



Replicated shape variation between simple and complex habitats in two estuarine fishes

CLIFTON B. RUEHL^{1*}, VIRGINIA SHERVETTE² and THOMAS J. DEWITT³

¹Department of Biology, East Carolina University, Greenville, NC 27858, USA

²Environmental Health Sciences, University of South Carolina, Columbia, SC 29208, USA

³Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX 77843-2258, USA

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A replicated pattern of habitat-associated morphology among different lineages may represent adaptive convergence. Deviation from the replicated (shared) pattern of diversification reflects unique (e.g. species specific) effects resulting from site- or species-specific selection, intrinsic factors (e.g. **G** matrix differences) or chance historical events (e.g. genetic drift). For two distantly-related estuarine fishes [*Lagodon rhomboides* (Sparidae; Linnaeus) and *Leiostomus xanthurus* (Sciaenidae; Lacepède)], we examined shared and unique instances of body shape variation between seagrass (complex) and sand (simple) microhabitats at four sites. We found extensive shape variation between microhabitats for both species. As a shared response, both species from sand had subterminal snouts and long caudal peduncles, whereas those from seagrass had terminal snouts and deep bodies. Unique responses involved a greater difference in *Lagodon rhomboides* head shape between microhabitats compared to *L. xanthurus*. Patterns of shape variation fit ecomorphological predictions for foraging in the respective microhabitats (simple versus complex) because deep bodies are expected for fish that must negotiate complex habitats and subterminal snouts facilitate benthic foraging common in barren habitats. Parallel differentiation between microhabitats simultaneously suggests that individuals of each species use a particular microhabitat within estuaries for development and the differentiation in shape represents adaptive convergence. Spatial variation in the magnitude of shape differences between microhabitats was an unexpected finding and suggests that phenotypic variation operates at multiple scales within estuaries. © 2011 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2011, 103, 147–158.

ADDITIONAL KEYWORDS: ecomorphology – ecophenotypy – geometric morphometrics – Gulf of Mexico – pinfish – shared and unique divergence – spot.

INTRODUCTION

Organisms respond to spatial and temporal variation in environmental factors through a variety of mechanisms. Repeated phenotype–environment associations across space and between species suggest that patterns are adaptive (Schluter, 1996; Losos *et al.*, 1998). Divergent natural selection produces such patterns through evolution of genetic polymorphism, phenotypic plasticity or a combination of these factors.

Genetic polymorphism is expected to evolve when environments are relatively constant and differ spatially among populations (Levins, 1968; Schlichting & Pigliucci, 1998; Leimar, Hammerstein & Van Dooren, 2006). Phenotypic plasticity is expected to evolve under temporal environmental variation when the pace of environmental change is coarse, allowing organisms to sense variation and produce appropriate phenotypes (Bradshaw, 1965; West-Eberhard, 2003; for evolution of plasticity in fine-grained environments, see Ruehl & DeWitt, 2005). Regardless of the mechanism of divergence, the pattern expected from replicated instances of divergent natural

*Corresponding author. E-mail: ruehlc1@ecu.edu

selection is replicated phenotypic divergence along environmental gradients, reflecting adaptive differentiation (Winemiller, 1991; Johnson & Belk, 2001). The concept of convergent-divergence (i.e. replicated patterns of phenotypic differentiation for different taxa between environments) has received considerable attention and appears common across many taxonomic scales (Robinson & Wilson, 1994; Relyea & Mills, 2001; Pigliucci, Pollard & Cruzan, 2003). However, every species has a distinct evolutionary history. The unique evolutionary history of a species includes past selection events, intrinsic factors such as variation in the genetic variance–covariance matrix (**G** matrix), and chance events such as genetic drift. Each of these factors can serve to constrain convergence toward an optimum phenotype or result in species arriving at a similar phenotypic solution to an environmental gradient through different mechanisms (Langerhans *et al.*, 2003). Therefore, when faced with similar selective regimes, species will typically exhibit both shared and unique responses (Langerhans & DeWitt, 2004; Langerhans, Knouft & Losos, 2006). Many studies testing for shared and unique components of phenotypic diversification focus on a particular clade, although the inclusion of multiple clades provides a test for the generality of shared phenotypic outcomes along common environmental gradients.

Obfuscating the role of divergent natural selection in driving phenotypic divergence along environmental gradients are biotic (ecology and life history) and abiotic (environmental heterogeneity) factors that do not pertain to the shared environmental gradient. Many species change habitats and diets over ontogeny that alters the magnitude and persistence of environmental influence on phenotypes (Robinson, Wilson & Shea, 1996; Svanback & Eklöv, 2002; Parker *et al.*, 2009). Population mixing through gene flow and migration can constrain phenotypic divergence among populations experiencing even strong divergent natural selection. Similarly, spatial and temporal variation in abiotic and biotic factors that are not associated with the shared environmental gradient can dilute the strength of natural selection and weaken shared phenotypic variation. Therefore, shared patterns of divergence between taxa that have complex life histories and occur in highly variable environments may be rare and consequently few studies have considered the possibility for such patterns.

