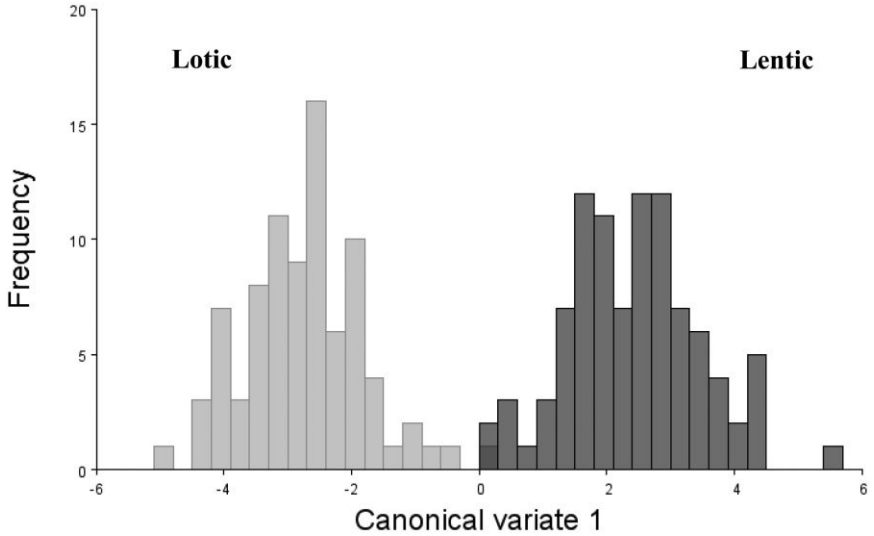

Figure 4. A plot of the canonical variate (CV) analysis results for *Goodea atripinnis*, with the CV scores of specimens on the *x*-axis and the frequency of the individuals on *y*-axis.

under different environmental conditions from a single genotype, and (2) genetic differentiation, in which the underlying genetic differences in individuals will be reflected by the phenotype of an individual (Stearns, 1989). The morphological responses as a result of phenotypic plasticity may allow for rapid responses to a changing environment (Robinson & Wilson, 1994; Crispo, 2008). Predicting such phenotypic plasticity is important for understanding the impacts of natural or anthropogenic ecosystem changes on organisms, and may allow for better risk protection of aquatic ecosystems (Maxwell *et al*., 2014). The morphological differences might have arisen as a result of divergent selection pressures of water flow differences, dissolved oxygen variation, or prey type/abundance variation between lotic and lentic habitats (Crispo & Chapman, 2010; Kekäläinen *et al*., 2010; Collin & Fumagalli, 2011).

Numerous biotic and abiotic factors have been shown to contribute to morphological divergence (Reznick & Endler, 1982; Reznick *et al*., 1997; Langerhans *et al*., 2003; Krabbenhoft *et al*., 2009) for a variety of fishes, including but not limited to characids (Langerhans *et al*., 2003), cichlids (Langerhans *et al*., 2003), cyprinids (Haas *et al*., 2010), poeciliids (Hankison *et al*., 2006), and atherinopsids (Krabbenhoft *et al*., 2009). Lotic environments tend to select for body shapes that reduce drag because a fusiform shape reduces resistance in aquatic environments, allowing effective propulsion and maintenance of velocity at a lower energy cost (Webb, 1984; Langerhans, 2008; Langerhans & Reznick, 2009). In the present study, both *G. atripinnis* and *C. jordani* exhibit a more fusiform body shape in lotic habitats (Figs 5B, 6B, Table 1). However, the morphological shifts between habitats are much more apparent in *G. atripinnis* than *C. jordani* (Table 1). This is possibly a result of the natural streamlined body shape of *C. jordani* because similar body shape differences have been recovered for other species of silversides in other divergent habitats (O'Reilly & Horn, 2004; Fluker, Pezold & Minton, 2011). In accordance with the steady–unsteady performance model of Langerhans (2008), a more fusiform shape and a narrow caudal peduncle enhance steady swimming. Both *G. atripinnis* and *C. jordani* had narrower caudal peduncles in lotic habitats, although *C. jordani* showed a minimal narrowing and elongation of the caudal peduncle.

