

ABSTRACT

BIEDERMAN, DIANE LYNNE. Phenotypic Divergence Across Biotic Gradients: Morphological Variation in the Tropical Killifish, *Rivulus hartii* in Response to Community Structure. (Under the direction of Dr. James F. Gilliam).

By studying convergent patterns across common selective gradients, we may be able to develop evolutionary-ecological paradigms that can be used to predict how local populations will be affected by changes in their environment. One pattern of convergence which has recently received considerable attention is morphological variation associated with the functional trade-off between steady- and unsteady-swimming ability in fish inhabiting different predation regimes. In this thesis I use landmark-based geometric morphometric methods to explore the morphological divergence among populations of the tropical killifish, *Rivulus hartii*, in the Northern Range Mountains of Trinidad, West Indies. In rivers of the Northern Range Mountains, *Rivulus* occur in three primary fish communities which can occur in the presence or absence of predatory prawns (*Macrobrachium spp.*): 1) High-Predation (HP) where large piscivorous fish are present, 2) Low-Predation (LP) where they coexist with guppies (*Poecilia reticulata*), a non-predatory fish, and 3) *Rivulus*-Only (RO) where they are the only fish species present. By comparing data from field collections of *Rivulus hartii* from six rivers, I investigated 1) whether *Rivulus* populations conform to the Langerhans and DeWitt (2004) ecomorphological paradigm of trade-offs in locomotor-associated morphology along the competition-predation gradient (i.e., unsteady-swimming morphology in high-predation vs. steady-swimming morphology in low-predation), 2) whether there is morphological variation between the two low-predation population categories (LP and RO) based on selection difference between intra- vs. interspecific competition, 3) whether the presence or absence of prawns affects how *Rivulus* respond to

their fish community, and 4) whether the ecomorphological paradigm could predict how *Rivulus* would respond to changes in their community structure. I did find that *Rivulus* populations conform to the ecomorphological paradigm, but only in the absence of prawns. When prawns are absent, HP *Rivulus* populations demonstrated unsteady-swimming morphology while LP and RO *Rivulus* populations demonstrated steady-swimming morphology. I also found a distinct divergence between RO and LP populations; RO populations were much more highly adapted for steady-swimming (i.e., more fusiform in morphology) than LP populations. When prawns are present, *Rivulus* populations are less divergent between community types and show the opposite pattern of divergence from prawns-absent communities (i.e., low-predation populations tended toward unsteady-swimming morphology while high-predation populations tended toward steady-swimming morphology). While prawns-absent *Rivulus* populations did demonstrate morphology consistent with the ecomorphological paradigm in three separate rivers, I was unable to use this pattern to predict how *Rivulus* would respond morphologically to recent and more established species invasions. While it was predicted RO populations invaded by guppies would shift towards LP morphology, the populations in fact became more “extreme RO-like” both in recent and more established invasions. Also, while the suggested trajectory of the predator-introduction population did suggest a shift from its historical LP morphology towards HP morphology as predicted, this population has not yet significantly diverged from a control LP population despite 30 years of predation.

Phenotypic Divergence Across a Biotic Gradient: Morphological Variation in the Tropical Killifish, *Rivulus hartii* in Response to Community Structure

by
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DEDICATION

For my mother.

BIOGRAPHY

Diane Lynne Biederman was born and raised in the Washington D.C. metro area. In 2002 she graduated from Eleanor Roosevelt High School in Greenbelt, MD and headed south to attend North Carolina State University in Raleigh, NC on an honor scholarship. In 2006 she graduated summa cum laude with a B.S. in Biology and a minor in Japan Studies. From 2006-2008 she lived in Toyama, Japan where she taught English at a public high school as part of the Japan Exchange and Teaching (JET) Programme. In 2008 she returned to the U.S. to attend graduate school at North Carolina State University to earn her Master of Science in Zoology.

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CHAPTER 1:

Phenotypic Divergence Across a Community Gradient

Abstract

It is often assumed that organisms facing similar selective gradients will develop convergent responses to common adaptive challenges. By studying convergent patterns across common selective gradients, we may be able to develop evolutionary-ecological paradigms which can be used to predict how local populations will be affected by changes in their environment. One pattern of convergence which has recently received considerable attention is the functional trade-off between steady- and unsteady-swimming morphology in fish inhabiting different predation regimes. In rivers of the Northern Range Mountains of Trinidad, the tropical killifish, *Rivulus hartii*, experiences three primary types of fish communities: High-Predation (HP) where large piscivorous fish are present, Low-Predation (LP) where they coexist with guppies (*Poecilia reticulata*), and *Rivulus*-Only (RO) where they are the only fish species present. By comparing data from field collections of *Rivulus hartii* populations from four rivers we investigated 1) whether *Rivulus* conform to the Langerhans and DeWitt (2004) ecomorphological paradigm of trade-offs in locomotor-associated morphology along the competition-predation gradient (i.e., unsteady-swimming morphology in high-predation vs. steady-swimming morphology in low-predation), and 2) whether there is morphological variation between the two low-predation population categories (LP and RO). We found that *Rivulus* do appear to follow the ecomorphological paradigm of unsteady-swimming morphology (i.e., small anterior region and deeper caudal peduncle) in high-predation environments and steady-swimming morphology (i.e., deeper anterior region and narrower caudal peduncle) in low-predation environments. We also found a distinct divergence between RO and LP populations with RO populations

demonstrating much more fusiform morphology compared to LP populations, suggesting strong intraspecific competition is a major driving force towards steady-swimming morphology. Additionally, this study found that while populations in three rivers of the Caroni River Drainage did follow the ecomorphological paradigm, low-predation populations in the Marianne River appeared “HP-like” in morphology and demonstrated less divergence than populations from the other rivers. We hypothesize that the observed shift to “HP-like” morphology in the Marianne River is due to the presence of predatory *Macrobrachium spp.* prawns.

Introduction

A central goal of evolutionary-ecological research is to better understand how ecological interactions create and maintain diversity, with the ultimate goal of using this knowledge to predict the outcome of future interactions. When organisms face similar selective gradients, it is often assumed that they may develop convergent responses to common adaptive challenges. By studying convergent patterns, or the lack thereof, across common selective gradients we may be able to develop evolutionary-ecological paradigms which can be used to predict how local populations will be affected by changes in their environment. The competition-predation gradient has long been considered a major driving force of evolution and therefore patterns of phenotypic divergence attributable to resource competition (Schoener 1983; Smith and Skúlason 1996; Andersson 2003; Andersson et al. 2006) and predation pressure (Endler 1995; Abjornsson et al. 2004; Vamosi 2005) have received considerable attention. Adaptations to the competition-predation gradient are typically associated with functional trade-offs (Schluter 2000; Blake 2004) that limit the range of possible phenotypes, and create a higher likelihood of convergent responses.

Fish morphology offers an ideal system for generating mechanistic models for phenotypic evolution along environmental gradients due to the constraints that movement in water places on overall body design (Langerhans and Reznick 2009; Langerhans 2010). A wealth of research exists on the relationship between morphology and swimming performance in fish (Webb 1982; Webb 1984; Domenici and Blake 1997; Sfakiotakis et al. 1999; Triantafyllou et al. 2000; Blake 2004). In particular, much attention has been given to the functional trade-off between steady- and unsteady-swimming. Steady-swimming is

constant, steady motion in a straight line as would often be used for foraging, searching for mates, seeking favorable abiotic conditions, or maintaining position in a current (Plaut 2001; Domenici 2003; Blake 2004). Unsteady-swimming refers to rapid changes in velocity or direction which are typical during predator-prey interactions (e.g., C- or S-start escape behavior) or while navigating through complex environments (Plaut 2001; Domenici 2003; Blake 2004). The successful performance of both of these swimming behaviors is highly dependent upon body shape. In general, steady-swimming is maximally achieved by fish with a streamlined, fusiform body with a deep anterior region (i.e., head to mid-body) and narrow caudal peduncle (i.e., tail region) which minimize energy loss to drag (Langerhans and Reznick 2009; Langerhans 2010). Generally, unsteady-swimming is maximally achieved by fish with a deep caudal peduncle and shallower anterior region which increase thrust and stability during abrupt turns (Langerhans and Reznick 2009; Langerhans 2010). Adaptation to either of these swimming modes could have a strong effect on the fitness of fish, but requires the development of opposite body forms. Therefore, it is argued that selection will favor swimming morphology optimally suited to the competition and predation levels imposed on the population (i.e., streamlined, fusiform bodies in low predation environments and smaller heads or mid-bodies, and deeper caudal regions in high predation environments).

Morphological variation associated with the presence or absence of predators has been investigated in many fish species and across multiple environments with fairly consistent results (Langerhans and DeWitt 2004; Andersson et al. 2006; Hendry et al. 2006; Zimmerman 2007; Domenici et al. 2008; Gomes and Monteiro, 2008; Burns et al. 2009;

Langerhans 2009). However, many of these studies do not specifically address the morphological divergence predicted by the functional trade-off in steady- vs. unsteady-swimming ability or test for the resulting divergence in locomotor ability. In addition, previous studies focused on the presence or absence of a strong predator, and did not address the effects of intraspecific versus interspecific competition.

In tropical rivers in the Northern Range Mountains of Trinidad, waterfalls act as natural barriers, filtering species so that large predatory fishes are restricted to the lower reaches while prey species can extend to relatively low-predation zones in the middle to upper reaches. The tropical killifish, *Rivulus hartii*, exhibits strong dispersal ability and is usually the only fish present in the extreme headwaters where they can be found in high abundance (Liley and Seghers 1975; Endler 1978; Gilliam et al. 1993); due to their exceptional dispersal ability, *Rivulus* occur in sites with various fish communities. Here, we compare *Rivulus* morphology occurring in three community types: high-predation (HP), low-predation (LP), and *Rivulus*-only (RO). HP sites are located at lower elevations and contain large piscivorous fishes. As elevation increases, barrier waterfalls block the movement of large piscivores creating LP zones where *Rivulus* coexist with guppies (*Poecilia reticulata*), a small competing species (Gilliam et al. 1993) and potential predator on newborn *Rivulus* (D.F. Fraser, B.A. Lamphere, and J.F. Gilliam, unpublished data). At the highest elevations, only *Rivulus* are able to breach the waterfall barriers, creating high-density RO zones where intraspecific competition for resources is high. The eel-like synbranchid, *Synbranchus marmoratus*, can also occur in the extreme headwaters, but only rarely and in very low densities, and therefore is not considered here. The consistency of the fish community

gradient in rivers of the Northern Range Mountains of Trinidad makes these rivers an excellent natural laboratory for evolutionary-ecological research. Each river can be treated as a natural replicate of evolution along a community gradient, thus providing insights into phenotypic diversification (Endler 1978; Reznick and Endler 1982; Endler 1995).