Estuaries represent ecosystems that vary spatially and temporally in a variety of abiotic (temperature, salinity, dissolved oxygen) and biotic (foraging and feeding) variables. Many fishes that inhabit estuaries have complex life histories that involve offshore breeding migrations, as well as ontogenetic diet and

habitat shifts. Microhabitats within estuaries occur as a patchwork of complex seagrass beds, oyster beds, and *Spartina* marshes interspersed with relatively simple sand and mud flats. These characteristics of estuaries and the fish species that live there suggest an environment with considerable population mixing and abiotic extremes that could dilute the effects of divergent natural selection. An alternative view of estuaries considers the patchwork of microhabitats that vary in resource types and quantities as an opportunity for divergent selection to operate on traits because these different habitats likely require different modes of feeding and locomotion. Variation in modes of feeding and locomotion between microhabitats creates trade-offs by placing different demands on traits associated with foraging and movement. Divergent natural selection between microhabitats might drive phenotypic variation within and between species that reflects the contrasting demands on acquiring resources and movement. As far as we know, no study has considered the potential for phenotypic diversity in fishes that co-occur between microhabitats in estuaries.

In the present study, we tested for habitat-associated morphology and shared differentiation in two common estuarine fish species, spot (*Leiostomus xanthurus*, Sciaenidae; Lacepède) and pinfish (*Lagodon rhomboides*, Sparidae; Linnaeus). The two species are distantly related (different families) but share life history patterns. Both species migrate offshore to breed each year and produce pelagic larvae that eventually settle and develop in seagrass and sand microhabitats within estuaries (Hansen, 1970; Chao & Musick, 1977). Their diets overlap, although *L. xanthurus* consumes more epifauna and infauna, whereas *L. rhomboides* consumes more algae and some vascular plants (Hansen, 1970; Chao & Musick, 1977). We made specific predictions about the morphology of these species collected from simple (sand) and complex (seagrass) microhabitats based on established ecomorphological patterns related to foraging and locomotion in fishes (Keast & Webb, 1966; Barel, 1983; Webb, 1984; Winemiller, 1991; Domenici, 2003; Langerhans & Reznick, 2009). We predicted that fishes collected from sand microhabitats would have subterminal snouts and long, narrow caudal peduncles because these fish likely forage by roaming over the relatively barren sand flat consuming invertebrates associated with the benthos (Table 1). However, in seagrass microhabitats that are much more complex, we predicted that fish would have deep mid-bodies and terminal snouts to facilitate fine-scale manoeuvring that allows picking food items from seagrass blades and capturing items in the water column between seagrass blades.

Table 1. Predicted fish morphologies and their functional significance between simple (sand) and complex (seagrass) habitats based on established ecomorphological patterns

Microhabitat	Description	Foraging and locomotion	Morphological expectation
Seagrass	Structurally complex; turtle and shoal grass	Picking prey and epiphytic algae; manoeuvring	Terminal snouts and deep mid-bodies
Sand flats	Structurally simple; sand and mud	Sifting sand and rooting; open-water cruising	Subterminal snouts and stream-lined bodies

MATERIAL AND METHODS

We collected *L. xanthurus* and *L. rhomboides* from four sites located in the Aransas and Upper Laguna Madre estuaries that occur along the Texas coast, USA (Aransas: 27°51'44"N, 97°04'31"W; Lydia Ann: 27°52'51"N, 97°03'24"W; Traylor: 27°55'18"N, 97°04'30"W; Packery: 27°37'16"N, 97°12'51"W). Both estuaries connect to the Gulf of Mexico, primarily through the Aransas Pass tidal-inlet, and they receive the majority of their freshwater from the Nueces River. All four sites were located along channels dredged for boat traffic. Spoil islands from dredging, black mangrove (*Avicennia germinans* Linnaeus), and spartina marshes (*Spartina alterniflora* Loisel.) border the channels. Shoal grass (*Halodule wrightii* Aschers.) and turtle grass (*Thalassia testudinum* Banks ex König) form seagrass meadows that occurred as patches among sand and oyster reefs along the shallow channel margins and in small bays stemming from the larger channels. At each site, we chose pairs of sand and seagrass microhabitats that were adjacent to each other and larger than 2700 m². Fishes likely moved between nearby sand and seagrass microhabitats but movement among sites was unlikely (Potthoff & Allen, 2003). We collected fishes in each microhabitat using a 1-m beam trawl and a beach seine (all mesh < 10 mm). Fishes were preserved in 10% formalin for 2 weeks, rinsed in water for at least 8 h, and stored in 70% ethanol. We collected 772 juvenile *L. xanthurus* and 1270 juvenile *L. rhomboides* for shape analysis.