Differences in prey type and abundance between lotic and lentic habitats may have given rise to morphological character diversification, such as mouth position and head size (Figs 5, 6). These traits have been attributed to differences in prey choice and feeding orientation within and between many fish species (Gatz, 1979; Winemiller, 1991; Hendry *et al*., 2002; Russo *et al*., 2008). *Chirostoma jordani* is primarily a zooplanktivorous species (Moncayo-Estrada, Lind & Escalera-Gallardo, 2010; Moncayo-Estrada, Escalera-Gallardo & Lind, 2011), whereas *G. atripinnis* is a filter feeder with a diet of zooplankton and green algae (Miller *et al*., 2005). Both species exhibit more of an upturned mouth in lentic environments (Figs 5, 6) and a more superior mouth has been shown to a trait common in surface feeding fishes (Winemiller, 1991, 1992). Based on this feature alone, this suggests *C. jordani* and *G. atripinnis* may be feeding higher in the water column in lentic

Table 2. The effect of habitat on shape was analyzed using both a permutation analysis of variance for each landmark coordinate separately and a canonical variate analysis (CVA) on residual shape data after an allometric regression against log centroid size for *Chirostoma jordani*

Landmarks	Canonical coefficients	*F* values	r^2	*P* values
x1	−25.166	0.127	0.001	0.690
y1	−11.755	2.334	0.013	0.150
x2	**32.998**	**6.105**	**0.034**	**0.011**
y2	**16.092**	**12.139**	**0.065**	**> 0.001**
x3	−0.402	1.582	0.009	0.213
y3	**−38.489**	**13.192**	**0.070**	**> 0.001**
x4	25.732	0.993	0.006	0.312
y4	**84.647**	**20.897**	**0.107**	**> 0.001**
x5	**−60.230**	**23.393**	**0.118**	**> 0.001**
y5	**16.932**	**6.900**	**0.038**	**0.011**
x6	**35.127**	**12.759**	**0.067**	**> 0.001**
y6	−39.046	0.0922	0.001	0.767
x7	**25.848**	**21.795**	**0.111**	**> 0.001**
y7	−34.224	2.722	0.015	0.082
x8	**−72.408**	**45.27**	**0.206**	**> 0.001**
y8	**94.555**	**10.089**	**0.055**	**0.003**
x9	39.002	3.085	0.017	0.087
y9	**−108.387**	**41.512**	**0.192**	**> 0.001**
x10	**−1.633**	**4.606**	**0.026**	**0.029**
y10	61.898	0.27985	0.002	0.613
x11	−29.587	0.547	0.003	0.473
y11	−52.358	1.146	0.007	0.251
x12	**30.719**	**13.247**	**0.070**	**> 0.001**
y12	**10.136**	**4.980**	**0.028**	**0.020**

The raw canonical coefficients based on the CVA of the allometric regression residuals. The *x*- and y-coordinates are given for each landmark and statistically significant values are indicated in bold.

Table 3. The effect of habitat on shape was analyzed using both a permutation analysis of variance for each landmark coordinate separately and a CVA analysis on residual shape data after an allometric regression against log centroid size for *G. atripinnis*. The raw canonical coefficients based on the canonical variate analysis (CVA) of the allometric regression residuals

Landmarks	Canonical coefficients	*F* values	r^2	*P* values
x1	**−24.966**	**15.802**	**0.079**	**0.002**
y1	**13.730**	**44.980**	**0.196**	**> 0.001**
x2	5.255	1.6381	0.009	0.193
y2	−59.857	0.1283	0.001	0.719
x3	**−46.665**	**202.680**	**0.523**	**> 0.001**
y3	**38.622**	**60.995**	**0.248**	**> 0.001**
x4	**−44.093**	**59.052**	**0.242**	**> 0.001**
y4	**−8.986**	**83.124**	**0.310**	**> 0.001**
x5	**52.734**	**45.060**	**0.196**	**> 0.001**
y5	**48.246**	**32.550**	**0.149**	**> 0.001**
x6	**−13.773**	**36.167**	**0.164**	**> 0.001**
y6	**−8.449**	**37.878**	**0.169**	**> 0.001**
x7	**36.377**	**16.620**	**0.082**	**> 0.001**
y7	**−77.416**	**106.940**	**0.366**	**> 0.001**
x8	**−46.029**	**80.872**	**0.304**	**> 0.001**
y8	**−21.963**	**62.047**	**0.251**	**> 0.001**
x9	**44.243**	**4.383**	**0.023**	**0.030**
y9	**−1.777**	**6.675**	**0.034**	**0.009**
x10	**70.084**	**66.357**	**0.263**	**> 0.001**
y10	60.772	2.158	0.011	0.127
x11	**7.444**	**11.802**	**0.059**	**0.003**
y11	19.511	0.2614	0.001	0.630
x12	−40.611	0.1969	0.001	0.659
y12	**−2.437**	**15.401**	**0.076**	**> 0.001**