Recent work on *Rivulus* life history (Walsh and Reznick 2008; Walsh and Reznick 2009; Walsh et al. 2011) and locomotion (Oufiero et al. 2011) suggest that morphological variation is present between *Rivulus* populations occurring in contrasting communities. *Rivulus* from HP environments demonstrated faster sprint swimming speeds (i.e., a measure of speed over short distances) and reduced critical swimming speeds (i.e., a measure of endurance) compared to fish from predator-absent populations (Oufiero *et al.* 2011). Additionally, HP fish were found to have longer tails than LP or RO fish. While this difference in tail length could account for the increased speed, Oufiero et al. (2011) found no significant statistical effect when tail length was included in their models, suggesting some other morphological or physiological reason for the difference in sprint speed. These results, combined with the fact that *Rivulus* from HP sites reach sexual maturity at a smaller size, have higher reproductive allotments, and produce smaller eggs than predator-absent sites (Walsh and Reznick 2009), suggest that fish community structure has a selective effect on *Rivulus* morphology.

By comparing data from field collections of *Rivulus hartii* from four rivers in the Northern Range Mountains of Trinidad we investigated 1) whether *Rivulus* conform to the Langerhans and DeWitt (2004) ecomorphological paradigm of trade-offs in locomotor-associated morphology along the competition-predation gradient (i.e., unsteady-swimming

morphology in high-predation vs. steady-swimming morphology in low-predation), and 2) whether there is morphological variation between the two low-predation population categories (LP and RO) due to selection differences between intra- and interspecific competition. The paradigm predicts that HP *Rivulus* will demonstrate smaller anterior regions and deeper caudal peduncles compared to LP and RO populations. Since there was no difference in swimming performance between LP and RO fish (Oufeiro et al. 2011), present evidence suggests that both LP and RO populations will demonstrate similar fusiform morphologies (i.e., deeper mid-bodies and narrow caudal peduncles) suited to steady-swimming ability.

Methods

Study Species

Rivulus hartii (Boulenger 1890) is a relatively large, non-annual killifish commonly found in freshwater habitats of eastern Colombia, northeastern Venezuela, and on the islands of Trinidad, Tobago, Margarita, and Grenada (Boeseman 1960; Huber 1996). *Rivulus* have a cigar-shaped body with a small dorsal fin and large, rounded caudal fin. They range in size from just a few millimeters at hatching to an observed maximum total length of about 10cm (Seghers 1978). There has been no previously reported difference in size or external morphology between the sexes, but *Rivulus* are sexually dimorphic in color pattern and mature fish >35 mm total length can be reliably sexed using caudal fin coloration (i.e., males' caudal fins are black with white edges dorsally and ventrally, while females' caudal fins are brown with a black edge around the entire circumference).

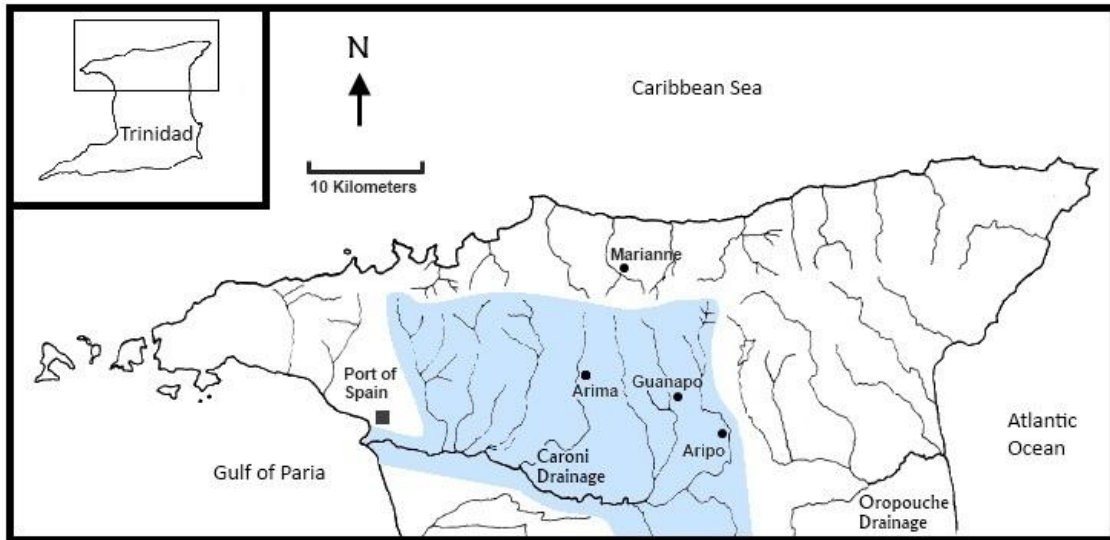


Figure 1.1: Map of Northern Range Mountains, Trinidad, West Indies. Blue region represents the Caroni Drainage.

Study Sites

We conducted field collections in four rivers in the Northern Range Mountains of Trinidad (Figure 1.1). The Aripo, Guanapo, and Arima rivers are part of the Caroni River Drainage which drains from the southern slope of the Northern Range Mountains west into the Gulf of Paria. The Marianne River is located on the northern slope and drains directly into the Caribbean Sea. Each of these rivers exhibits the HP-LP-RO community gradient, though there is some variation in species present. HP sites in the Caroni River Drainage contain common South American predatory species including cichlids (*Crenicichla sp.* and *Andinoacara pulcher*), trahiras (*Hoplias malabaricus*), and characids (*Astyanax bimaculatus* and *Hemibrycon dentatum*) (Reznick 1982; Gilliam et al. 1993; Fraser et al. 1999). The Marianne River's HP site, on the other hand, contains marine-derived species including three

species of sleepers (*Eleotris pisonis*, *Gobiomorus dormitor*, and *Dormitator maculatus*) and mountain mullet (*Agnostomus monticola*) (Endler 1983; Reznick et al. 1996). Fish populations in the LP and RO sites are consistent except for the catfish, *Rhamdia sebae*, which have been observed near the Guanapo LP site (Gilliam et al. 1993).

Sample Collection

We conducted field collections between July 4, 2008 and July 20, 2008. We collected forty individuals ranging in size from roughly 20mm to 90mm using baited minnow traps or by dip net from each population in RO, LP, and HP sites in the Arima, Aripo, Guanapo, and Marianne rivers. After collection, we transported the fish back to the field station where they were held overnight. Each fish was euthanized with an overdose of pH-buffered tricaine methanesulfonate (MS-222) then photographed laterally using a digital camera mounted on a tripod above a standard plastic background with ruler. Each specimen was then preserved in 95% ethanol (EtOH) for use in a separate study.

Geometric Morphometric Techniques

We assessed *Rivulus* morphology using landmark-based geometric morphometric methods (Rohlf and Marcus, 1993). Due to the lack of biological landmarks between the eye and dorsal fin, we superimposed a standardized overlay grid over each image to provide an estimate of curvature between biological landmarks (Fig. 1.2). The grid consists of a horizontal line running parallel to the body from the center of the orbit to the distal tip of the hypural plate (i.e., where the hypural plate ends and caudal fin rays begin) and four vertical



Figure 1.2: Male *Rivulus hartii* digitized with overlay grid and 19 landmarks.

lines representing 0, 30, 50, and 90% of the distance from the center of the orbit to the distal tip of the hypural plate. We digitized nineteen landmarks to each image using TpsDig software (version 2.14, Rohlf, 2009a). The nineteen landmarks correspond to: 1) anterior point along the body outline where the upper and lower maxilla touch, 2) ventral indentation of the lower maxilla, 3) dorsal indentation of upper maxilla, 4) most dorsal point of orbit along the intersecting gridline, 5) center of orbit, 6) most ventral point of orbit along the intersecting gridline, 7) point along ventral body outline intersected by the gridline passing through the center of the orbit, 8) point where the most anterior region of the operculum intersects with the ventral body outline, 9) most ventral point along the outline of the body where the gridline representing 30% of the distance from the center of the orbit to the distal tip of the hypural plate intersects, 10) most dorsal point along the outline of the body where the gridline representing 30% of the distance from center of the orbit to the distal tip of the hypural plate intersects, 11) most anterior point where the anal fin connects to the outline of the body, 12) most posterior point where the anal fin connects to the outline of the body, 13)

most anterior point where the dorsal fin connects to the outline of the body 14) most posterior point where the dorsal fin connects to the outline of the body, 15) most ventral point along the outline of the body where the gridline representing 90% of the distance from center of the orbit to the distal tip of the hypural plate intersects, 16) most ventral point along the outline of the body where the gridline representing 90% of the distance from center of the orbit to the distal tip of the hypural plate intersects, 17) point where the caudal fin connects to the ventral body outline and 18) point where the caudal fin connects to the dorsal body outline, and 19) distal tip of the hypural plate.

We aligned the digitized landmarks by generalized least squares superimposition using TpsRelw (version 1.46; Rohlf 2008). This superimposition method rotates, translates, and scales landmark coordinates thereby removing differences in size and position so that overall shape can be compared (Bookstein 1991; Zelditch et al. 2004). Once the specimens were aligned, we calculated centroid size, partial warps, and uniform components. Centroid size is the square root of the sum of squared distances of landmarks to their centroid. Centroid size is used in geometric morphometric methods as an estimate of overall size. Since allometric effects remain after superimposition, centroid size can be included as a covariate in statistical models to investigate shape variation independent of allometry. Shape variation is broken down into partial warps which represent the nonaffine shape components (i.e., localized deformation) and uniform components which represent affine shape components (i.e., whole body stretching or skewing). When combined, these variables allow overall body shape variation to be investigated using multivariate statistical methods.

