

Differing evolutionary patterns underlie convergence on elongate morphology in endemic fishes of Lake Waccamaw, North Carolina

TREVOR J. KRABBENHOFT^{1*}†, MICHAEL L. COLLYER^{2‡} and JOSEPH M. QUATTRO¹

¹Department of Biological Sciences, Marine Science Program, University of South Carolina, Columbia, SC, 29208 USA

²Department of Ecology, Evolution, and Organismal Biology, and Department of Statistics, Iowa State University, Ames, IA, 50011 USA

Received 5 January 2009; accepted for publication 17 May 2009

An extensive body of research has recently demonstrated patterns of parallel and/or convergent evolution that arise from divergent natural selection pressures exerted across environmental gradients. These studies, although providing some of our best empirical evidence for natural selection, have focused on rather narrow phylogenetic scopes, more often than not comparing patterns of morphological change among closely-related taxa within a single genus. Organisms in replicated populations in these studies are often assumed to have accomplished convergence via similar underlying processes. However, such assumptions cannot be made when looking at evolution across broader phylogenetic and ecological spectra. In the present study, we assessed morphological change across a much broader scale to test whether similar evolutionary and developmental patterns underlie convergence. Specifically, we studied morphological change that has occurred in a novel lake environment (Lake Waccamaw, North Carolina, USA) where three phylogenetically-disparate fishes representing different orders have speciated and independently evolved streamlined morphologies relative to their deeper-bodied progenitors occupying nearby streams and coastal regions. Geometric morphometric analyses revealed that, although the bulk of shape change between environments is similar across taxa, significant species-specific responses, concordant with differing expectations based on the ecologies of these taxa, were also found. Moreover, allometry analyses indicated that the developmental patterns underlying this change also differ across taxa. The present study provides evidence that, within a common environment, convergence can be achieved by different evolutionary and developmental patterns in phylogenetically- and ecologically-disparate taxa. Finally, these results contradict the commonly-held hypothesis that fishes should be more streamlined in streams than lakes and emphasize the need to also consider other environmental characteristics, such as water clarity and physical complexity, in studies of divergence. © 2009 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2009, 98, 636–645.

ADDITIONAL KEYWORDS: allometry – convergent evolution – geometric morphometrics – morphological divergence – speciation.

INTRODUCTION

Divergent natural selection has long been implicated as a major driving force for speciation and has been

invoked as a causal factor for evolutionary change in many lineages. Unlike purifying or directional selection, divergent selection results in an increase in variance in trait space and thus plays an important creative role in evolution (Gould, 2002). Recent research assessing the effects of divergent selection across a variety of environmental gradients is extensive. In fishes, for example, sticklebacks (Taylor & McPhail, 2000; Rundle, Vamosi & Schluter, 2003; Vamosi & Schluter, 2004), coregonid whitefishes

*Corresponding author. E-mail: krabbent@unm.edu

†Current address: Department of Biology and Museum of Southwestern Biology, MSC03-2020, 1 University of New Mexico, Albuquerque, NM, 87131 USA.

‡Current address: Department of Biology, Stephen F. Austin State University, SFA Station, Nacogdoches, TX, 75962 USA.

(Bernatchez *et al.*, 1996; Pigeon, Chouinard & Bernatchez, 1997), and poeciliids (Reznick & Endler, 1982; Reznick *et al.*, 1997) have clearly demonstrated that differences in biotic and abiotic factors among populations (e.g. water flow, temperature, and predation regime) can drastically affect morphological and life history attributes, potentially resulting in 'ecological speciation' (Schluter, 1996; McKinnon *et al.*, 2004). In many cases, such morphological differences have been shown to have direct genetic underpinnings (Foster & Baker, 2004; Shapiro *et al.*, 2004; Colosimo *et al.*, 2005) and functional significance (e.g. higher burst swimming speeds and greater survival of predatory encounters in guppy populations with predatory fish than without; Ghalambor, Reznick & Walker 2004). Research in this vein provides some of our richest insights into organism–environment interactions. Much of the recent work on the effects of divergent selection has focused on comparing patterns of change (i.e. convergence) among replicated environments across a narrow phylogenetic scope (e.g. anoles, sticklebacks, guppies, etc.). In these cases, replicated populations are likely to accomplish convergence in similar ways, either because the underlying genetic architecture or the ecologies of replicated populations are similar. Conversely, when convergent evolution occurs across phylogenetically- and ecologically-disparate taxa, it is not clear whether it is achieved in similar or different ways, and how extensive species-specific patterns of morphological change will be. In the present study, we tested whether similar evolutionary and developmental patterns underlie shape change in phylogenetically- and ecologically-disparate fishes that independently evolved elongate morphologies in a novel environment.

A particularly well-suited system for investigating the dynamics of recent convergent evolution is Lake Waccamaw, a bay lake located on the Atlantic coastal plain in North Carolina, USA. Lake Waccamaw has relatively clear water and high (nearly neutral) pH as a result of its location overlying the limestone Waccamaw Formation, and supports a diverse fish community comprised of approximately 42 species (Shute, Shute & Lindquist, 1981). Using geological and sedimentary core data, Stager & Cahoon (1987) estimated the lake was formed relatively recently, perhaps 15 000 and 32 000 years ago. Despite this recent origin, the lake is unique in having a suite of endemic species (Hubbs & Raney, 1946; Shute *et al.*, 1981), including three fishes that form the focus of the present study: *Etheostoma perlongum*, *Fundulus waccamensis*, and *Menidia extensa*. These fishes are phylogenetically distant, belonging to disparate taxonomic orders (Perciformes, Cyprinodontiformes, and Atheriniformes, respectively) that likely diverged during the Mesozoic or before (Steinke, Salzburger &

Meyer, 2006). The lake endemics also differ in ecology: *M. extensa* is a pelagic, schooling zooplanktivore; *E. perlongum* is a near-shore, solitary, benthic insectivore; and *F. waccamensis* is a mid-water shoaling species but feeds in the benthos.