MORPHOMETRICS

We used geometric morphometric methods to analyze shape variation (Rohlf & Marcus, 1993). All fish were X-rayed in the lateral perspective. Radiographs are desirable in morphometric studies because they reveal the insertion of fins and locations of bones that allow for precise placement of landmarks. We digitally scanned radiograph film at a resolution of 31.5 pixels mm⁻¹ and used tpsDig (Rohlf, 2006) software to digitize 11 homologous landmarks for each species (Fig. 1). Landmark coordinates (11x + 11y = 22

coordinates/fish) were adjusted for position, orientation, and scale by generalized Procrustes superimposition using TPSRELW (Rohlf, 2005b). Relative warps (principal components of shape) were calculated from the aligned landmark constellations with a principal components analysis that placed components of shape into decreasing order. The last four components were null because of the superimposition procedure (two for translation, one for orientation, and one for scaling), which left 18 components to serve as shape variables for analysis. Centroid size was retained for use as a covariate to characterize and statistically adjust for general and species-specific allometries. Centroid size is the square root of the sum of squared distances between the landmarks and the centroid of the landmarks and is highly correlated with standard length but is fundamentally less correlated with shape (Bookstein, 1991; Zelditch *et al.*, 2004)

STATISTICAL ANALYSIS

To examine variation in size, we tested for differences in centroid size between species, microhabitats, and among sites with analysis of variance. For a preliminary examination of the major factors contributing to shape differences, we plotted the first two (of 18) principal components of shape. After this initial survey of shape variation, we developed a multivariate analysis of covariance (MANCOVA) model that included all 18 of the shape variables to quantify shared and unique aspects of shape variation between microhabitats for the two species. Shape variables served as dependent variables testing for effects of microhabitat (shared shape variation between sand and seagrass), species (shape differences between species), habitat-by-species (unique or species-specific shape differences between microhabitats), site (local shape variation), and all other two-way and three-way interactions. Centroid size and two-way, three-way, and four-way interactions (tests for heterogeneity of slope) served as covariates to estimate and statistically adjust for multivariate allometry (i.e. the change in shape with size). We estimated effect sizes (relative importance) in multivariate models by calculating Wilks' partial η^2 .

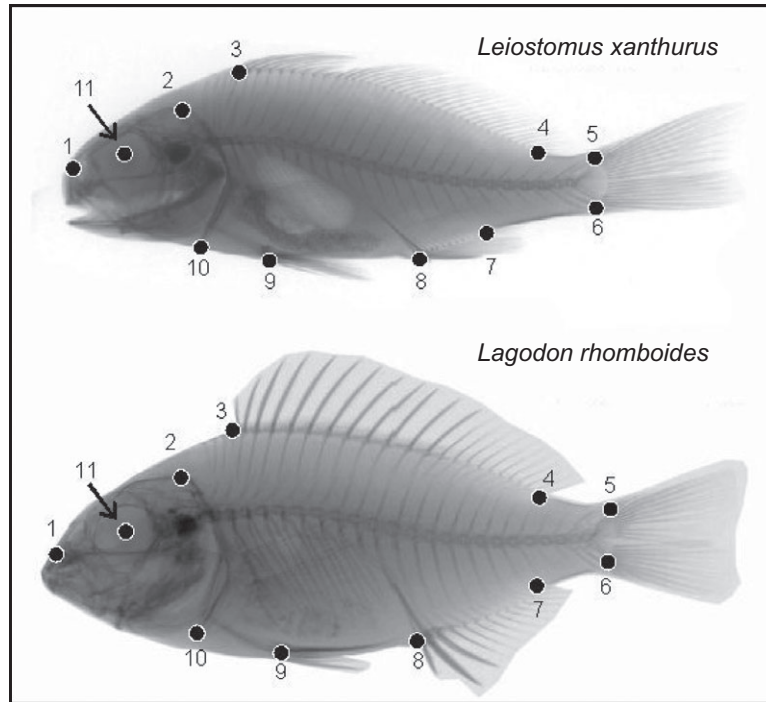


Figure 1. Radiographs depicting landmarks used in morphometric analysis. Landmarks: 1, dorsal tip of ethmoid; 2, dorsal tip of the supraoccipital; 3, 4, anterior and posterior insertion of the dorsal fin; 5, 6, dorsal and ventral insertion of the caudal fin; 7, 8, posterior and anterior insertion of the anal fin; 9, anterior insertion of the pelvic fin; 10, ventral tip of the cleithrum; 11, center of the orbital cavity.

To visualize and interpret shared and unique shape variation across microhabitats, we used TPSREGR to produce vectors of phenotypic variation that depicted differences in shape between microhabitats (Rohlf, 2005a). We used the design matrix for the statistical model that included all covariates and interactions to visualize shape variation between microhabitats. Thus, the vectors produced for the microhabitat effect represent the difference in shape between sand and seagrass at the same time as accounting for all of the other variables and covariables in the model. Shared shape variation was depicted using a dataset with both species, whereas unique aspects of shape variation were illustrated using species-specific datasets.