Statistical Analysis and Shape Visualization

We performed all statistical analyses using SAS JMP software (version 8.0.2, SAS Institute Inc., Cary, NC). We determined shape variation using multivariate analysis of covariance (MANCOVA). We tested the shape variables (16 partial warps and 2 uniform components) for effects due to centroid size (size covariate), community type, river, and interactions between these variables. Since centroid size was included as a size covariate, we tested for heterogeneity of slopes (i.e., the interaction between the size covariate and main factors) for all of the main factors. Non-significant interaction terms were then removed from the model.

We generated canonical variates which correspond to morphological variation optimally associated with a given factor from the MANCOVA. We used these canonical scores to create thin-plate spline deformation grids in TpsRegr (version 1.37; Rohlf 2009b) depicting morphological variation at the two extremes of the canonical axes. We estimated the relative contribution of each factor to the total morphological variation using Wilks' partial η^2 (see Langerhans and DeWitt 2004). Wilks' partial η^2 is a measure of the explanatory ability of a given factor relative to unexplained variation. Since Wilks' partial η^2 is a measure of partial variance [$SS_{\text{effect}} / (SS_{\text{effect}} + SS_{\text{error}})$] the sum of all variables will not equal one.

We also performed Discriminant Function Analysis (DFA) using the shape variables (partial warps and uniform components) as the dependent variables and community type as the independent variable. We assessed the relative magnitude of divergence between the community types by comparing how successful the DFA was at placing fish into their correct

community type. Misclassified fish were analyzed in more depth to discover whether any underlying pattern existed.

Results

The MANCOVA found significant effects for all factors and their interactions except for the interaction terms of sex x river and sex x centroid size which were subsequently removed from the model (Table 1.1). For this study, the factor which explained the largest portion of the variance was the size covariate (i.e., centroid size, 77.7% of partial variance explained). This result was expected based on the wide range of size classes sampled including juvenile fish. In regard to the main focus of this study, *Rivulus* do appear to have a

Table 1.1: Results of the MANCOVA. F values were approximated using Wilks' λ for all factors except for centroid size which is exact F. Partial variance explained was calculated using Wilks' partial η^2 .

Factor	F	Df	P	Partial Variance Explained (%)
centroid size	43.5355	34, 425	<0.0001	77.69
River	9.0274	102, 1273.5	<0.0001	41.90
community	7.0928	68, 850	<0.0001	36.20
sex x centroid	4.5317	68, 850	<0.0001	26.61
community x river	3.5562	204, 2524.9	<0.0001	22.06
river x centroid	2.5376	102, 1273.5	<0.0001	16.86
community x centroid	2.436	68, 850	<0.0001	16.31
Sex	1.8985	68, 850	<0.0001	13.19

strong and consistent response to their community structure (36.2% of partial variance) regardless of their river of origin. While historical factors unique to each river (e.g., founder effects, genetic drift, etc.) explain the largest portion of partial variance after the size covariate (accounting for 41.9%), their effect on how *Rivulus* respond morphologically across communities (i.e., the community x river interaction term) is limited compared to the general response pattern (22.1% of partial variance explained compared to 36.2%).

Investigation of the canonical axes derived from the community type term from the MANCOVA indicated significant morphological divergence between populations where large piscivorous predators are present (HP sites) and where they are absent (LP and RO sites) regardless of river of origin (Canonical Axis 1, Figure 1.3). Thin-plate splines of individuals representing the maximum observed positive and negative canonical scores revealed that fish living in the presence of large predators (Figure 1.3b) tended to have a smaller head and deeper caudal peduncle than fish in predator-free zones (Figure 1.3a). Additionally, the canonical plot showed distinct morphological divergence between the two predator-absent zones (Canonical Axis 2, Figure 1.3). In LP sites where *Rivulus* interact with guppies, fish tended to be deeper bodied and have hypural plates which were extended further past the caudal fin inserts (i.e., landmark 19 vs. 17 and 18; Fig. 1.3c) than in areas where *Rivulus* exist alone (Fig. 1.3d). When river of origin is considered, an additional pattern is revealed. The three Caroni Drainage rivers were much more highly diverged between populations than the Marianne River. While the Marianne populations showed a similar divergent pattern to the other rivers, they were generally more “HP-like” in morphology and exhibited reduced divergence compared to other rivers.

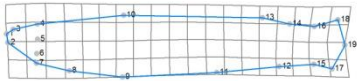


Figure 1.3a: Maximum Observed Positive CA1 Score

Figure 1.3b: Maximum Observed Negative CA1 Score

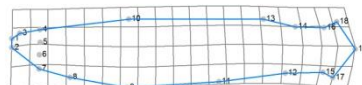
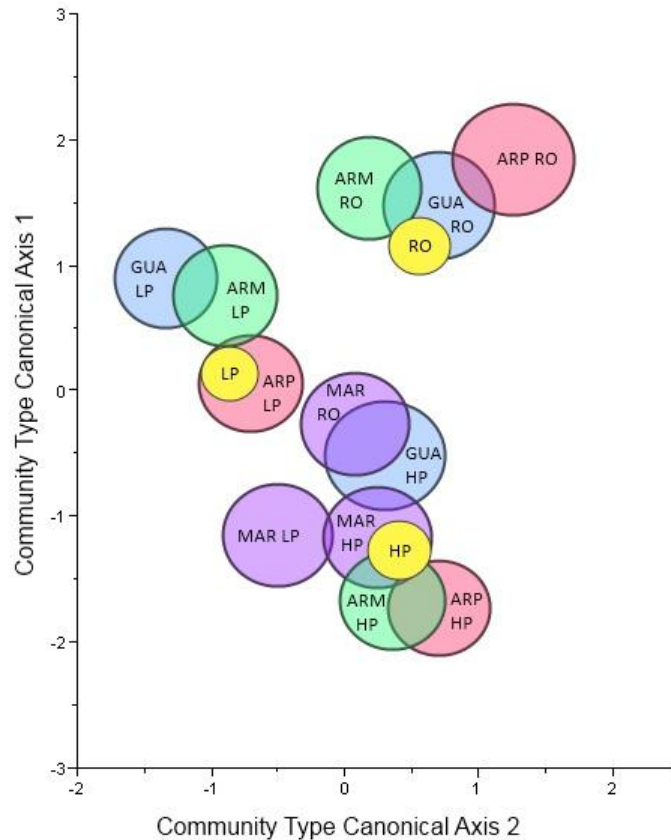


Figure 1.3c: Maximum Observed Negative CA 2 Score

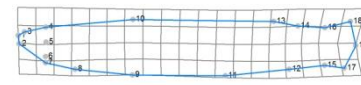


Figure 1.3d: Maximum Observed Positive CA2 Score

Figure 1.3: Canonical Centroid Plot of Morphological Variation Attributable to Community Type. The axes represent canonical variates from the MANCOVA optimized to reflect variation attributable to community type [i.e., Rivulus-Only, (RO); Low-Predation, (LP); and High-Predation, (HP)] in four Trinidadian rivers [i.e., Aripo, (ARP); Guanapo, (GUA), Arima, (ARM); and Marianne, (MAR)]. Solid yellow ellipses represent the 95% confidence limit for the mean of the given community type. Red, blue, green, and purple ellipses represent the 95% confidence limit for the mean of each community type in the Aripo, Guanapo, Arima, and Marianne rivers respectively. Figures 1.3a-1.3d represent thin-plate spline grids showing the maximum observed positive and negative scores along each canonical axis.

Discriminant function analysis (DFA) of community type correctly classified 327 fish or 68% compared to the null hypothesis of 33% by chance (Wilks' $\lambda = 0.45$, $df = 68, 888$, $P < 0.0001$), indicating significant divergence. When the 153 misclassified fish are broken down into river of origin, 31 are from the Arima, 29 from the Aripo, 37 from the Guanapo, and 56 from the Marianne. This corresponds to 25%, 24%, 31%, and 47% of the total population respectively. A closer analysis of the Marianne fish revealed that half of the fish from low-predation populations were misclassified (20 from LP and 21 from RO) and of these, 76% were misclassified as HP fish. This is strikingly high compared to 23% from the Arima, 21% from the Aripo, and 14% from the Guanapo misclassified as HP when in fact from a RO or LP population. When Marianne fish are removed from the analysis, the number of fish correctly classified increased to 76% compared to the null hypothesis of 33% by chance (Wilks' $\lambda = 0.28$, $df = 68, 648$, $P < 0.0001$).

Investigation of the canonical plot depicting morphological variation attributable to the community type x river interaction term (Figure 1.4) further revealed the unusual nature of morphological variation in the Marianne River. All of the rivers in the Caroni Drainage had a distinct separation between populations depending upon the presence or absence of predators. Caroni Drainage predator-absent populations tended to score more positively on the first canonical axis and more negatively on the second canonical axis while predator-present populations scored more negatively on the first axis and positively on the second axis. The opposite pattern was observed in the Marianne River: LP and RO populations clustered with the Caroni Drainage HP populations while the HP population clustered with the Caroni LP and RO populations.