In 1946, Hubbs and Raney described the endemic Lake Waccamaw fishes and hypothesized that they share sister relationships with three widely-distributed, allopatric riverine/coastal species: *Menidia beryllina* (Cope), *Etheostoma olmstedii* Storer, and *Fundulus diaphanus* Lesueur. Subsequent phylogenetic studies have generally supported these relationships (Shute, 1984; Wiley & Mayden, 1985; J. M. Quattro, unpubl. data). All three endemics are morphologically similar to their respective sister species with the exception of having 'stretched' morphologies (long and slender bodies) with concomitant higher longitudinal meristic features (e.g. vertebrae and lateral scale counts). Although the adaptive significance (if any) of this long, slender morphology is unknown, Hubbs & Raney (1946) posited that the Waccamaw endemics are adapted to evading predators in the clear, open waters of the lake and are thus more elongate, terete, and streamlined. Evidence from the functional morphology literature suggests that such morphology would be conducive to relatively-high sustained swimming speeds (Webb, 1978; Blake, 1983; Videler, 1993), as might be expected in open, physically homogeneous, clear-water environments. Similarly, Wiley & Mayden (1985) hypothesized that the endemics evolved as peripheral isolates of their widespread sister species as a result of local adaptation to the unique conditions in Lake Waccamaw. However, the occurrence of relatively streamlined body shapes in lakes compared to streams is perplexing because streamlining is typically expected in flowing waters (McLaughlin & Grant, 1994; McGuigan *et al.*, 2003; McGuigan, Chenoweth & Blows, 2005).

Lake Waccamaw presents a unique evolutionary 'experiment' that affords the opportunity to study the dynamics of convergent evolution. The existence of large numbers of endemics despite the lake's recent formation comprises evidence that this is a novel environment with attendant unique selective regimes for many taxa beyond fishes. In the present study, we analysed shape variation in endemic Waccamaw fishes and their stream and coastal progenitors aiming to better understand the evolutionary processes underlying the origin of this unique fauna and to gain insight into the dynamics of natural selection across phylogenetically- and ecologically-disparate taxa. Our primary goals were to test whether the streamlining noted by Hubbs and Raney was accomplished in a similar manner in the three Lake Waccamaw endemics, and whether allometric patterns

differed across the three genera. Specifically, we were interested in testing whether particular regions of the body changed in similar ways across taxa or, alternatively, whether they each responded uniquely to the novel lake environment. Similar responses across species that vary strongly in phylogeny, behaviour, and life history would provide strong evidence for selective pressures arising from environmental differences between stream and lake environments. Failure to detect similar patterns of divergence would suggest that other factors such as constraint (functional, developmental, etc.) or historical contingency might have played an important role in the evolution of this unique lacustrine fauna (Gould & Woodruff, 1990; Losos *et al.*, 1998; Langerhans & DeWitt, 2004). Finally, to test whether the patterns underlying body elongation in the lake were similar among genera, we compared allometric trajectories across environments and taxa. We tested whether stream and lake species differed in the rate and/or covariance of allometric change (i.e. whether the developmental patterns underlying body elongation were similar across taxa).

MATERIAL AND METHODS

Lateral body-shape of stream-lake pairs was analysed using landmark-based geometric morphometrics (Bookstein, 1991; Rohlf & Marcus, 1993) on preserved specimens from the North Carolina State Museum of Natural History. For the stream species, whenever possible, we used specimens from the Waccamaw River, a tributary of Lake Waccamaw. However, sufficient material was not always available and, in those instances, specimens from nearby drainages were used. Specific collection locality data are available from the corresponding author on request. Because the lake endemics evolved recently as peripheral isolates of the widely-distributed stream/coastal sister species (Wiley & Mayden, 1985), the stream species were considered the ancestral shapes. Two-dimensional images of the left side of 868 adult fishes were captured with a Nikon Coolpix 5400 digital camera. The TPS software series (available at: <http://life.bio.sunysb.edu/morph/>) was used for extracting and processing shape data for 13 landmarks (Fig. 1). Generalized Procrustes analysis (GPA) was performed to remove variation in landmark configurations arising from differences in position, size, and orientation (Rohlf & Slice, 1990). Shape data consisting of partial warps and two uniform scores were calculated and used for statistical analyses. Shape data were first collected for each genus aligned separately, and then for all three genera together in a combined analysis.

Principal components analysis (PCA) was conducted on shape data to visualize overall patterns of shape variation, first for each of the three genera separately to summarize within-genus patterns of variation, and then on the combined dataset to compare patterns of variation among genera. Next, a two-factor multivariate analysis of variance (MANOVA) was conducted using R (<http://www.r-project.org>) to compare the variation associated with factors potentially influencing shape. The linear model included genus (G), environment (E; lake or stream), and genus by environment interaction ($G \times E$; i.e. species-specific effects) as fixed effects. The $G \times E$ interaction essentially measures whether at least one genus has a unique pattern of shape change.

However, a significant interaction is difficult to interpret, as it is not readily obvious if the variance associated is the result of differences in the magnitude or direction of shape change among genera in the multivariate shape space. Accordingly, vector magnitudes were measured as the Euclidian distance (D_E) between stream-lake pairs of species by genus and converted to generalized Mahalanobis distance (D_M) to assess significance (Legendre & Legendre, 1998). Magnitudes of divergence were compared pairwise with the test statistic: $\bullet\bullet$ where D_{E_i} and D_{E_j} are the Euclidian distances between lake and stream species in genus i and j , respectively. To determine whether the lake endemics have diverged in a similar direction in morphospace (i.e. parallel shape divergence), vector correlations (VC) were calculated and converted to angular difference (θ) with the formula (Cheverud & Leamy, 1985; Klingenberg & Leamy, 2001):

$\theta = \cos^{-1}\left(\frac{\Delta\bar{Y}_i^T \cdot \Delta\bar{Y}_j}{D_{E_i} \cdot D_{E_j}}\right)$, where Y is the matrix of partial warps scores and uniform component. Finally, the 'residual randomization' procedure of Collyer & Adams (2007) was employed to determine the probability that the observed test statistics $|D_{E_i} - D_{E_j}|$ and θ were greater than would be expected from random pairs of vectors sampled from a null distribution. This procedure randomizes residuals from a 'reduced' model (that only contains G and E effects, but lacks the $G \times E$ interaction) and re-estimates parameters from the 'full' models many times to produce empirical null distributions of vector length contrasts and vector correlations. We ran this procedure with 10 000 random permutations.