We used Procrustes distance to estimate the amount of shared shape variation and unique shape variation between microhabitats. Procrustes distance is the standard metric for quantifying shape dissimilarity in geometric morphometrics (Bookstein, 1996). We calculated Procrustes distance as the Euclidian distance between the mean superimposed landmark configurations for sand and seagrass with both species together for shared effects and each species separately for unique effects. We used a bootstrapping technique to resample the shape dataset and iteratively calculate the Procrustes distance between

microhabitats 1000 times. This process yielded a mean \pm SD of shape distance between microhabitats.

Discriminant function analyses (DFA) with the shape variables as dependents provided an intuitive measure of the relative strength of different factors based on how well individuals can be classified into categories based on their morphology. Discriminant analyses tested for shape difference between species (unique differences), microhabitats (shared differences), and combinations of species and microhabitats (four groups: shared and unique). All statistical analyses were conducted with SAS software, version 9.3 (SAS Institute).

RESULTS

SIZE

We found a gradient of size between species, sites, and microhabitats (Table 2). *Lagodon rhomboides* from sand at Lydia Ann were the largest fish, followed by *L. rhomboides* from sand at Aransas and Traylor; *L. xanthurus* from both microhabitats at Lydia Ann and from seagrass at Traylor were the smallest fish collected (Fig. 2). Despite these differences at either end of the size distribution, there was considerable

Table 2. Analysis of variance testing for differences in size

Effect	d.f.	F	P	η^2
Microhabitat (M)	1,2026	77.26	< 0.0001	0.031
Species (Sp)	1,2026	128.28	< 0.0001	0.052
M × Sp	1,2026	33.3	< 0.0001	0.013
Site (S)	3,2026	9.65	< 0.0001	0.012
M × S	3,2026	33.88	< 0.0001	0.041
Sp × S	3,2026	32.2	< 0.0001	0.039
M × Sp × S	3,2026	5	0.0019	0.006

Centroid size served as the dependent variable defined as the square root of the sum of squared distances between the landmarks and the centroid of the landmarks. Centroid size is highly correlated with standard length. Effect size was estimated by calculating eta squared (η^2).

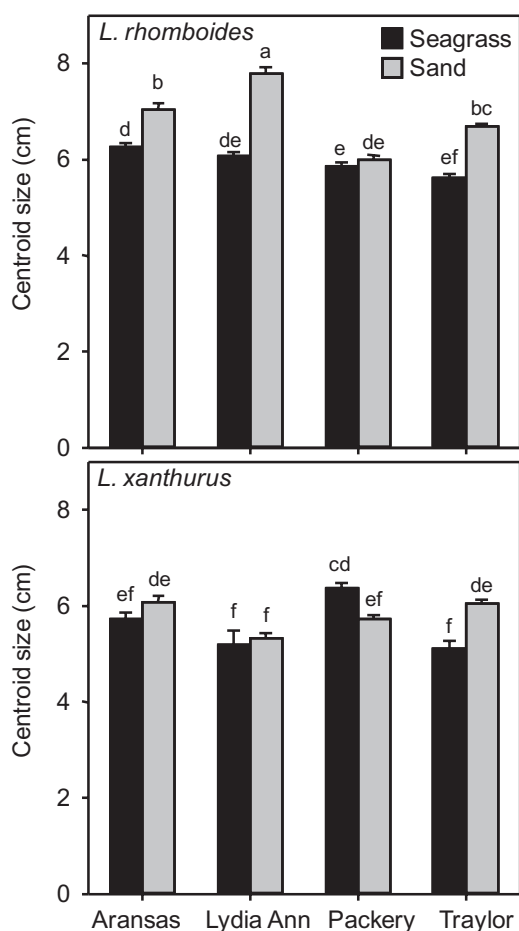


Figure 2. Size variation (mean \pm SE) among sites for *Lagodon rhomboides* (top) and *Leiostomus xanthurus* (bottom). Centroid size is a multivariate measure of size analogous to standard length used as a covariate in shape analysis. Bars with the same letters are not different from each other (Tukey > 0.05).

overlap in size among combinations of species, sites, and microhabitats.

SHAPE

After accounting for size with the Procrustes superimposition, plots of the first two (of 18) principal components of shape revealed that species separated along the first component and the second component largely separated microhabitats (Fig. 3). These plots illustrate that the nature of shape variation between microhabitats was generally similar for all sites and species; however, the extent of overlap between individuals collected from sand and seagrass varied among sites and *L. rhomboides* appeared to exhibit greater shape differences between microhabitats than *L. xanthurus*. Therefore, we expected further statistical analyses with all eighteen shape components to reveal strong differences between species and the magnitude of shape variation between microhabitats would vary among sites (site and microhabitat-by-site effects) in addition to our a priori expectations of shared and unique shape variation between microhabitats.

Our MANCOVA that included all 18 principal components of shape captured the major differences between species and site-specific shape differences between microhabitats illustrated in the principal component plots (Table 3). The partial eta squared estimates of effect size confirmed that these factors were major axes of shape variation in the data. The statistical analysis also revealed that *L. xanthurus* and *L. rhomboides* shared aspects of shape variation and exhibited unique aspects of shape variation between microhabitats. On the basis of the partial eta squared estimates of effect size, shared shape variation (microhabitat effect) and unique shape variation (M \times Sp effect) were similar in magnitude.