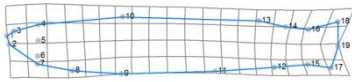


Figure 1.4a: Maximum Observed Positive CA1 Score

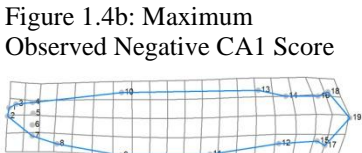


Figure 1.4b: Maximum Observed Negative CA1 Score

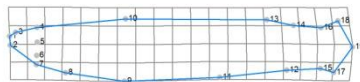


Figure 1.4c: Maximum Observed Negative CA2 Score



Figure 1.4d: Maximum Observed Positive CA2 Score

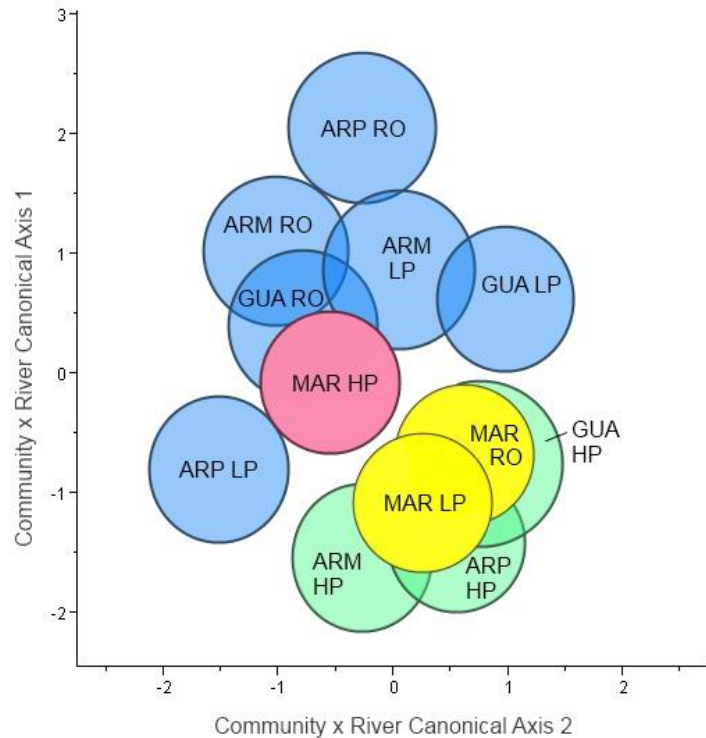


Figure 1.4: Canonical Centroid Plot of the Effect of River of Origin on Morphological Divergence by Community Type. The axes represent canonical variates from the MANCOVA optimized to reflect variation attributable to the interaction term community type [i.e., Rivulus-Only, (RO); Low-Predation, (LP); and High-Predation, (HP)] x river [i.e., Aripo, (ARP); Guanapo, (GUA), Arima, (ARM); and Marianne, (MAR)]. Ellipses represent the 95% confidence limit for the specified population's mean. Blue ellipses represent predator-absent populations and green ellipses represent predator-present populations in the Caroni Drainage. The Marianne River has been distinguished from the other rivers using yellow ellipses for its predator-absent populations and red for its predator-present population. Figures 1.4a-1.4d represent thin-plate spline grids showing the maximum observed positive and negative scores along each canonical axis.

Discussion

The results of this study indicate that *Rivulus* do appear to follow the ecomorphological paradigm of unsteady-swimming morphology (i.e., small anterior region and deeper caudal peduncle) in high-predation environments and steady-swimming morphology (i.e., deeper anterior region and narrower caudal peduncle) in low-predation environments. In this study system, the contrast between Caroni Drainage HP and RO environments (Figure 1.3, Canonical Axis 1) was the most similar to previous studies on other fish species (Langerhans and Reznick 2009). While LP populations in the Caroni Drainage did demonstrate the fusiform morphology consistent with their demonstrated steady-swimming ability (Oufiero et al. 2011), they were less divergent than RO populations and more similar to the consensus shape. This difference may reflect guppy predation pressure on juvenile *Rivulus* (Walsh et al. 2011) which would select for some level of escape ability early in life. Resource levels are also more abundant in LP environments due to the lower density of *Rivulus*. Therefore, LP fish may face less selection pressure to optimize their morphology towards competitive ability.

We also found significant differences between RO and LP populations despite their similarity in swimming performance (Figure 1.3, Canonical Axis 2). While both predator-absent populations demonstrated steady-swimming morphology as expected from their high critical swimming speeds (Oufiero et al. 2011), our results indicate that LP fish tend to have deeper abdomens than RO fish. While a deeper anterior body region minimizes recoil energy loss resulting in more efficient thrust (Blake 2004), LP fish did not have any noticeable advantage in swimming performance (Oufiero et al. 2011).

Deeper abdomens may also reflect phenotypic responses to predation differences between LP and RO environments. When faced with a gape-limited predator, deeper bodied fish can avoid predation by exceeding their predator's gape (Domenici et al. 2008; Zimmerman 2007). Female guppies, which pose the greatest threat to juvenile *Rivulus*, rarely exceed 32mm (Rodd and Reznick 1997) whereas *Rivulus* can reach nearly 10cm in many populations (Seghers 1978). In LP environments, juvenile *Rivulus* with deeper abdomens may have a better rate of survival against the severely gape-limited guppy. In RO environments, deeper bodied fish might receive less selective advantage since *Rivulus* likely cannot escape cannibalism until well after sexual maturity. Alternatively, this difference in body depth may just be an artifact of the higher per capita resource availability in LP sites resulting in fatter fish, but further studies are needed to test this hypothesis.

Additionally, this study found that while the Caroni Drainage populations conformed to their expected patterns of morphological divergence, the Marianne River appears fundamentally different. Both of the canonical plots and the DFA suggest that predator-absent populations in the Marianne are more "HP-like" in morphology. The three community types are also less diverged in the Marianne River than the Caroni Drainage (Figure 1.3). While the difference could be due to differences in genetic background (e.g., due to founder effects), a simpler explanation for the "HP-like" morphology in "predator-absent" populations is indeed the presence of a predator in these populations. North Slope rivers like the Marianne have highly dense populations of predatory *Macrobrachium spp.* prawns throughout their reaches (Endler 1976; Reznick et al. 1996; Magurran 2005). These amphidromous prawns were once present throughout the entire Northern Range Mountains,

but have recently (circa 1960s) been extirpated from the Caroni Drainage by anthropogenic modifications at lower elevations (Guillozet, 1999). Since prawns are not blocked by waterfall barriers, they could represent a significant predation threat throughout the entire reach of a river. This could explain the morphological differences observed in the Marianne River, but further studies are needed to test whether the observed difference between prawns-present and prawns-absent populations is replicated across multiple rivers.

The results of this study add further support to the ecomorphological paradigm of divergence in swimming morphology between predator-present and predator-absent populations. The consistency of results both in this study and in previous studies suggest that community gradients induce common patterns of divergence in fish species regardless of the specific predators present, type of habitat, or evolutionary lineage of the prey species. The generality of this paradigm suggests that it is possible to predict some aspects of phenotypic divergence in fish populations based on community structure. However, while we did find that overall patterns were largely consistent, the Marianne River showed a muted pattern of divergence in the opposite direction from predicted patterns. This discrepancy shows that while the ecomorphological paradigm can be a useful tool to predict phenotypic divergence in many cases, factors not considered in the assumptions of the paradigm may affect the general pattern. Further studies are needed to determine what factors invalidate the predicted outcomes of the paradigm.

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CHAPTER 2:

Rapid Phenotypic Divergence in Response to Release from a Predator

Abstract

Recently, the functional trade-off between steady- and unsteady-swimming morphology in prey fish in the presence or absence of predatory fish has received considerable attention, revealing common patterns of divergence in fish species regardless of the specific piscine predators present, type of habitat, or evolutionary lineage of the prey species. While this simple model appears to be broadly applicable, it fails to account for how invertebrates could structure morphology via their effect on the availability of resources, or their predation potential on small fish. In rivers of the Northern Range Mountains of Trinidad, the tropical killifish, *Rivulus hartii*, experiences three primary fish communities: High-Predation (HP) where large piscivorous fish are present, Low-Predation (LP) where they coexist with guppies (*Poecilia reticulata*), and *Rivulus*-Only (RO) where they are the only fish species present. Additionally, *Rivulus* populations can occur in the presence or absence of predatory prawns (*Macrobrachium spp.*). While prawns were once present throughout the Northern Range, they were extirpated from the Caroni Drainage rivers roughly 50 years ago resulting in novel prawns-absent environments. In this study, we investigate whether the presence of prawns affects *Rivulus* morphology in general, and whether the presence or absence of prawns affects how *Rivulus* populations respond morphologically to their fish community. We found that regardless of their fish community structure, *Rivulus* living in the presence of prawns are much more laterally narrow bodied, have larger heads, and narrower caudal peduncles than prawns-absent *Rivulus*. Prawns also appear to constrain *Rivulus* morphology, resulting in less divergence between community types and divergence in the opposite direction compared to prawns-absent populations. While

the goal of mechanistic models is to be generalized with only a few simplified assumptions, our results indicate that future studies testing this ecomorphological paradigm in other species and systems should carefully consider the predation or competition potential of other organisms.

Introduction

Whether it is the shape of a bird's bill or the thickness of a plant's cuticle, evolutionary biologists have come to recognize that convergence of phenotypic traits in organisms experiencing similar selective regimes is common in nature (Niemi 1985; Fleger and Henrickson 1997; Grenier and Greenberg 2005; Langerhans and DeWitt 2004). Such patterns of convergence suggest that if we can develop a deep enough understanding of the selective forces acting on a given organism, then we should be able to predict how changes in those forces will affect its evolution and by corollary the evolution of other organisms experiencing similar selective regimes. One pattern of convergence which has recently received considerable attention is the functional trade-off between steady and unsteady-swimming morphology in fish inhabiting different predation regimes (Langerhans and Reznick 2009; Langerhans 2010). Steady-swimming morphology (i.e., fusiform bodies with a narrow tail region) allows fish to swim more efficiently at a steady pace, giving such fish a competitive advantage when foraging. Unsteady-swimming morphology (i.e., narrow anterior region and deeper tail) produces greater thrust and stability during abrupt turns thereby allowing such fish to better escape predators. Since these two swimming morphologies cannot be optimized at the same time, it is expected that natural selection will shift phenotypes in a given population to reflect the selective pressures experienced (i.e., steady-swimming morphology in high-competition environments and unsteady-swimming morphology in high-predation environments). This ecomorphological paradigm has been investigated in many fish species and across multiple environments with fairly consistent results (Langerhans and DeWitt 2004; Andersson et al. 2006; Hendry et al. 2006;

Zimmerman 2007; Domenici et al. 2008; Gomes and Monteiro 2008; Burns et al. 2009; Langerhans 2009). While this simple model linking fish morphology to locomotor ability and subsequently to competitive ability or predator avoidance against other fish appears to be broadly applicable, it fails to account for how invertebrates could affect the availability of both food (Pringle et al. 1999; Crowl et al. 2001; March et al. 2002; Rudnick and Resh 2005) and habitat resources (Rahel and Stein 1988; Light 2005), or their predation potential on small fish (Guillozet 1999; Taylor and Soucek 2010). Since amphidromous prawns are common throughout the entire reach of many tropical island rivers and make up a large portion of their biomass, it is important to consider prawns in models of community structure effects.