The advantage of this method is that it randomizes residuals across $G \times E$ interactions without discarding the information contained in the significant fixed effects. Strongly positively-correlated vectors of shape divergence among genera would indicate consistent responses to the novel lake environment (i.e. conver-

1

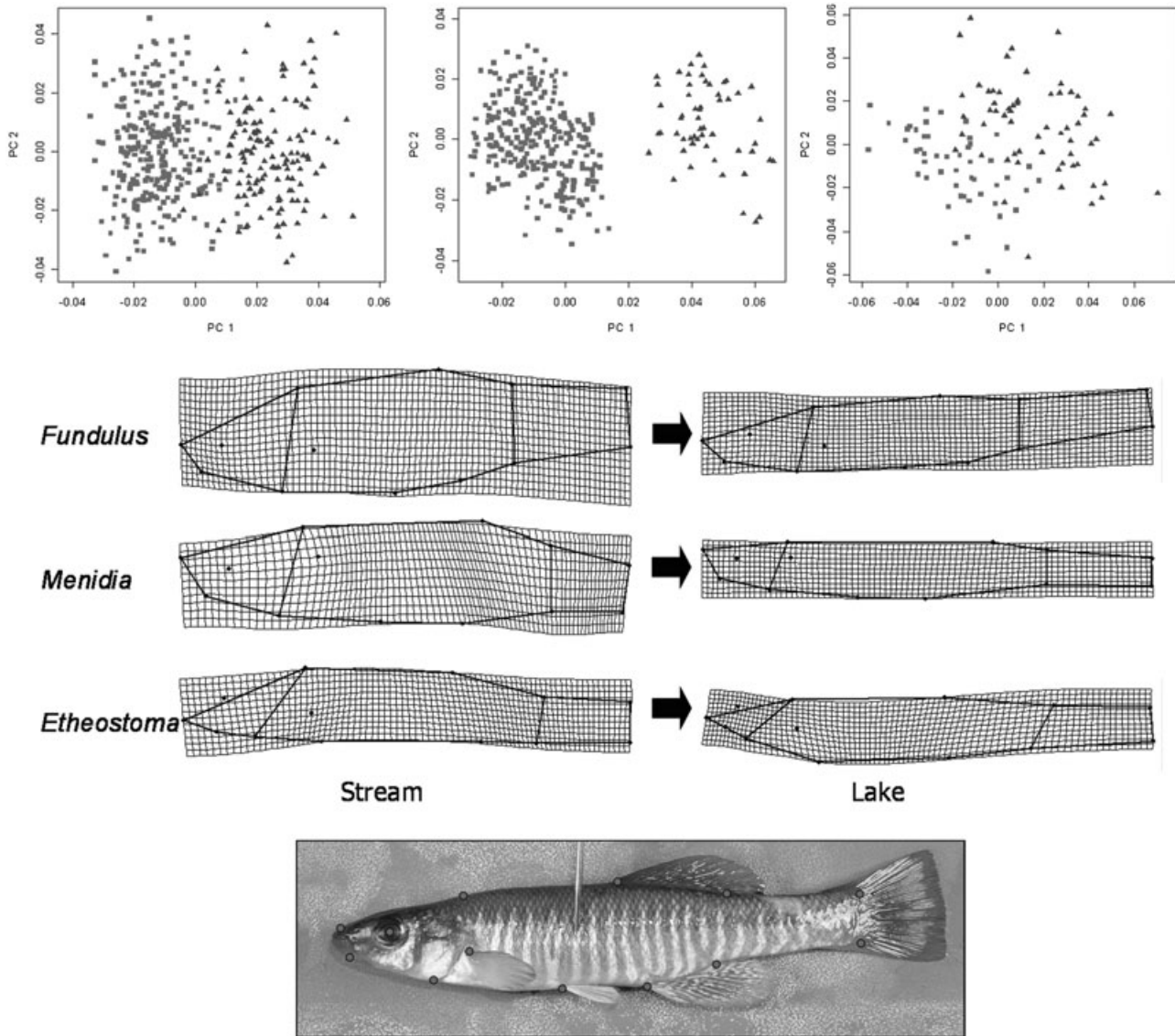


Figure 1. Top panel: principal components analysis plots for three Lake Waccamaw endemics (red/squares) and their respective stream-dwelling sister taxa (blue/triangles) for (from left to right) *Fundulus*, *Menidia*, and *Etheostoma*. The first two principal components account for between 46.0% and 58.7% of overall shape variation. Middle panel: deformation grids depicting mean shape for the three stream species (left) and the respective lake species (right). Shape differences are magnified three times for ease of comparison. Note similar overall patterns of shape divergence among the three genera, but also the presence of genus-specific differences. Bottom panel: locations of 13 landmarks used in the present study, primarily consisting of fin origins and insertions.

gence) and would suggest that underlying selective pressures are pervasive. Conversely, weakly or negatively-correlated vectors would suggest that other factors such as historical contingency or constraint have played a relatively greater role in the evolution of body shape in these species.