The *x*- and *y*-coordinates are given for each landmark and statistically significant values are indicated in bold.

environments than in lotic environments. The differences in mouth position for *C. jordani* and *G. atripinnis* may be a response to changes in available prey within the divergent environments (McEachran, Boesch & Musick, 1976; Ellis, Pawson & Shackley, 1996; Platell, Sarre & Potter, 1997). Further data on diet and prey abundance for both species would be necessary to identify whether the observed changes in mouth and head morphology can be definitively attributed to prey differences in the divergent habitats.

Head size variation previously has been shown to be a phenotypic response to predation pressure (Walker, 1997; Vamosi & Schluter, 2002; Langerhans *et al*., 2004), where fish populations in the presence of predators exhibited larger caudal regions, smaller heads, and more elongate bodies (Langerhans *et al*., 2004). Such changes may be advantageous for predator evasion because they increase unsteady or burst swimming as a result of the enlarged musculature in the caudal region and the smaller, more fusiform anterior region (Langerhans *et al*., 2004). Similarly, the present study found that *G. atripinnis* displayed a larger caudal peduncle and shortened, smaller head in lentic habitats, although testing the effect of predation on body shape would require additional predator data from the field.

Based on the data that we have on hand, it appears as though phenotypic plasticity is the driving force behind the morphological divergence observed across habitats for both species. Gene flow would only constrain morphological adaptation but not phenotypic plasticity (Scheiner, 1993; Hendry *et al*., 2002). However, it is possible that large mixing of populations across habitat types would constrain genetic diversification and phenotypic plasticity, lessening or

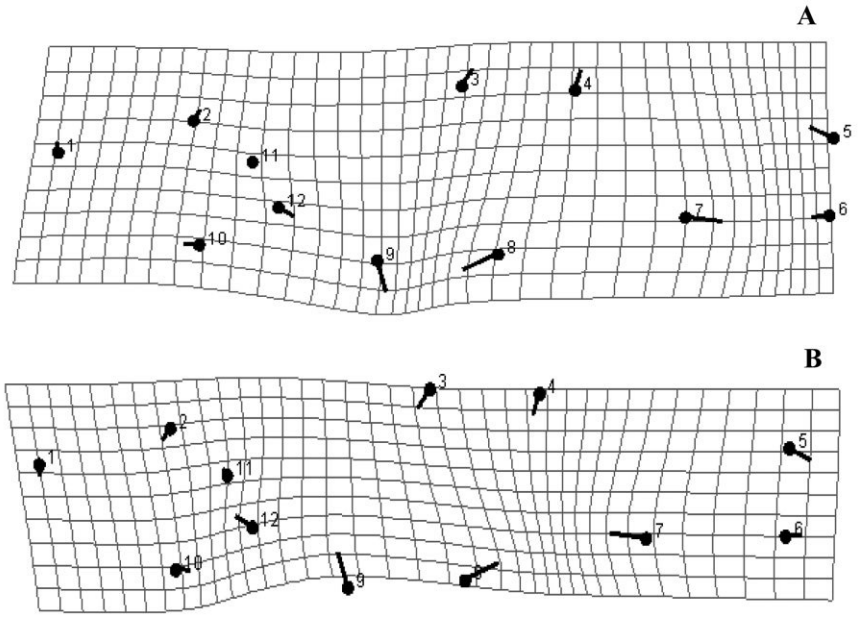

Figure 5. Transformation grids illustrating the shape changes between a consensus shape of each habitat type and a mean shape of all specimens, using the residual data for *Chirostoma jordani*. The lines point in the direction of the shape change for each landmark, where A denotes specimens collected from lentic habitats and B denotes specimens from lotic habitats.