In the Northern Range Mountains of Trinidad, the tropical killifish *Rivulus hartii* (Boulenger 1890) experiences three primary types of fish communities: high-predation (HP) where large piscivorous fish are present, low-predation (LP) where they co-occur with guppies (*Poecilia reticulata*), and *Rivulus*-only (RO) where they are the only fish species present and occur in high abundance (Liley and Seghers 1975; Endler 1978; Gilliam et al. 1993). We have recently demonstrated that *Rivulus* collected from the Caroni River Drainage conform to the ecomorphological paradigm of unsteady-swimming morphology in high-predation environments and steady-swimming morphology in low-predation environments (see Chapter 1). However, a muted pattern in the opposite direction was observed in fish collected from the Marianne River. While it is possible that the difference in morphology is due to underlying genetic differences between the populations (e.g., genetic drift), a simpler answer for the “HP-like” morphology in LP and RO populations in this river

is the presence of a predator. While the fish assemblages are consistent throughout the region, one notable difference between rivers is the presence or absence of the predatory prawns *Macrobrachium crenulatum*, *M. carcinus*, *M. faustinum* and *M. heterochirus* (Endler 1978; Endler 1983; Magurran 2005; Guillozet 1999). These amphidromous prawns were once present throughout the entire Northern Range Mountains, but have recently (circa 1960s) been extirpated from the Caroni Drainage by anthropogenic modifications at lower elevations (Guillozet 1999).

Though not initially considered, our results (see Chapter 1) combined with previous work (Guillozet 1999), suggest that *Macrobrachium* prawns may have a significant effect on *Rivulus* ecology. *Macrobrachium* prawns are opportunistic predators which are known to prey on guppies (Endler 1976; Reznick et al. 1996; Magurran 2005), tadpoles (Downie et al. 2001), and other decapods (Crowl and Covich 1994). *Rivulus* have demonstrated avoidance of prawns in both field and laboratory tests, shifting habitat use from shallow to deep water where benthic prawns cannot reach them or to isolated ephemeral pools which prawns are less likely to colonize due to their higher dissolved oxygen requirement (Guillozet 1999). Since prawns are not blocked by waterfall barriers, they represent a significant predation threat throughout the entire reach of a river. In HP environments *Rivulus* usually avoid larger predatory fish by seeking shallow water, but in prawns-present environments this behavior would leave them vulnerable to benthic prawns. *Rivulus* in these environments would only be freed from this constant predation threat by colonizing isolated ephemeral side-pools. *Rivulus* in LP and RO environments are likely to be highly vulnerable to prawns since these sites occur at higher elevations where the rivers are shallower and isolated side-pools are less

common. Even though *Rivulus* could shift to deeper water in LP and RO environments, they still must remain exposed to predation by prawns when they forage for benthic invertebrates, a major portion of their diet.

In this study we investigate the effect of prawns on *Rivulus* morphology in HP, LP, and RO environments. By comparing fish collected from two prawns-present and two prawns-absent rivers, we attempt to determine whether the pattern observed in our 2008 sample for the Marianne River is present in other prawns-present rivers and indeed represents an opposite pattern of divergence to the pattern observed in the Caroni River Drainage. We predict that *Rivulus* in prawns-present habitats will demonstrate unsteady-swimming morphology regardless of fish community type due to the increased predation threat posed by prawns occupying the benthic environments where *Rivulus* primarily forage. Furthermore, we predict that prawns-present HP populations will demonstrate a stronger shift towards ideal unsteady-swimming morphology than prawns-absent HP populations due to the loss of shallow water refugia when prawns are present, forcing *Rivulus* to occupy deeper, open water where they will come into more contact with large predators compared to prawns-absent environments.

Methods

Study Sites

We conducted field collections in four rivers in the Northern Range Mountains of Trinidad (Figure 2.1). The Aripo and Guanapo rivers are part of the Caroni River Drainage

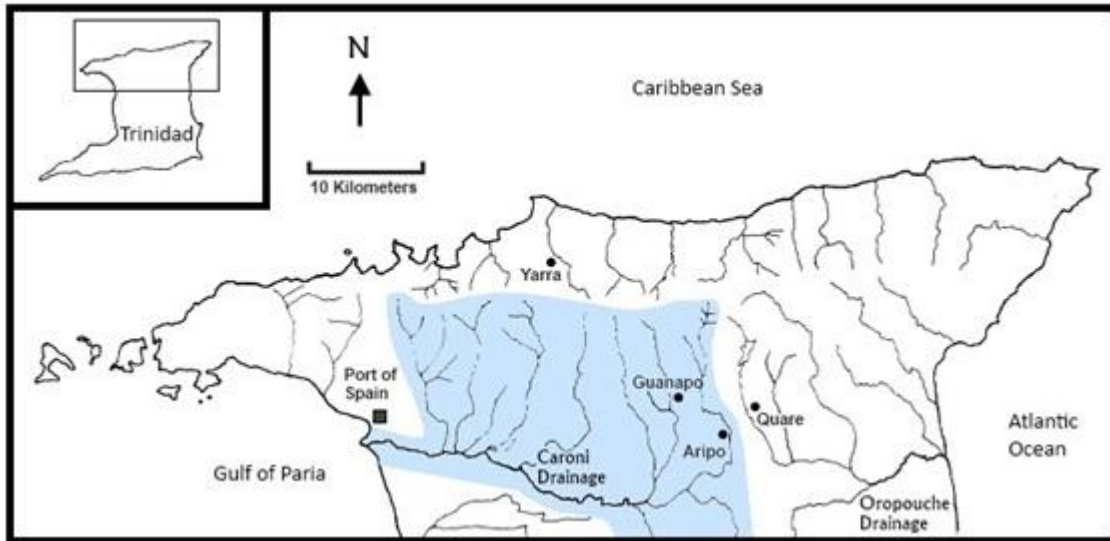


Figure 2.1: Map of Northern Range Mountains, Trinidad, West Indies. Blue region represents the Caroni River Drainage where prawns are absent.

which drains from the southern slope of the Northern Range Mountains west into the Gulf of Paria. The Yarra River is located on the northern slope and drains directly into the Caribbean Sea. The Quare River is part of the eastern Oropouche River Drainage which empties into the Atlantic Ocean. Each of these rivers exhibits the HP-LP-RO community gradient, though there is some variation in species present. All HP sites contain large piscivorous fish. On the South Slope (i.e., Caroni and Oropouche Drainages) common South American predatory species including cichlids (*Crenicichla sp.* and *Adinoacara pulcher*), trahiras (*Hoplias malabaricus*), and characids (*Astyanax bimaculatus* and *Hemibrycon dentatum*) are present (Reznick 1982; Gilliam et al. 1993; Fraser et al. 1999). North Slope rivers contain marine-derived species including three species of sleepers (*Eleotris pisonis*, *Gobiomorus dormitor*, and *Dormitator maculatus*) and mountain mullet (*Agnostomus monticola*) (Endler 1983;

Reznick et al. 1996). Fish species in the LP and RO sites are consistent across rivers except for the catfish, *Rhamdia sebae*, which have been observed near the Guanapo LP site (Gilliam et al. 1993). Amphidromous *Macrobrachium* prawns are present throughout the entire reach of North Slope and Oropouche Drainage rivers, but are absent from the Caroni Drainage due to recent anthropogenic modifications (Guillozet 1999).

Sample Collection

To investigate the effects of prawns, we sampled two prawn-present rivers (i.e., the Yarra and Quare) and two prawn-absent rivers (i.e., the Guanapo and Aripo) between January 24, 2011 and January 30, 2011. We collected between 30 and 50 sexually mature *Rivulus* (i.e., fish >35mm total length with visible secondary sexual characteristics) ranging in size from roughly 35mm to 95mm total length from HP, LP, and RO sites in each river using minnow traps and dip nets. However, it must be noted that the Yarra River's RO site is not a true *Rivulus*-only site, but rather a LP site with an extremely low density of guppies (a total of 8 guppies observed over a roughly 200m stretch). Extensive searching of upstream tributaries yielded only dry river beds and a prawn/tadpole-only site. The "RO" stretch of the river had suitable habitat for guppies, yet contained very few individuals. Since guppies are prolific breeders, it is likely that the site does not maintain a permanent population of guppies, and that the guppies observed were immigrants which were able to bypass the man-made barrier wall that separates the standard LP site from the low-guppy site. Due to the extremely low density of guppies and likely stochastic nature of the interaction, the low-guppy site was treated as a RO site for this study.

After collection, we transported the fish back to the field station where they were held overnight. Each fish was anesthetized using pH-buffered tricaine methanesulfonate (MS-222), weighed and measured, then photographed laterally using a digital camera mounted on a copystand above a standard glass background with ruler. Fish were then either transferred to a tank for recovery and subsequent return to their sample location or euthanized using an overdose of MS-222 if return was not possible. Final sample sizes for each site (Table 2.1) were reduced due to mortalities and/or unsatisfactory image quality.

Table 2.1: Summary of fish collected. Population values represent final count after the photos were screened for image quality.

River	Prawns Present/Absent	Community Type	<i>N</i>
Yarra	Present	HP	30
		LP	29
		RO	21
Quare	Present	HP	26
		LP	39
		RO	48
Guanapo	Absent	HP	38
		LP	32
		RO	30
Aripo	Absent	HP	33
		LP	21
		RO	30

Geometric Morphometrics and Statistical Analysis

We used landmark-based geometric morphometrics to assess *Rivulus* body shape (Rohlf and Marcus 1993; Zelditch et al. 2004). We placed an overlay grid over each image to supplement the lack of biological landmarks between the eye and dorsal fin. We then digitized the same nineteen landmarks used in the 2008 sample (see Figure 1.2) to each image using TpsDig software (version 2.14, Rohlf 2009a). We aligned the digitized images by generalized least squares superimposition using TpsRelw (version 1.46; Rohlf 2008) and calculated centroid sizes, partial warps, and uniform components. In order to investigate the effects of prawns on *Rivulus* morphology, we subjected the shape variables (partial warps and uniform components) to a nested MANCOVA with the factors centroid size (size covariate), sex, community type, river nested in prawns, prawns, and all interactions between these factors. Non-significant interaction terms were subsequently removed from the model. We used canonical variates generated from the MANCOVA to create thin-plate spline deformation grids in TpsRegr (version 1.37; Rohlf 2009b) depicting morphological variation at the two extremes of the canonical axes for each factor of interest. We estimated the relative contribution of each factor in the model to the observed morphological variation using Wilks' partial η^2 (see Langerhans and DeWitt 2004). We also conducted Discriminant Function Analyses (DFAs) using the shape variables (partial warps and uniform components) as the dependent variables to determine the relative strength of the prawns and community type factors on overall morphology based on the degree to which the DFAs could successfully place each fish into its correct grouping.