ALLOMETRY ANALYSIS

In addition to considerations of shape differences, we analysed variation in shape allometries (i.e. change in

shape associated with body size). These analyses are important for discerning whether shape differences result from different allometric trajectories. For these analyses, we used a linear model that contained the G, E, and $G \times E$ effects mentioned previously, but also log centroid size (CS) as a covariate (as well as interactions between log CS and main effects). Centroid size was calculated as the square root of the summed, squared distances of each landmark about the centroid of the landmark configuration (Book-

Table 1. Euclidian (D_E) and generalized Mahalanobis (D_M) distances between endemic Lake Waccamaw fishes and their respective sister species

Comparison	D_E	D_M	N_1	N_2	d.f.	F	P -value
<i>Menidia extensa</i> / <i>Menidia beryllina</i>	0.0539	8.659	285	55	22, 317	147.37	< 0.0001
<i>Fundulus waccamensis</i> / <i>Fundulus diaphanus</i>	0.0371	5.642	274	143	22, 394	129.43	< 0.0001
<i>Etheostoma perlongum</i> / <i>Etheostoma olmstedi</i>	0.0410	4.788	51	60	22, 88	23.20	< 0.0001

stein, 1991). Significant variation associated with log CS indicates that shape change is associated with fish growth. Significant variation associated with the interaction between main effects and log CS indicates that groups differ in their patterns of shape change associated with fish growth.

Differences in shape allometries (as described by the vectors of regression coefficients for different genus–environment groups) can manifest two different ways. First, the magnitude of allometry vectors can differ. This indicates that groups differ in the amount of shape change associated with similar changes in body size (i.e. different rates of shape change). Second, allometry vectors can differ in orientation as a result of differences in covariation of shape variables. For example, two genus–environment groups might exhibit similar amounts of shape change per unit body size, but one group's change is expressed more so by tail elongation than body elongation.

We used a slight variation of the residual randomization method described above to test for differences in shape allometries. The reduced model was the same (i.e. contained only G and E effects), but the full model also contained parameters that estimated the allometric slopes for every genus–environment group (i.e. additionally contained the $G \times E$ interaction, log CS, and the interaction between log CS and all other factors). Shape allometries were estimated as the vector of regression coefficients of shape variables on log CS for every genus–environment group, which were recalculated with every random permutation. Differences in the length of these vectors indicate differences in the amount of shape change associated with fish growth. Differences in vector directions indicate differences in the covariances of shape variables associated with fish growth. We created null distributions of vector length contrasts and vector correlations for allometry vectors from 10 000 random permutations to statistically test these attributes of shape allometry.

RESULTS

PCA revealed distinct patterns of shape variation between lake and stream species within each genus

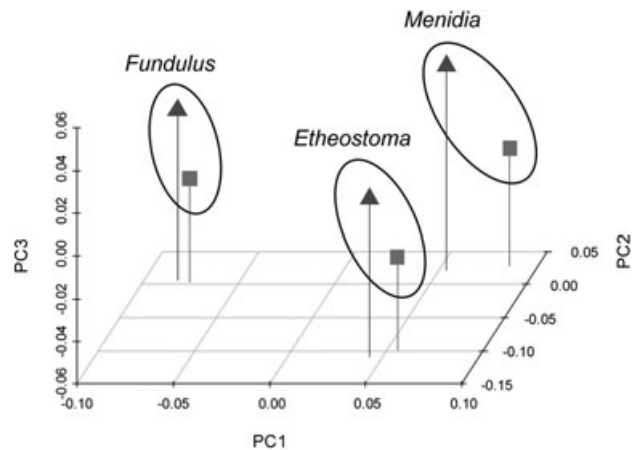


Figure 2. Three-dimensional principal components analysis plot depicting mean shape for each of the three lake endemics (squares) and the respective stream species (triangles). Only species means are shown. Not surprisingly, the first two principal components largely account for variation among the three genera. The third principal component, however, reveals striking environmental patterns, with lake species having smaller mean values in all three cases.

(Fig. 1). In all three comparisons, stream and lake species occupy distinct portions of morphospace, with little overlap in *Menidia* and *Fundulus*, and slightly more overlap in *Etheostoma*. The concomitant generalized Mahalanobis distances were all highly significant (Table 1), indicating significant morphological divergence between environments for each of the three genera.

PCA conducted on the combined dataset also yielded interesting results (Fig. 2). Not surprisingly, the among-genus component of shape variation was large, with scatter along the first two principle axes largely resulting from inter-generic differences. Of greater interest is the third principal component, where the lake endemics have smaller mean scores than their stream counterparts, suggesting that variation along the longest axis of 'non-intergeneric' shape variation is primarily associated with differences between environments. That is, shape differences between environments appear to be correlated among genera. However, inferences about shape divergence in reduced dimen-

Table 2. Pairwise comparisons of vectors of shape divergence for three lake-stream species pairs

Vector comparison	Vector correlation	θ	P_{rand}	$ D_{E_i} - D_{E_j} $	P_{rand}
<i>Etheostoma</i> / <i>Menidia</i>	0.773	39.4°	< 0.0001	0.0129	< 0.0001
<i>Etheostoma</i> / <i>Fundulus</i>	0.493	60.4°	< 0.0001	0.0039	0.0780
<i>Menidia</i> / <i>Fundulus</i>	0.725	43.5°	< 0.0001	0.0168	< 0.0001

θ is the angular difference between two vectors, whereas $|D_{E_i} - D_{E_j}|$ represents the absolute value of the difference in lengths between vectors i and j . P -values represent the probability of finding a larger test statistic under a null distribution.