We used phenotypic variation vectors to illustrate the nature of shared shape differentiation between microhabitats. Regardless of species, fish collected from seagrass had deep mid-bodies and terminal snouts, whereas those from sand had subterminal snouts with streamlined bodies (Fig. 4). Species-specific, or unique, shape variation consisted of differences in magnitude and direction. Head morphology was more labile in *L. rhomboides* than *L. xanthurus*. *Lagodon rhomboides* from sand had subterminal snouts compared to those from seagrass. By contrast, *L. xanthurus* exhibited a small difference in head morphology that involved longer snouts in fish collected from sand microhabitats. The main shift in morphology between microhabitats for *L. xanthurus* involved a more streamlined body in sand compared to seagrass microhabitats. Procrustes distance

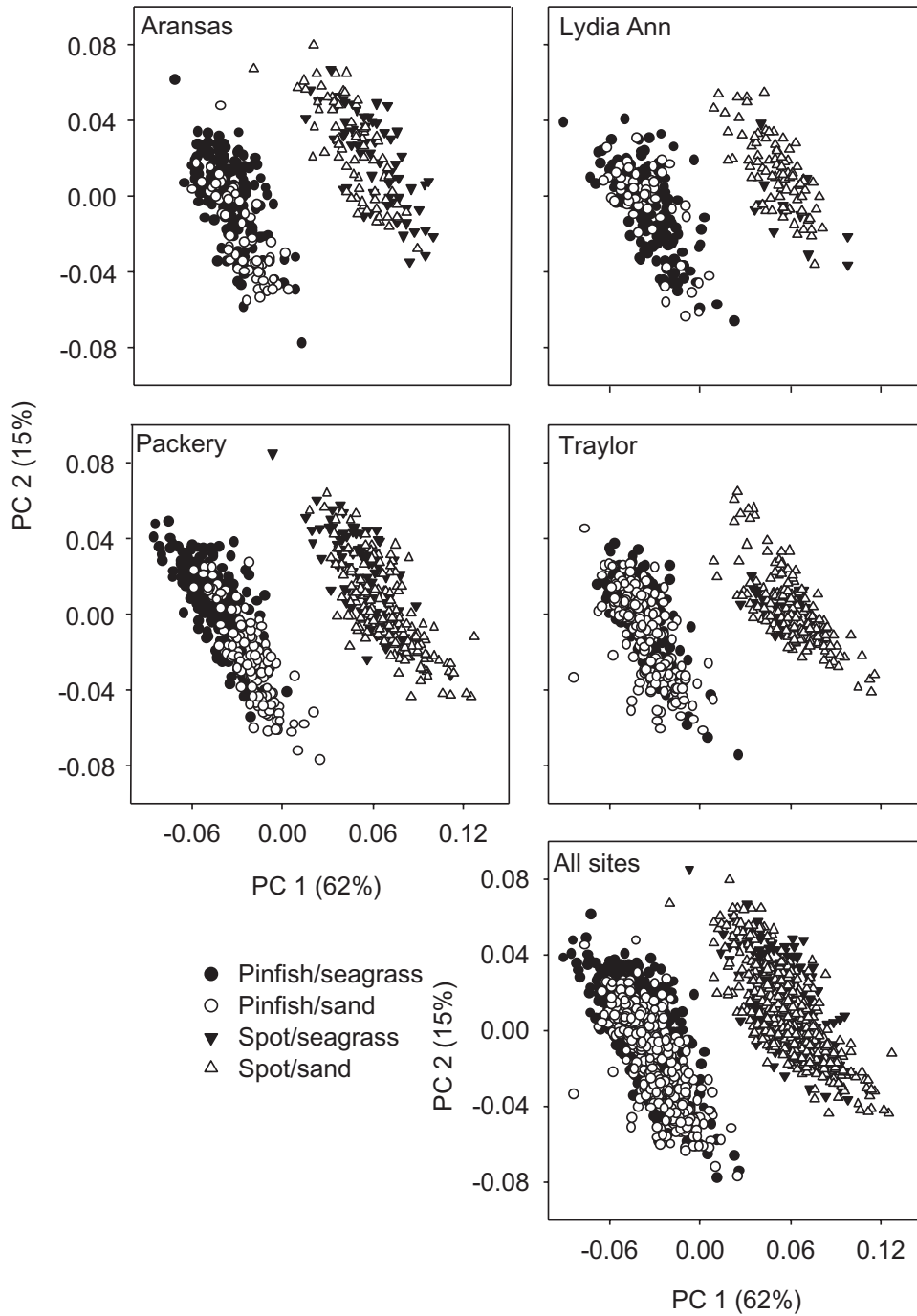


Figure 3. Principal component (PC) plots from a single principal components analysis that included *Leiostomus xanthurus* and *Lagodon rhomboides* collected from sand and seagrass habitats at four sites. PC1 explained 62% and PC2 explained 15% of the total variation in the data. Components are divided by site.

between seagrass and sand microhabitats was greater for *L. rhomboides* than the distance for *L. xanthurus*, indicating that *L. rhomboides* exhibited greater shape variation between microhabitats.