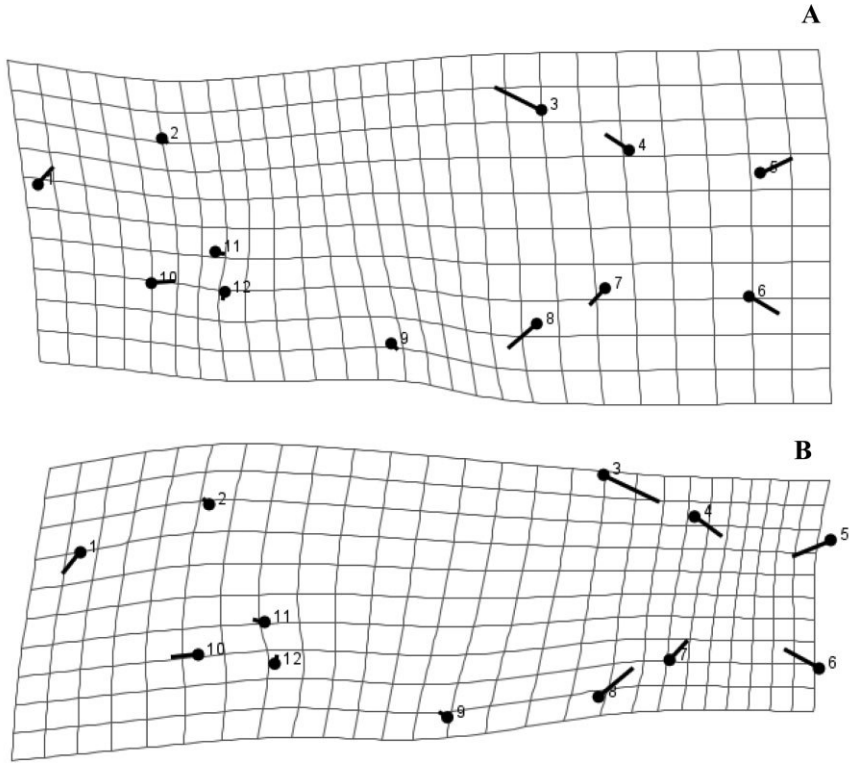

Figure 6. Transformation grids illustrating the shape changes between a consensus shape of each habitat type and a mean shape of all specimens, using the residual data for *Goodea atripinnis*. The lines point in the direction of the shape change for each landmark, where A denotes specimens collected from lentic habitats and B denotes specimens from lotic habitats.

preventing morphological diversification. Neotropical silversides are an economically important component of Mexican fisheries (Lyons *et al*., 1998), and *C. jordani* is the most widely distributed silverside species in Central Mexico (Barbour, 1973; Miller *et al*., 2005). With regard to *C. jordani*, the weaker morphological difference between the lentic and lotic populations could be attributed to undocumented introductions of individuals among habitats. However, further data based on fisheries and the movement of individuals between habitats would be necessary to test this hypothesis.

Conclusions

In summary, the body shape variation observed in the present study most accurately reflects the steady–unsteady swimming performance model (Langerhans, 2008; Langerhans & Reznick, 2009). Both species developed a deeper body shape suitable for unsteady swimming in lentic environments and streamlined body shapes in lotic habitats. Additionally, *G. atripinnis* developed a smaller head size and wider elongated caudal peduncle in lentic habitats and a larger head size and shallow/narrow caudal peduncle in lotic habitats, further supporting the steady–unsteady swimming performance model (Langerhans, 2008; Krabbenhoft *et al*., 2009; Langerhans & Reznick, 2009).

Clearly, the general influence of the different habitat regimes has played a role in the body shape differences recovered in the present study and highlights similar adaptive morphological responses of the two distantly-related sympatric species to similar environmental gradients. With the lack of intraspecific genetic variation, phenotypic plasticity is the likely mechanism for the morphological divergence seen in the present study (Stearns, 1989). The morphology of head and mouth regions may be related to differences in prey or environmental differences within each habitat type, although additional research is needed to disentangle the main driving forces behind the morphological divergence. Our results are consistent with the evolutionary hypothesis that divergent habitats drive intraspecific phenotypic diversification, and are important for predicting adaptive responses of freshwater fish species to divergent habitats and anthropogenic stream modification.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Thin spline plates using the residuals of the allometric regression for *Goodea atripinnis* females with lines pointing in the direction of the shape change for each landmark, where A denotes specimens collected from lentic habitats and B denotes specimens from lotic habitats.

Figure S2. Thin spline plates using the residuals of the allometric regression for *Goodea atripinnis* males with lines pointing in the direction of the shape change for each landmark, where A denotes specimens collected from lentic habitats and B denotes specimens from lotic habitats.

Table S1. Locality information and habitat designation for *Goodea atripinnis* (Goodeidae).

Table S2. Locality information and habitat designation for *Chirostoma jordani* (Atherinopsidae).

Table S3. NP-MANOVA results testing for the species-specific body shape divergence in lentic and lotic habitats using the matrix of residuals for *G. atripinnis*.