sionalities should be regarded with caution because correlations along particular principal axes might differ from correlations for the full dataset. As such, we calculated VC using the entire matrix of PC scores. In all three comparisons, vectors were positively correlated with VC in the range 0.493–0.773. However, angular differences between vectors were significantly different from null expectations in all three cases (Table 2). The magnitude of shape divergence also differed for two of the three pairwise comparisons (Table 2). Vector lengths differed significantly between *Menidia* and *Fundulus* ($|D_{E_i} - D_{E_j}| = 0.0168$; $P_{\text{rand}} < 0.0001$), as well as between *Menidia* and *Etheostoma* ($|D_{E_i} - D_{E_j}| = 0.0129$; $P_{\text{rand}} < 0.0001$), with the *Menidia* vector being longer in both instances. By contrast, *Fundulus* and *Etheostoma* vectors did not significantly differ in length ($|D_{E_i} - D_{E_j}| = 0.0039$; $P_{\text{rand}} = 0.0780$). As expected, MANOVA revealed highly significant genus (Wilks' $\Lambda = 0.02$, $P < 0.0001$) and environment (Wilks' $\Lambda = 0.08$, $P < 0.0001$) effects, as well as smaller, but significant $G \times E$ interactions (Wilks' $\Lambda = 0.64$, $P < 0.0001$).

Deformation grids confirm that the entails majority of shape change across environments entails 'stretching' along the anterior–posterior axis (i.e. long, slender bodies in the lake forms) (Fig. 1). Interestingly, the three lake species appear to become streamlined in similar ways: all three species have relatively shorter and more slender heads (particularly in *Menidia* and *Etheostoma*), longer and shallower caudal peduncles, and more slender bodies. However, as previously stated, both the direction and differences in the magnitudes of vectors differ from null expectations, implying the presence of genus-specific responses. These differences are also evident in the deformation grids. For example, the stretching appears to be more extreme in *Menidia* and *Fundulus* than in *Etheostoma*. This could be a result of *Etheostoma* already being more slender-bodied in the stream form. Additionally, lake *Etheostoma* appear to be less 'benthic' than their stream counterparts. The lake forms have relatively up-turned heads and straighter dorsal profiles, whereas the stream forms have down-turned heads

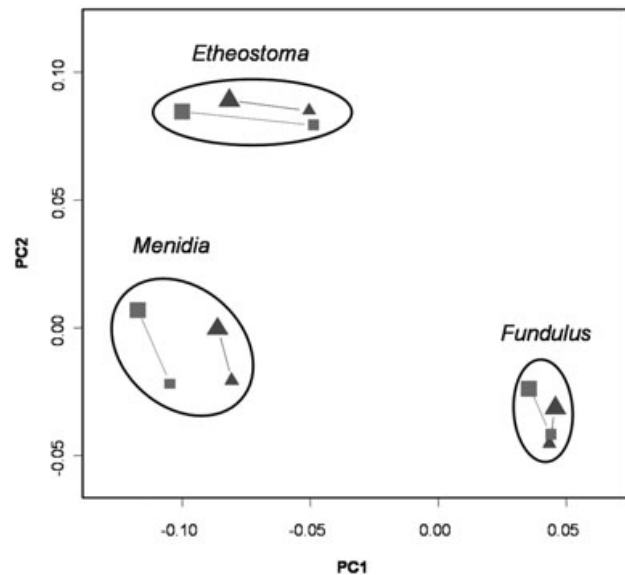


Figure 3. Principal components analysis plot depicting allometric trajectories for three Lake Waccamaw endemics (squares) and their sister taxa (triangles). Squares and triangles reflect locations of predicted shapes for the smallest (small symbols) and largest (large symbols) individuals.

and 'arched' dorsal profiles. These differences are not evident in the pelagic *Menidia* and *Fundulus*.

Allometry analyses also revealed genus-specific patterns (Fig. 3). *Etheostoma* had a significantly higher rate of allometric change in the lake compared to streams (0.075 versus 0.048; $P < 0.01$), but allometry vectors did not differ in direction across environments (43.6°; $P = 0.528$). *Menidia* differed in direction (75.1°; $P = 0.020$) but not rate (0.055 versus 0.051; $P = 0.913$) of allometric change across environments. *Fundulus* differed neither in rate (0.039 versus 0.043; $P = 0.337$), nor direction (31.0°; $P = 0.088$) across environments. Figure 4 illustrates the predicted shapes for the smallest and largest specimens of each species. Largely overlapping distributions of centroid size across species suggest that allometric differences