Based on shape, our first DFA, with a null expectation of correctly classifying 50% of individuals,

placed all 2042 fishes into the correct species category accounting for the obvious shape differences between species. The second DFA, also with a null expectation of 50%, classified 1422 of 2042 (70%) individuals to their microhabitat of origin regardless of species, signifying that a majority of fishes were sorted into their

Table 3. Multivariate analysis of covariance examining shared and unique components of lateral shape (18 principal components) in *Lagodon rhomboides* and *Leiostomus xanthurus*

Effect	Wilks' λ	F	d.f.	P	η_p^2
Microhabitat (M)	0.96	4.48	18,1993	< 0.001	0.039
Species (Sp)	0.06	1660.15	18,1993	< 0.001	0.937
M \times Sp	0.97	3.18	18,1993	< 0.001	0.028
Site (S)	0.58	21.90	54,5939.17	< 0.001	0.165
M \times S	0.87	5.38	54,5939.17	< 0.001	0.046
Sp \times S	0.81	7.95	54,5939.17	< 0.001	0.067
M \times Sp \times S	0.90	4.03	54,5939.17	< 0.001	0.035

Significant interactions with the size covariate were included in this model but are not reported for brevity. Effect size was estimated by Wilks' partial variance explained (η_p^2).

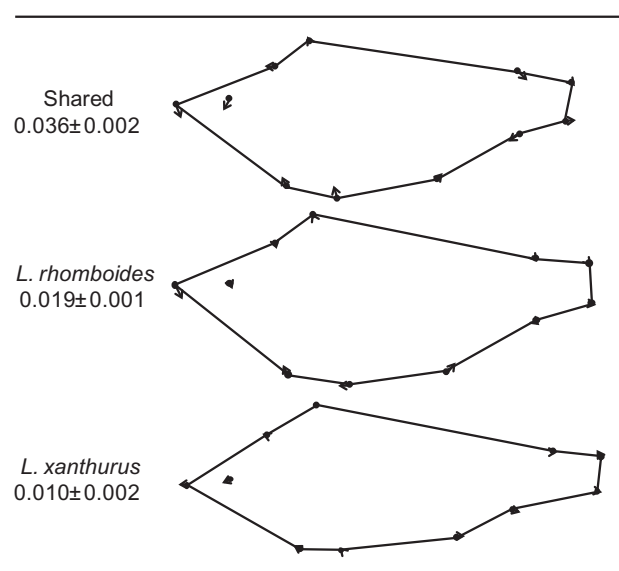


Figure 4. Phenotypic change vectors depicting shared and unique shape variation in *Lagodon rhomboides* and *Leiostomus xanthurus* between sand and seagrass microhabitats. The landmark configuration is the mean shape of individuals from seagrass and the vectors represent the shape of individuals from sand as a deformation from seagrass. Vectors were magnified 5 \times to illustrate shape differences. The magnitude of shape variation between microhabitats based on Procrustes distance (mean \pm SD) is reported under the heading. Mean \pm SD for Procrustes distances were based on 1000 bootstraps of pairwise distances.

proper microhabitat based on body morphology alone. Our third DFA examined microhabitat-by-species combinations (four combinations), with the null expectation of correctly classifying 25% of individuals. It assigned 1373 of 2042 (67%) individuals to their correct microhabitat–species category. Adding unique aspects of shape variation (i.e. species-specific variation) between microhabitats did not improve the

number of fishes correctly classified (67% versus 70%).

DISCUSSION

Phenotypic divergence within species is well known across a wide range of vertebrate taxa (Meyer, 1990; Wimberger, 1992; Robinson & Wilson, 1994; Smith & Skulason, 1996; Johnson & Belk, 2001; Jonsson & Jonsson, 2001; Kassam *et al.*, 2003; Langerhans *et al.*, 2003; Chapman *et al.*, 2007). A replicated pattern of phenotypic divergence between two environments that is shared among species is evidence for adaptive convergence and is well documented among closely-related species (Harvey & Pagel, 1991; Schluter, 2000; Ruzzante *et al.*, 2003; Langerhans *et al.*, 2004). We collected two distantly-related estuarine fish species (i.e. different families within the order Perciformes) from adjacent simple (sand) and complex (seagrass) microhabitats at four sites in a large estuary. Despite high connectivity between microhabitats and the phylogenetic distance between species, there was evidence for shared shape divergence between microhabitats that matched ecomorphological predictions related to foraging and manoeuvring. Species-specific (i.e. unique) aspects of differentiation between microhabitats were also prevalent and included greater variation in snout position between microhabitats in *L. rhomboides*. The magnitude of shape divergence between microhabitats varied among sites suggesting that site-specific processes altered phenotypic divergence, which was an unexpected finding.