Results

The MANCOVA found significant effects for all factors except for the interaction terms of sex x river nested in prawns and sex x community type which were subsequently removed from the model (Table 2.2). For this study, the factor which explained the largest portion of the variance was the size covariate (centroid size, 84.3% of partial variance explained) which was expected based on the wide range of size classes sampled. While sex

Table 2.2: Results of the MANCOVA. F values were approximated using Wilks' λ for all factors marked †. All others are exact F. Partial variance explained was calculated using Wilks' partial η^2 .

Factor	F	df	P	Partial Variance Explained (%)
Centroid	50.9349	34, 323	<0.0001	84.3
Sex	15.2381	34, 323	<0.0001	61.6
Prawns	11.493	34, 323	<0.0001	54.7
community x prawns	7.6089†	68, 646	<0.0001	44.5
river[prawns]	7.1334†	68, 646	<0.0001	42.9
Community	5.872†	68, 646	<0.0001	38.2
community x river[prawns]	3.6988†	136, 1288.4	<0.0001	28.0
centroid x sex	2.8642	34, 323	<0.0001	23.2
centroid x community	2.2798†	68, 646	<0.0001	19.4
centroid x river[prawns]	2.1736†	68, 646	<0.0001	18.6
centroid x prawns	2.0381	34, 323	0.0009	17.7
prawns x sex	1.8999	34, 323	0.0025	16.7

was a minor factor in 2008 (only explaining 13.9% of the partial variance), in the 2011 sample it was the second largest factor, explaining 61.6% of the partial variance. Inspection of the TPS grids derived from this factor revealed that males have larger anal fins and deeper caudal peduncles than females, suggesting that unlike previously thought, *Rivulus* are in fact sexually dimorphic in body shape. This large discrepancy in partial variance explained by sex between the two samples is likely an effect of sampling methods. Canonical axes represent linear combinations of the response variables which maximize the differences between the levels of a given factor. Since roughly a third of the fish sampled in 2008 were juvenile, they likely masked the slight differences between mature males and females since ontogenetic effects would be more significant. While sex turned out to account for a large portion of partial variance, separate analyses of the sexes did not reveal any difference in response patterns to community type. While females do experience stronger effects for all factors (i.e., had higher partial variance values), their pattern is the same as males and only minor differences were observed in canonical plots (i.e., both sexes demonstrated the same pattern of divergence, only males were more negative on the first canonical axis due to their deeper caudal peduncles). Since the focus of this study is how *Rivulus* in general respond morphologically to their environment, all plots depict the total population. Of the factors of interest for this study, the presence vs. absence of prawns was the most important factor explaining 54.7% of the partial variance followed by the interaction term of prawns x community type (44.5%), river nested in prawns (42.9%), and finally community type (38.2%).

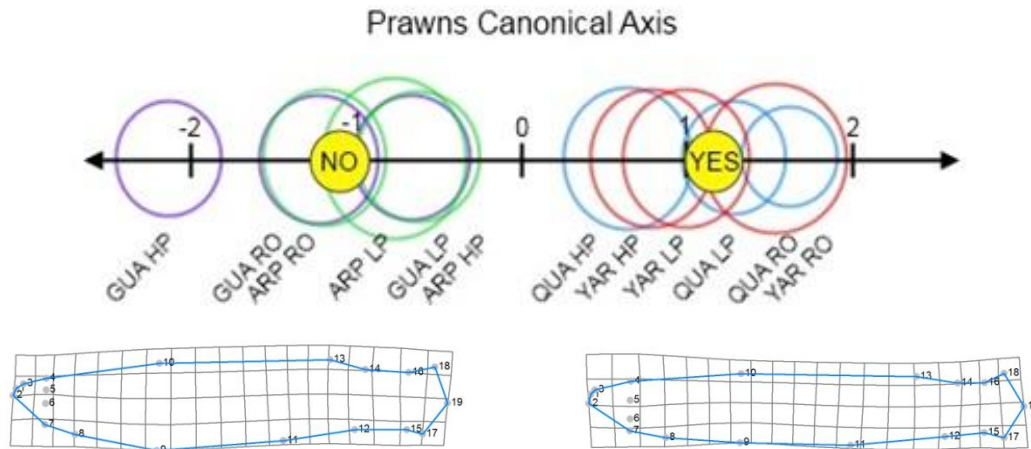


Figure 2.2a: 2x Maximum Observed Negative Canonical Score

Figure 2.2b: 2x Maximum Observed Positive Canonical Score

Figure 2.2: Canonical Centroid Plot of Morphological Variation in the Presence or Absence of Prawns. The axis represents the canonical variates from the MANCOVA optimized to reflect variation attributable to the presence or absence of prawns. Solid yellow ellipses represent the 95% confidence limit for the mean of prawns-present (YES) or prawns-absent (NO) populations. Red, blue, green, and purple ellipses represent the 95% confidence limit for the mean for each community type in the Yarra, Quare, Aripo, and Guanapo rivers respectively. Figures 5a and 5b represent thin-plate spline grids depicting 2x the observed range of morphological variation to emphasize differences.

Canonical variates derived from the prawns factor in the MANCOVA showed that *Rivulus* in prawns-absent communities (Figure 2.2a) have smaller heads, deeper bodies (particularly in the anterior region), and smaller anal fins (i.e., landmarks 11 and 12) than prawns-present communities (Figure 2.2b) regardless of community type. Canonical variates depicting how prawns affect *Rivulus* response to their fish community type (i.e., the community type x prawns interaction term) revealed opposite patterns of divergence (Figure 2.3). The first canonical axis appears to be consistent with the ecomorphological paradigm

of divergence between high-predation and low-predation fish communities already demonstrated in the Caroni Drainage during the 2008 sample. In RO populations in the absence of prawns (i.e., NO RO), *Rivulus* have larger heads, larger dorsal fins (i.e., landmarks 13 and 14), smaller anal fins, and a narrower caudal peduncle (Figure 2.3a) compared to HP populations in the absence of prawns (i.e., NO HP; Figure 2.3b). Prawns-present RO and HP populations (i.e., YES RO and YES HP), however, showed the opposite pattern and were much less divergent in morphology. LP populations for both prawns-present and prawns-absent communities were intermediate in morphology between their respective RO and HP communities. The second community type x prawns canonical axis describes the divergence between LP and RO/HP communities. In prawns-absent RO communities, *Rivulus* are deeper bodied and have a more upturned mouth (Figure 2.3d) compared to prawns-absent LP populations (Figure 2.3c) while prawns-absent HP populations are intermediate between the two morphologies. In prawns-present populations the pattern is opposite with LP populations having more upturned mouths and deeper bodies than RO populations. Unlike their prawns-absent counterpart, prawns-present HP fish are even more diverged from prawns-present LP populations than the RO populations.

DFA of the prawns factor correctly classified 330 fish or 86% compared to the null hypothesis of 50% by chance (Wilks' $\lambda = 0.45$, $df = 34, 342$, $P < 0.0001$) indicating a strong effect of prawns on overall *Rivulus* morphology. DFA of community type correctly classified 252 fish or 67% compared to the null hypothesis of 33% by chance (Wilks' $\lambda =$

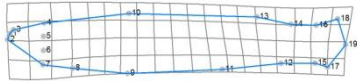


Figure 2.3a: 2x Maximum Observed Positive CA1 Score

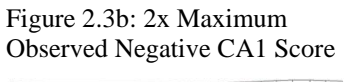


Figure 2.3b: 2x Maximum Observed Negative CA1 Score

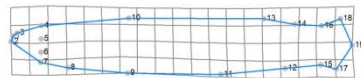
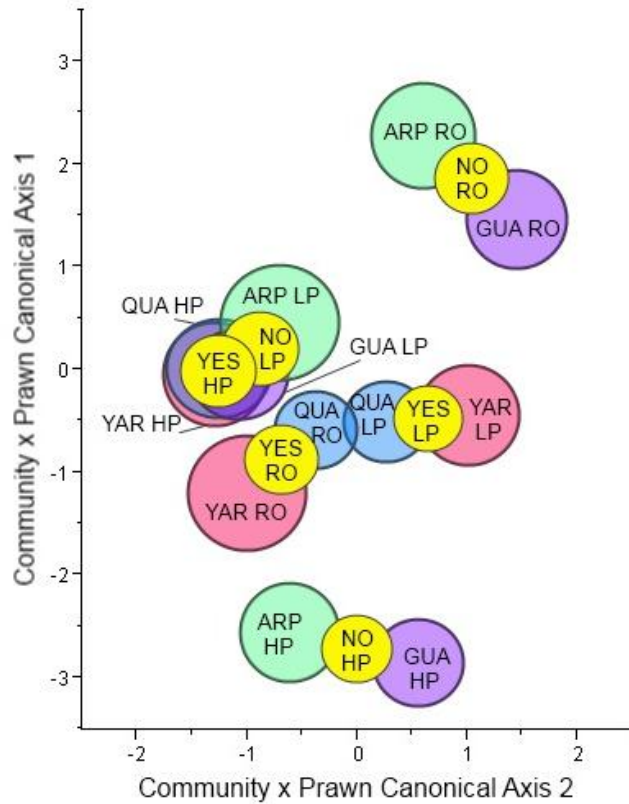


Figure 2.3c: Maximum Observed Negative CA2 Score



Figure 2.3d: Maximum Observed Positive CA2 Score

Figure 2.3: Canonical Centroid Plot of the Effect of Prawns on Morphological Variation Between Community Types. The axes represent canonical variates from the MANCOVA optimized to reflect variation attributable to the interaction term community type x prawns. Solid yellow ellipses represent the 95% confidence limit for the centroid for the given community type [i.e., high-predation (HP), low-predation (LP), and Rivulus-only (RO)] in the presence (YES) or absence (NO) of prawns. Red, blue, green, and purple ellipses represent the 95% confidence limit for each community type in the Yarra, Quare, Aripo, and Guanapo rivers respectively. Figures 2.3a-d represent thin-plate spline grids depicting morphological variation along each canonical axis. Figures 2.3a and 2.3b have been magnified 2x the observed range to emphasize morphological differences. Figures 2.3c and 2.3d are the observed range.