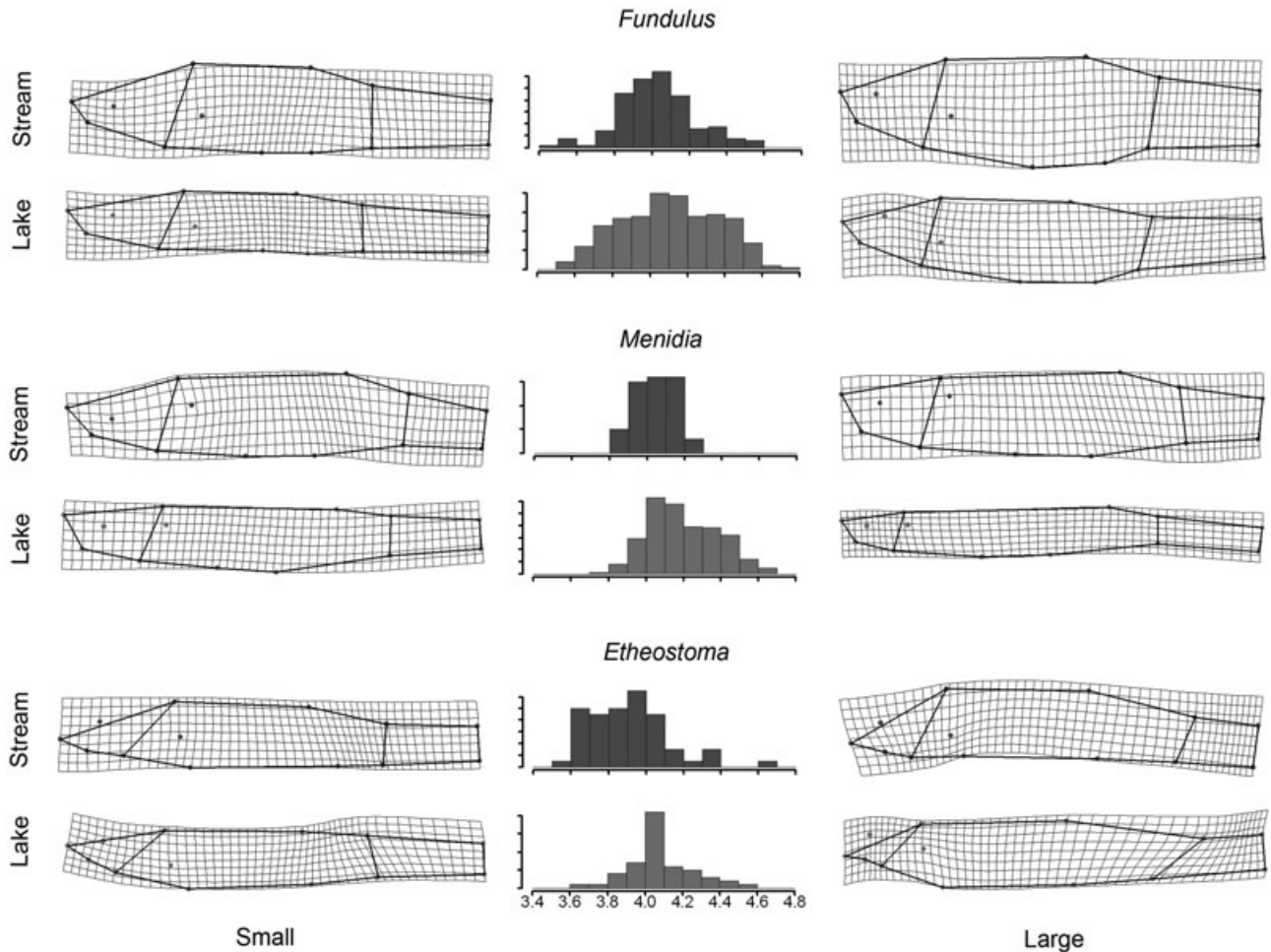


Figure 4. Comparison of body shape in relation to log centroid size in three Lake Waccamaw endemics (red/squares) and their sister taxa (blue/triangles). Deformation grids illustrate least-squares predicted landmark configurations for the smallest (left column) and largest (right column) specimen examined for each species. The center column consists of histograms depicting log centroid size of specimens in this study, demonstrating largely overlapping distributions of body size, even across genera.

did not simply result from size differences. Finally, of the six species, only lake *Menidia* appears to become proportionally more elongated through ontogeny.

DISCUSSION

The endemic fishes of Lake Waccamaw afford a unique opportunity to study morphological divergence across two different environments and broad phylogenetic and ecological scopes. Within the lake, three endemic fish species have independently evolved more elongate morphologies relative to their respective sister species. Additionally, positively-correlated vectors of shape divergence suggest that the underlying patterns of shape change are similar. Shape differences between environments are largely the result of overall differences in body depth, as noted by Hubbs & Raney

(1946), with all three lake endemics being longer and more slender than their stream progenitors (Fig. 1). More importantly, shape differences are not simply a result of uniform stretching along the horizontal axis, but are centered on particular regions of the body. For example, two of the endemics have shorter heads, and all three have substantially longer caudal peduncles than the stream species. Similar morphological changes are unlikely to arise repeatedly by chance, suggesting an adaptive component to this divergence (Schluter, 2001). This morphology (short head, slender body, and elongated caudal peduncle) is consistent with theoretical expectations for fishes adapted for high sustained swimming speeds (Webb, 1978; Blake, 1983; Videler, 1993). Alternatively, such shape differences could be the result of dietary differences across environments. This hypothesis is

unlikely because the slender-bodied lake fishes have higher vertebral and lateral scale counts in all three cases (Hubbs & Raney, 1946), suggesting phenotypic canalization. Another hypothesis, that temperature differences between the lake and streams account for higher vertebral counts and, thus more elongate shape, is also unlikely. 'Jordan's Rule' states that, in fishes, meristic counts such as vertebrae often increase with decreasing temperature during development (Jordan, 1891; Bailey & Gosline, 1955; Yamahira, Lankford & Conover, 2006), which is precisely the opposite pattern to that observed in the relatively warm Lake Waccamaw.

Relatively more streamlined morphologies are generally expected in streams because it reduces drag in flowing water (McLaughlin & Grant, 1994; McGuigan *et al.*, 2003; McGuigan *et al.*, 2005). Paradoxically, the opposite is observed in the Lake Waccamaw endemics, suggesting the presence of other unknown selective pressures in the lake. This finding underscores the need to consider environmental features other than flow, such as behaviour, microhabitat preferences, water clarity, and the physical complexity of the environment. Lake Waccamaw has significantly clearer water and large expanses of open water with a sandy bottom and little physical structure (trees, rocks, etc.). By contrast, the streams and coastal habitats of the progenitor species tend to have darkly-coloured or turbid water with poor visibility and are physically heterogeneous, with copious woody debris and vegetation. Such environmental differences affect the behaviour of fishes and dynamics of natural selection, with lake endemics tending to form large schools (in *Fundulus* and *Menidia*) in open water and engaging in more constant swimming. Thus, the expectations for deep-bodied versus streamlined morphotypes depends not only on habitat type, such as 'lake' or 'stream', but also on the physical, chemical, and biotic characteristics of those environments.