Simple and complex habitats pose a trade-off for traits related to foraging and manoeuvring in fishes. Streamlined bodies and subterminal snouts facilitate carangiform swimming for cruising and retrieval of prey in or near the benthos in simple microhabitats. Conversely, deep bodies and terminal snouts aid in precise manoeuvring to pick prey items from structure in complex habitats (Keast & Webb, 1966;

Webb, 1982; 1984; Barel, 1983; Motta, 1988; Wine-miller, 1991; Domenici, 2003; Ruehl & DeWitt, 2005; Langerhans & Reznick, 2009). In estuaries, fishes feeding in simple environments such as sand flats likely consume infauna and epifauna from the benthos requiring them to winnow through substrates for prey items and cover substantial open territory in search of prey or to avoid predation. Alternatively, feeding in complex environments, such as seagrass meadows, requires manoeuvrability and picking prey items from seagrass blades and gaps between blades. We predicted that fishes collected from sand would have streamlined bodies and subterminal snouts, whereas those collected from seagrass would have deep bodies and terminal snouts. We found evidence supporting these ecomorphological predictions for both species. The principal component plots, MANCOVA, DFA, and Procrustes distance calculations all indicated the presence of shared and unique shape variation between microhabitats, although other factors also contributed to shape variation in the dataset. Despite large differences in shape between species and among sites, DFA, for example, correctly classified 70% of the fishes to their microhabitat of origin. As a shared response, *L. rhomboides* and *L. xanthurus* collected from seagrass had terminal snouts and deep bodies, whereas those from sand microhabitats had streamlined bodies and subterminal snouts. These findings imply that both species incorporate similar foraging and swimming modes when they occur in either sand or seagrass microhabitats based on ecomorphological principles.

The unique evolutionary and environmental history of every species interacts with natural selection to produce phenotypic variation along shared environmental gradients. The effects of history can have strong implications for the products of selection (Gould & Woodruff, 1990; Price *et al.*, 2000). Species-specific evolutionary histories within clades may produce distinct phenotypes in response to common environmental gradients or produce similar phenotypes as a result of the shared evolutionary history of the clade depending on the age of divergence and the rate of clade diversification. Many studies considering shared and unique effects of phenotypic diversification have focused efforts within a particular clade (Langerhans & DeWitt, 2004; Langerhans *et al.*, 2006). We increased the phylogenetic breadth in the present study by considering shared and unique components of phenotypic diversification between two families: sparids and sciaenids. Unique, or species-specific, shape variation between sand and seagrass was similar in magnitude to shared shape variation according to the MANCOVA, although adding unique effects to the microhabitat DFA did not improve the number of individuals correctly classified and Pro-

crustes distance was greater for shared effects compared to unique shape effects. Therefore, unique (species-specific) effects of diversification between microhabitats were not as strong as shared effects.

Species-specific shape variation emerged from differences in the magnitude and direction of change between species. *Lagodon rhomboides* displayed greater shape variation than *L. xanthurus* based on the Procrustes distance of shape configurations between microhabitats. Specifically, *L. rhomboides* exhibited much greater variation in snout location between microhabitats, a response that we expected to be strong in both species. Diet and morphological traits associated with foraging likely explain the species-specific differences in the magnitude of shape variation between sand and seagrass. *Leiostomus xanthurus* includes zooplankton in their diet, although they primarily consume invertebrates that live in or near the benthos (Chao & Musick, 1977). Conversely, *L. rhomboides* are omnivorous; they consume algae, detritus, and vascular plants in addition to invertebrates (Hansen, 1970). Therefore, the unique evolutionary history related to a more specialized diet of consuming benthic invertebrates may limit the magnitude of phenotypic variation in *L. xanthurus*, whereas the general diet in *L. rhomboides* may have resulted in the potential for greater phenotypic variation in this species.

MECHANISMS OF SHAPE VARIATION

The shared shape variation in *L. xanthurus* and *L. rhomboides* between sand and seagrass microhabitats revealed in the present study implies replicated divergent selection for habitat-specific morphologies in these two distantly-related species. Such phenotypic variation could result from genetic differentiation, phenotypic plasticity, recent phenotype- or genotype-dependent mortality or habitat choice. Genetic polymorphism is expected to evolve when environments vary spatially among populations or temporally at fine scales (Levins, 1968; Schlichting & Pigliucci, 1998; DeWitt & Scheiner, 2004; Leimar *et al.*, 2006). Differential survival of phenotypes between microhabitats (i.e. selection) might produce the observed phenotypic variation through the evolution of genetic polymorphism, although it is unlikely to be the only source. Adults of both species migrate out of estuaries each year and breed offshore; larvae wash into estuaries with tidal flow and eventually settle in a variety of microhabitats including sand and seagrass (Hildebrand & Cable, 1930; Caldwell, 1957; Chao & Musick, 1977; Warlen & Chester, 1985). Genetic differences could arise if there was assortative mating by populations and offspring returned to their parents' natal habitat during ontogeny, reflecting a combination of