0.46, $df = 68, 682, P < 0.0001$). Of the misclassified fish, 70% were from prawns-present sites (51 from Quare and 37 from Yarra compared to 23 from Guanapo and 14 from Aripo), but there was no pattern for how the fish were misclassified.

Discussion

The results of this study indicate that the presence of *Macrobrachium* prawns has a strong effect on *Rivulus* morphological divergence. In our MANCOVA model, the presence or absence of prawns had a much larger effect on *Rivulus* morphology (explaining 54.7% of the partial variance) than the populations' fish community type (38.2% of partial variance) or unique historical backgrounds (42.9% of partial variance). While *Rivulus* do appear to follow the ecomorphological paradigm of unsteady-swimming morphology (i.e., small anterior and deep caudal peduncle) in high-predation environments and steady-swimming morphology (i.e., deeper anterior and narrow caudal peduncle) in low-predation environments, this pattern only occurs in the absence of prawns. Regardless of their fish community structure, *Rivulus* living in the presence of prawns have a smaller mid-body region, larger heads, and narrower caudal peduncles than prawns-absent *Rivulus*. This pattern seems to suggest that *Rivulus* in the presence of prawns are more adapted for steady-swimming than those in the absence of prawns. While prawns do pose a significant predation threat to *Rivulus* throughout the entire river, their benthic lifestyle and inability to give chase makes them an inefficient predator. Steady-swimming morphology would therefore prove advantageous for *Rivulus* living in prawns-present habitats since they can more efficiently maintain their position in deeper water while visually scanning for prawns.

Prawns also appear to constrain *Rivulus* morphology, resulting in less divergence between community types and in the opposite direction compared to prawns-absent populations (Figure 2.3). In LP and RO environments in the presence of prawns, *Rivulus* develop unsteady-swimming morphology similar to prawns-absent HP communities (Figure 2.3b). This may be due to the fact that prawns reach very high abundance in LP and RO environments where they too are released from predation pressure by large piscivorous fish. In these environments, *Rivulus* would be faced with an abundant and inescapable predator since LP and RO zones occur at higher elevations where water levels are lower and there are few side-pools to offer refuge. Faced with a ubiquitous predator, natural selection would favor unsteady-swimming ability in these environments to increase survival ability. However, since prawns are only a threat in close proximity, RO and LP fish do not need to be able to dart away as quickly as they would if they were being chased by a larger fish and therefore can retain some steady-swimming ability to enable them to forage more efficiently.

While we predicted that prawns-present HP *Rivulus* would be under increased predation threat since they would face predation by larger fish in deep water and predation by prawns in shallow water, this appears not to be the case. Instead, prawns-present HP *Rivulus* are similar to prawns-absent LP fish in morphology. This shift towards steady-swimming ability seems counterintuitive, especially since LP and RO fish shifted towards unsteady-swimming in the presence of prawns. However, this pattern may be explained by predatory fish selectively preying on prawns due to their lower escape ability, subsequently resulting in decreased predation on *Rivulus*. This decrease in predation threat would free *Rivulus* from

the need to develop higher performance fast-start escape ability and would instead allow them to maximize their foraging ability.

This opposite pattern of divergence along community gradients in prawns-present versus prawns-absent *Rivulus* populations warrants further investigation. While the Caroni Drainage shows similar divergent responses to many other species experiencing similar predation environments (Langerhans and Reznick 2009), their divergent pattern likely represents recent phenotypic divergence and is not typical for Trinidadian populations. It is unclear exactly when prawns were extirpated from the Caroni Drainage, but based on local reports, it likely occurred within the last 50 years. While it is impossible to know what morphological pattern was present in Caroni Drainage populations when prawns were present, the results of our study suggest that they were probably very similar to the prawns-present populations sampled in the Oropouche Drainage and North Slope rivers. If this assumption is true, then *Rivulus* populations in the Caroni Drainage have undergone rapid phenotypic divergence to reflect optimal swimming abilities for their given environments.

If Caroni Drainage populations diverged as rapidly as our results suggest, this would indicate that *Rivulus* might be maladapted to their fish communities in other drainages. Since the Caroni Drainage and Oropouche Drainage share the same fish assemblages, the “ideal” morphological response to selection pressures exerted by their fish community should be the same, yet these populations show opposite patterns. The rapid divergence of the Caroni populations after release from prawns suggests that prawns act as a much stronger selective force on *Rivulus* morphology than fish community type and prevents *Rivulus* from optimally responding to their fish community.

Currently it is unknown whether these morphological differences represent phenotypic plasticity or genetic divergence. Regardless of the underlying mechanism, divergence occurred fairly rapidly despite some level of gene flow. If this is the case, rapid divergence may soon be observed in the opposite direction. Several small *Atya spp.* shrimp have recently been observed in the Guanapo River. Like *Macrobrachium* prawns, these shrimp are amphidromous and are frequently observed in North Slope and Oropouche rivers. The presence of these shrimp in the Guanapo River suggests that the Caroni Drainage may be in the process of becoming hospitable to amphidromous species once again. If this is the case, it may be possible to observe morphological shifts within Caroni Drainage populations as these changes to community structure occur.

While the pattern of phenotypic divergence along community gradients in the presence or absence of prawns is striking, it is possible that the difference in morphology may be an artifact of differences in genetic backgrounds. Recent analysis of *Rivulus* mtDNA haplotypes and nuclear microsatellite variation (Walter et al. 2011) suggests that populations on the southern slope of the Northern Range Mountains were probably derived from a single founding event, and that southern and northern slope populations do not share a recent common ancestor. Walter et al. also found that unlike guppies (Suk and Neff 2009), there is no evidence to suggest that Oropouche and Caroni Drainage *Rivulus* populations are closely related despite their relatively close proximity. This study also found evidence of gene flow between North Slope and Oropouche populations. Fish collected in the Turure River demonstrated a mixture of both northern and southern haplotypes. While the Quare River was not sampled by Walter et al., it is likely similar in genetic makeup to the Turure River

since it is immediately adjacent. Due to the similarity of haplotypes between North Slope and Oropouche Drainage rivers, the observed pattern of phenotypic variation in the presence or absence of prawns may just be coincidental, and observed morphological differences may in fact represent some unique underlying aspect of their physiologies. Further work is needed to determine to what extent different genetic backgrounds affect morphological divergence in *Rivulus* populations.

While further studies are needed, our results suggest that *Rivulus* in the Caroni Drainage have undergone rapid phenotypic divergence between community types after being released from prawn predation. While the general ecomorphological paradigm of trade-offs in locomotor-associated morphology along a competition-predation gradient does appear to hold true for Caroni Drainage populations, our results indicate that other ecological interactions must also be considered. Crustaceans are often overlooked in ecological studies despite their effects on resource availability (Pringle et al. 1999; Crowl et al. 2001; March et al. 2002; Rudnick and Resh 2005; Rahel and Stein 1988; Light 2005) or predation potential on small fish (Guillozet 1999; Taylor and Soucek 2010). Our results offer compelling evidence that, at least for *Rivulus hartii*, the presence or absence of prawns is a much more important determinant factor for phenotypic diversity than fish community structure or river of origin. While the goal of mechanistic models is to be generalized with only a few simplified assumptions, our results indicate that future studies testing this ecomorphological paradigm in other species and systems should carefully consider the predation or competition potential of other organisms.

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CHAPTER 3:

Phenotypic Responses to Anthropogenic Species Range Expansions

Abstract

Human-induced environmental change through climate change, introduction of non-native species, and habitat fragmentation have resulted in selective regimes shifting much more rapidly than organisms typically experienced in times past. This raises the question of whether the outcomes of such rapid phenotypic shifts to environmental changes can be predicted based on prior understanding of the selective forces involved. Using the ecomorphological paradigm of the functional trade-off between steady- and unsteady-swimming morphology in fish inhabiting different predation regimes, we attempt to predict the morphological response of the tropical killifish, *Rivulus hartii*, to changes in its fish community structure. Previous work has shown that *Rivulus hartii* diverges morphologically among its three primary fish communities: High-Predation (HP) where large piscivorous fish are present, Low-Predation (LP) where they coexist with guppies (*Poecilia reticulata*), and *Rivulus*-Only (RO) where they are the only fish species present. Using previous species-introduction experiments as a model of natural species range expansions, we attempt to predict how *Rivulus* populations will respond to invasion by a predator (*Crenicichla sp.*) or a competitor (*Poecilia reticulata*). While the suggested morphological trajectory in the predator introduction site does suggest a shift from its historical LP morphology to its new HP status as predicted, it was not significantly different from the LP control population. Two of the guppy introduction sites did significantly diverge from their RO control population, but instead of shifting to reflect their new LP status, they became more “extreme RO-like” in morphology. This failure of the paradigm to predict morphological trajectories contrasts

with recent work which has shown rapid evolution of some life history traits in the predicted direction and endpoints.

Introduction

All organisms experience a complex mosaic of selection pressures which vary over space and time (Thompson 2005). As populations become isolated, they begin to adapt to their specific selective regime creating patches of phenotypic variation across their natural range. Since adaptations to the competition-predation gradient are often associated with functional trade-offs (Schluter 2000), the expansion of a species' range into a region where it was previously absent will typically exert strong selection pressure on the resident species and vice versa as the two species interact. While such shifts in selective regimes are common in nature, human-induced environmental change through climate change, introduction of non-native species, and habitat fragmentation have resulted in selective regimes shifting much more rapidly than organisms typically experienced in times past (Palumbi 2001). Many recent studies have demonstrated that organisms experiencing environmental change can undergo rapid phenotypic divergence within a few generations and across distances which they can normally disperse (Thompson 1998; Schluter 2000; Hendry and Kinnison 1999; Reznick and Ghalambor 2001; Gilchrist et al. 2004; Hargeby et al. 2004; Skelly 2004; Phillips and Shine 2006; Urban 2010). This raises the question of whether the outcomes of such rapid phenotypic shifts to environmental changes can be predicted based on prior understanding of the selective forces involved.