Although the majority of shape variation is consistent across genera as predicted by Hubbs & Raney (1946), we also found significant species-specific responses to the novel lake environment that only became evident through the use of geometric morphometrics. Angles between vectors of shape divergence differ significantly from 0° in all comparisons, suggesting that, although the majority of shape divergence is consistent across genera, statistically and perhaps biologically meaningful differences in shape change among genera are also present. That is, the three genera appear to differ subtly in their patterns of shape divergence, suggesting not only the importance of novel selective pressures in the lake (i.e. convergent evolution), but also the importance of taxon-specific factors. Such differences might reflect the fine-tuning of morphology to the unique ecologies

of the endemics. For example, as stated previously, the stretching appears to be more extreme in *Menidia* and *Fundulus* than in *Etheostoma*, and the *Menidia* vector is significantly longer than both the *Fundulus* and *Etheostoma* vectors. This is not unexpected, and it is reasonable to assume that selection for high sustained swimming would act more strongly on the pelagic *Menidia* and *Fundulus* than on the benthic *Etheostoma*. Additionally, lake *Etheostoma* have relatively upturned heads and straighter dorsal profiles (i.e. less 'benthic') compared to their stream counterpart, which comprises a pattern that is not seen in the pelagic *Fundulus* and *Menidia*. This difference could reflect a release from pressures on *Etheostoma* to maintain close association with the current boundary layer in flowing water, in lieu of a more pelagic morphology in the lake. Whether such differences in morphological divergence arise as a result of historical contingency, constraint, and/or species-specific selective pressures remains known. We speculate that differences in feeding ecologies and behaviour underlie genus-specific patterns of divergence and might play a role in constraining the evolution of shape, particularly in the head region.

Additionally, our allometry analyses suggest that the evolutionary and developmental patterns underlying body elongation differ among the three genera. *Etheostoma* has a significantly faster rate of allometric change in the lake, but does not differ in direction of allometric change between environments. *Menidia* has different directions of allometric change, but not different rates. *Fundulus* species differed neither in rate, nor direction of allometries. These data suggest that, even in a common environment, phylogenetically- and ecologically-disparate taxa can accomplish convergence in different ways.

Given the presence of streamlined morphology in the three endemics, a natural question is whether the other approximately 39 fish species in Lake Waccamaw show similar morphological patterns. A fourth endemic from the lake, '*Notropis waccamanus*', was described (Fowler, 1942), but later synonymized with *Notropis petersoni*, a stream species (Frey, 1951). Shape comparisons of *N. waccamanus* and *N. petersoni* show a similar, albeit less extreme morphological pattern compared to that seen in the three endemics (T. J. Krabbenhoft, unpubl. data). In this case, almost all of the difference in shape is located in the caudal peduncle, with *N. waccamanus* having a longer tail than stream *N. petersoni*. Although detailed shape analyses are lacking, other species in the lake do not appear to have evolved elongated morphologies. Many of these are infrequent visitors to the lake and are more abundant in the Waccamaw River, a distributary of Lake Waccamaw. Contemporary gene flow exists between the lake and river populations in these

species, potentially masking selection for elongate morphology in the lake.

Lake Waccamaw presents a previously unexploited opportunity to examine the effects of divergent natural selection arising from novel environmental conditions on phylogenetically-disparate fishes. The combination of high rates of endemism and the recent formation of these lakes suggests that substantial selective pressures underlie this divergence. Additionally, this research demonstrates that Lake Waccamaw, although small, is a rather unique source of aquatic biodiversity and represents a novel lacustrine system worthy of conservation focus.

ACKNOWLEDGEMENTS

We thank D. Adams for his insight into this project and for providing laboratory space for the first author. W. Starnes and G. Hogue provided access to specimens at the North Carolina Museum of Natural Sciences. J. Kenney-Hunt and D. Strickland reviewed an earlier version of this manuscript. We are also indebted to C. Anderson, T. Mousseau, T. Munroe, K. Oswald, M. Roberts, T. Turner, and D. Vogt for their assistance. The first author was supported by Graduate Student Fellowship and Elsie Tabor Fellowship from the University of South Carolina and benefited from participation in the NSF-sponsored Iowa State University Geometric Morphometrics Workshop.