habitat preference and genetic polymorphism. Studies are emerging that suggest natural selection can drive phenotypic divergence despite large-scale larval dispersal and substantial gene flow (Conover *et al.*, 2006; Hemmer-Hansen *et al.*, 2007; Larsen *et al.*, 2007), although this remains an open question for *L. xanthurus* and *L. rhomboides*. There is at least some potential for local adaptation. Population structure in red drum (*Sciaenops ocellatus*, Linnaeus), a species with similar reproductive biology to *L. xanthurus* and *L. rhomboides*, follows a pattern of isolation by distance with the greatest gene flow occurring between adjacent estuaries (Gold, Burridge & Turner, 2001). However, extensive gene flow probably occurs through the simultaneous migrations of many populations of both species to a common breeding area overwhelming directional selection in any one population, an effect known as gene swamping (Haldane, 1948; Endler, 1977; Lenormand, 2002; Hendry & Taylor, 2004). Therefore, large amounts of gene flow likely negate selection for the evolution of genetic polymorphism.

Phenotypic plasticity is ubiquitous in nature and often confers an advantage to individuals that produce phenotypes that match environmental cues (West-Eberhard, 2003; DeWitt & Scheiner, 2004). Plasticity is expected to evolve when the grain of environmental variation is coarse enough for organisms to respond developmentally to the environmental cue by producing a suitable phenotype. Recent research with sticklebacks has revealed that habitat complexity produces morphological differences through phenotypic plasticity likely associated with foraging mode (Garduño-Paz, Couderc & Adams, 2010). In estuaries, many abiotic and biotic variables change rapidly depending on tidal stage. However, the distribution and persistence of simple (sand) and complex (seagrass) microhabitats in estuaries are relatively constant over time scales relevant to the residence of most estuarine fishes including *L. xanthurus* and *L. rhomboides*. Therefore, the evolution of phenotypic plasticity in response to sand and seagrass microhabitats appears likely for estuarine fishes as long as they exhibit fidelity toward a particular microhabitat after settlement. Studies have found that many estuarine fishes demonstrate site fidelity after they transition from their planktonic larval stage and settle in estuaries (Lotrich, 1975; Weinstein & O'Neil, 1986; Able & Hales, 1997; Able, Hales & Hagan, 2005). Few studies have examined microhabitat use at a site, although those that have suggest juvenile fishes choose microhabitats within sites (Irlandi & Crawford, 1997; Potthoff & Allen, 2003). Therefore, we suggest that many fish in estuaries settle in a particular microhabitat as larvae and produce phenotypes that match local environmental

cues yielding the shared shape divergence across microhabitats observed in the present study.

SCALES OF PHENOTYPIC DIVERSITY

In addition to the a priori predictions of phenotypic variation in estuaries, our survey of estuarine fish shape in two species revealed differences in the magnitude of phenotypic variation between microhabitats among sites. Spatial variation in the degree of shape disparity between sand and seagrass was similar in strength to shared and unique components of shape variation based on Wilks' η^2_P . Therefore, morphology for these two species varies at multiple spatial scales within estuarine systems and signifies the importance of documenting the extent of phenotypic variation along common environmental gradients across space. Indeed, a mosaic of induced shape differences might be common in ecosystems characterized by substantial environmental heterogeneity, although few studies have considered the potential for phenotypic variation in such ecosystems. Variation in the magnitude of phenotypic diversity between microhabitats among sites could arise from differences in the genetic structure (**G** matrix) of a species at a site or the strength of selection at a site. Similarly, spatial and temporal variation in the strength and persistence of environmental cues could produce disparity in the magnitude of phenotypic differences produced through plasticity. For example, freshwater snails vary the magnitude of their response to fish predator cues depending on the spatial proximity of the predator and the age of predator cues (Turner & Montgomery, 2003). Future studies should consider the spatial extent of induced phenotypes along relatively open abiotic and biotic environmental gradients. On the basis of the results obtained in the present study, we hypothesize that gradients of morphology will be common in nature.

The present study documents a replicated pattern of parallel shape divergence in estuarine fish species. Regardless of species, fishes collected from simple (sand) microhabitats had streamlined bodies and subterminal snouts, whereas those from complex (seagrass) microhabitats had deep mid-bodies and terminal snouts. These morphological responses to simple and complex microhabitats fit established ecomorphological principles. Unique components of shape were similar in magnitude to shared components despite the great phylogenetic distance between species. Species-specific shape responses included greater differences in snout morphology for the more omnivorous *L. rhomboides*. We argue that trade-offs associated with foraging mode and locomotion between sand and seagrass microhabitats produced the observed patterns of phenotypic divergence

primarily through phenotypic plasticity, although a role for genetic polymorphism cannot be dismissed. Regardless of the mechanism, shared phenotypic diversity between these two disparately-related species between sand and seagrass provides evidence of convergence in shape and warrants further study.

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