REFERENCES

- Bailey RM, Gosline WA. 1955.** Variation and systematic significance of vertebral counts in the American fishes of the Family Percidae. Miscellaneous Publications of the Museum of Zoology of Michigan, No. 93. Ann Arbor, MI: Museum of Zoology of Michigan.
- Bernatchez L, Vuorinen JA, Bodaly RA, Dodson JJ. 1996.** Genetic evidence for reproductive isolation and multiple origins of sympatric trophic ecotypes of whitefish (*Coregonus*). *Evolution* **50**: 624–635.
- Blake RW. 1983.** *Fish locomotion*. Cambridge: Cambridge University Press.
- Bookstein FL. 1991.** *Morphometric tools for landmark data: geometry and biology*. New York, NY: Cambridge University Press.
- Cheverud JM, Leamy LJ. 1985.** Quantitative genetics and the evolution of ontogeny. III. Ontogenetic changes in correlation structure among live-body traits in random-bred mice. *Genetical Research* **46**: 325–335.
- Collyer ML, Adams DC. 2007.** Analysis of two-state multivariate phenotypic change in ecological studies. *Ecology* **88**: 683–692.
- Colosimo PF, Hosemann KE, Balabhadra S, Villarreal G Jr, Dickson M, Grimwood J, Schmutz J, Myers RM, Schluter D, Kingsley DM. 2005.** Widespread parallel evolution in sticklebacks by repeated fixation of Ectodysplasin alleles. *Science* **307**: 1928–1933.
- Foster SA, Baker JA. 2004.** Evolution in parallel: new insights from a classic system. *Trends in Ecology and Evolution* **19**: 456–459.
- Fowler HW. 1942.** Descriptions of six new fresh-water fishes (Cyprinidae and Percidae) from the southeastern United States. *Notulae Naturae* **107**: 1–11.
- Frey DG. 1951.** The fishes of North Carolina's bay lakes and their intraspecific variation. *Journal of the Elisha Mitchell Scientific Society* **67**: 1–44.
- Ghalambor CK, Reznick DN, Walker DN. 2004.** Constraints on adaptive evolution: the functional trade-off between reproduction and fast-start swimming performance in the Trinidadian guppy (*Poecilia reticulata*). *American Naturalist* **164**: 38–50.
- Gould SJ. 2002.** *The structure of evolutionary theory*. Cambridge, MA: Harvard University Press.
- Gould SJ, Woodruff DS. 1990.** History as a cause of area effects – an illustration from *Cerion* on Great Inagua, Bahamas. *Biological Journal of the Linnean Society* **40**: 67–98.
- Hubbs CL, Raney EC. 1946.** Endemic fish fauna of Lake Waccamaw, North Carolina. Miscellaneous Publications of the University of Michigan Museum of Zoology No. 65. Ann Arbor, MI: Museum of Zoology of Michigan.
- Jordan DS. 1891.** Relations of temperature to vertebrae among fishes. *Proceedings of the United States National Museum* **14**: 107–120.
- Klingenberg CP, Leamy LJ. 2001.** Quantitative genetics of geometric shape in the mouse mandible. *Evolution* **55**: 2342–2352.
- Langerhans RB, DeWitt TJ. 2004.** Shared and unique features of evolutionary diversification. *American Naturalist* **164**: 335–349.
- Legendre P, Legendre L. 1998.** *Numerical ecology*, 2nd edn. Amsterdam: Elsevier Press.
- Losos JB, Jackman TR, Larson A, de Queiroz K, Rodríguez-Schettino L. 1998.** Contingency and determinism in replicated adaptive radiations of island lizards. *Science* **279**: 2115–2118.
- McGuigan K, Chenoweth SF, Blows MW. 2005.** Phenotypic divergence along lines of genetic variance. *American Naturalist* **165**: 32–43.
- McGuigan K, Franklin CE, Moritz C, Blows MW. 2003.** Adaptation of rainbow fish to lake and stream habitats. *Evolution* **57**: 104–118.
- McKinnon JS, Moori S, Blackman BK, David L, Kingsley DM, Jamieson L, Chou J, Schluter D. 2004.** Evidence for ecology's role in speciation. *Nature* **429**: 294–298.
- McLaughlin RL, Grant JWA. 1994.** Morphological and behavioural differences among recently-emerged brook charr, *Salvelinus fontinalis*, foraging in slow- vs. fast-running water. *Environmental Biology of Fishes* **39**: 289–300.
- Pigeon D, Chouinard A, Bernatchez L. 1997.** Multiple modes of speciation involved in the parallel evolution of sympatric morphotypes of lake whitefish (*Coregonus clupeaformis*). *Evolution* **51**: 196–205.

- Reznick DN, Endler JA. 1982.** The impacts of predation on life-history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution* **36**: 160–177.
- Reznick DN, Shaw FH, Rodd FH, Shaw FG. 1997.** Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). *Science* **275**: 1934–1937.
- Rohlf FJ, Marcus LF. 1993.** A revolution in morphometrics. *Trends in Ecology and Evolution* **8**: 129–132.
- Rohlf FJ, Slice DE. 1990.** Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Zoology* **39**: 40–59.
- Rundle HD, Vamosi SM, Schluter D. 2003.** Experimental test of predation's effect on divergent selection during character displacement in sticklebacks. *Proceedings of the National Academy of Sciences of the United States of America* **100**: 14943–14948.
- Schluter D. 1996.** Ecological speciation in postglacial fishes. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* **351**: 807–814.
- Schluter D. 2001.** Ecology and the origin of species. *Trends in Ecology and Evolution* **16**: 372–380.
- Shapiro MD, Marks ME, Peichel CL, Blackman BK, Nereng KS, Jonsson BJ, Schluter DS, Kingsley DM. 2004.** Genetic and developmental basis of evolutionary pelvic reduction in threespine sticklebacks. *Nature* **428**: 717–723.
- Shute JR. 1984.** A systematic evaluation of the Waccamaw darter, *Etheostoma perlongum* (Hubbs and Raney), with comments on relationships within the subgenus *Boleosoma* (Percidae : Etheostomatini). MS Thesis, University of Tennessee.
- Shute JR, Shute PW, Lindquist DG. 1981.** Fishes of the Waccamaw River drainage. *Brimleyana* **6**: 1–24.
- Stager JC, Cahoon LB. 1987.** The age and trophic history of Lake Waccamaw, North Carolina. *Journal of the Elisha Mitchell Scientific Society* **103**: 1–13.
- Steinke D, Salzburger W, Meyer A. 2006.** Novel relationships among ten fish model species revealed based on a phylogenomic analysis using ESTs. *Journal of Molecular Evolution* **62**: 772–784.
- Taylor EB, McPhail JD. 2000.** Historical contingency and ecological determinism interact to prime speciation in sticklebacks, *Gasterosteus*. *Proceedings of the Royal Society of London Series B, Biological Sciences* **267**: 2375–2384.
- Vamosi SM, Schluter D. 2004.** Character shifts in the defensive armor of sympatric sticklebacks. *Evolution* **58**: 376–385.
- Videler JJ. 1993.** *Fish swimming*. London: Chapman and Hall.
- Webb PB. 1978.** Fast-start performance and body form in seven species of teleost fish. *Journal of Experimental Biology* **74**: 211–226.
- Wiley EO, Mayden RL. 1985.** Species and speciation in phylogenetic systematics, with examples from the North American fish fauna. *Annals of the Missouri Botanical Gardens* **72**: 596–635.
- Yamahira K, Lankford TE Jr, Conover DO. 2006.** Intra- and interspecific latitudinal variation in vertebral number in *Menidia* spp. (Teleostei : Atherinopsidae). *Copeia* **2006**: 431–434.