While evolutionary biologists would ideally like to understand all of the selective forces acting on an organism and the evolutionary framework of all observed traits, this would be nearly impossible. Even if this monumental task were possible, it would be difficult to decide which organisms to focus our efforts on since anthropogenic modifications

to the environment are so widespread. Even in well studied populations it is difficult to predict all of the environmental fluctuations which can affect an organism (Grant and Grant 2002). Rather than trying to understand all aspects of selection and evolution within a single species, it is useful to identify patterns of divergent selection along common selective gradients which can be used to develop general models of phenotypic evolution. Examples of replicated patterns of phenotypic evolution along common selective gradients have been identified in a range of taxa (Reznick et al. 1996; Losos et al. 1998; Schluter 2000; Van Buskirk 2002; Langerhans and DeWitt 2004). One pattern of convergent evolution which has recently received considerable attention is the functional trade-off between steady- and unsteady-swimming morphology in fish inhabiting different predation regimes (Langerhans and Reznick 2009; Langerhans 2010). Steady-swimming morphology is characterized by a streamlined, fusiform body with a narrow tail region to reduce drag. Fish exhibiting this body type can swim more efficiently at a steady pace, giving them a competitive advantage while foraging or maintaining position in flowing water. Unsteady-swimming morphology is characterized by a smaller anterior region and deeper tail. This deeper tail region produces greater thrust and stability during abrupt turns which allows fish to better escape predators. While both of these swimming morphologies have a strong effect on the fitness of fish, they require the opposite pattern of divergence. Therefore, it is expected that natural selection will shift phenotypes in a given population to reflect the selective pressures experienced (i.e., steady-swimming morphology in high-competition environments and unsteady-swimming morphology in high-predation environments). This paradigm has been demonstrated in multiple fish species occupying different environments (Langerhans and DeWitt 2004;

Andersson et al. 2006; Hendry et al. 2006; Zimmerman 2007; Domenici et al. 2008; Gomes and Monteiro 2008; Burns et al. 2009; Langerhans 2009) suggesting a strong and predictable pattern of convergent evolution in fish. We recently demonstrated that this pattern also holds true in populations of the tropical killifish, *Rivulus hartii*, in the Northern Range Mountains of Trinidad (Chapter 1).

Tropical streams in the Northern Range Mountains of Trinidad have long been recognized as ideal natural replicates of evolution along ecological gradients due to the consistency of their fish communities and the similarity of environmental conditions (Haskins and Haskins 1951; Endler 1978; Reznick and Endler 1982; Endler 1995). Each river is divided by a series of waterfall barriers into three primary community types: High-Predation (HP) where large predatory species are present, Low-Predation (LP) where *Rivulus* coexist with guppies (*Poecilia reticulata*), and *Rivulus*-Only (RO) where no other fish species are present. Comparisons of guppy and *Rivulus* populations along this community gradient have revealed patterns of divergence in life history, coloration, and behavior in both species (Seghers 1978; Endler 1980; Endler and Reznick 1982; Endler 1995; Reznick 1996; Reznick et al. 1996; Magurran 2005; Walsh and Reznick 2009). Many researchers have also taken advantage of the replicated structure of these rivers to conduct experimental introductions of species in a field setting (Haskins and Haskins 1954; Endler 1980; Reznick 1996). Four of these introduction experiments offer useful models for invasion of *Rivulus* communities by fish previously blocked by natural barriers. In 1976, guppies collected from a HP section of the main stem of the Aripo River were introduced into a previously RO section of a side tributary (Endler 1980), creating a new LP zone. In 1981, a large predator,

Crenicichla sp., was introduced above a waterfall barrier into a previously LP site on the main stem of the Aripo River located just downstream of Endler's guppy introduction tributary (D.N. Reznick, personal communication). Then in 2008, guppies from a HP section of the Guanapo River were introduced into previously RO sites in two upstream tributaries, designated as the Lower La Laja and Upper La Laja streams.

In this study we take advantage of these four introduction experiments in rivers of the Northern Range Mountains of Trinidad to test the effects of recent and more established range expansions of guppies, a small, competing species (Gilliam et al. 1993) and possibly a predator on new born *Rivulus* (Walsh and Resnick 2009) and a large predator, *Crenicichla sp.*, on the morphology of the resident *Rivulus* populations. Based on our previous work on *Rivulus* morphology along community gradients, we would predict that RO populations exposed to guppy invasions will shift their morphologies to reflect their new LP status, becoming less fusiform compared to natural RO communities. We also predict that the LP *Rivulus* population exposed to predator invasion will shift from steady-swimming morphology (i.e., fusiform body with narrow tail) to unsteady-swimming morphology (i.e., smaller anterior region and deeper tail) to reflect their new HP status.

Methods

Study Sites and Sample Collection

We conducted field samplings in natural and experimental species-introduction populations in the Guanapo and Aripo rivers in the Caroni Drainage of the Northern Range Mountains of Trinidad in January and March 2011. Study sites in the Guanapo River

included the two guppy introduction sites in the Upper La Laja and Lower La Laja tributaries (hereafter UI and LI respectively), their natural RO control populations (hereafter UC and LC), and natural LP and HP sites on the main stem of the Guanapo. The UI and LI sites are immediately downstream of their control populations. These sites are separated by man-made barriers constructed between the downstream introduction sites and the upstream control sites prior to the introduction to prevent the introduced guppies from invading the upstream control population. Additionally, canopy cover was thinned in the UI and UC sites to simulate light regimes experienced at lower elevations. Study sites in the Aripo River included Endler's guppy-introduction site (EI), natural RO and LP sites on the same tributary as the EI site, and the *Crenicichla* introduction site (CI) and a natural HP site on the main stem of the Aripo, downstream of Endler's Tributary.

We collected between 25 and 40 mature *Rivulus* (i.e., fish with visible secondary sexual characteristics) ranging in size from about 35mm to 95mm total length from each site using baited minnow traps and dip nets. After collection, we transported the fish back to the field station where they were held overnight. We then anesthetized each fish using pH-buffered tricaine methanesulfonate (MS-222), weighed and measured them, then photographed them laterally using a digital camera mounted on a copystand above a standard glass background. After photographing, each fish was transferred to a tank for recovery and subsequent return to its sample location. Final sample sizes (Table 3.1) were reduced due to mortalities and/or unsatisfactory image quality. Field conditions (flooding) prevented the EI and CI populations from being collected with their natural counterparts in late January 2011 as originally planned. Instead, these two populations were collected mid-March 2011.

Table 3.1: Summary of Fish Collected. Population values represent final count after the photos were screened for image quality.

River	Community	Other Species Present	<i>N</i>
Guanapo (main stem)	HP	Large predators	38
	LP	Guppies	32
Lower La Laja (Guanapo Tributary)	LC	None	30
	LI	Guppies (introduced in 2008)	30
Upper La Laja (Guanapo Tributary)	UC	None	30
	UI	Guppies (introduced in 2008)	33
Aripo (main stem)	HP	Large predators	33
	CI	<i>Crenicichla</i> (introduced in 1981)	19
Endler's Tributary (Aripo Tributary)	RO	None	27
	EI	Guppies (introduced in 1976)	19
	LP	Guppies	28

Geometric Morphometrics and Statistical Analysis

We assessed morphological variation in natural versus “invaded” populations using landmark-based geometric morphometric methods (Bookstein 1991; Rohlf and Marcus 1993; Zelditch et al. 2004). We superimposed a standardized grid and digitized nineteen landmarks to each image using TpsDig software (version 2.14, Rohlf 2009a) as described previously (Chapter 1). We aligned the digitized landmarks by generalized least squares superimposition using TpsRelw (version 1.46; Rohlf 2008), and calculated centroid size, partial warps, and uniform components. In order to investigate the effects of recent species range expansions on *Rivulus* morphology, we subjected the shape variables (partial warps

and uniform components) to separate MANCOVAs testing for effects due to centroid size (size covariate), sex, community of origin, and two-way interactions between these variables using SAS JMP software (version 8.0.2, SAS Institute Inc., Cary, NC). Non-significant interaction terms were subsequently removed from the models.

We estimated the relative contribution of each factor to the total morphological variation within each river using Wilks' partial η^2 (see Langerhans and DeWitt 2004). We assessed morphological divergence between introduced populations and their natural counterparts using canonical variates generated from the community of origin factor of the MANCOVA. We then created thin-plate spline deformation grids depicting morphological variation at the two extremes of the canonical axes using TpsRegr (version 1.37; Rohlf 2009b).

Results

Short-term Response: the Guanapo River Sites

For the Guanapo River data, the MANCOVA found significant effects for all factors (Table 3.2). Like the larger 2011 data set (see Chapter 2), centroid size (the size covariate) and sex accounted for the greatest portions of partial variance (accounting for 88.2% and 74.6% respectively). Again, while sex accounted for a large portion of morphological variation, males and females did not differ in their response pattern to community of origin so all plots show the total population. In the Guanapo River, community of origin accounted for 56.5% of the partial variance. Inspection of the canonical axes derived from the community factor of the MANCOVA (Figure 3.1) revealed that the Upper La Laja

introduction (UI) site has significantly diverged from its control population (UC). However, rather than shifting from their previously RO morphology towards their new LP status, the UI population shifted towards an even higher positive value on the first canonical axis, making them “even more RO-like” than the RO control population. The Lower La Laja introduction (LI) suggests a qualitatively similar but not statistically significant shift from its control (LC), on the first canonical axis. There was no significant divergence on the second canonical axis for either population. While the second axis did not reveal any difference between the introduction and control populations, it does seem to suggest a difference in morphology between this study and the results from our previous study. While the first canonical axis depicts the ecomorphological paradigm of steady- vs. unsteady-swimming morphology and is consistent with the 2008 community type canonical axis 1 (see Chapter 1, Figure 1.3), the second axis is different from the 2008 sample. In 2008, LP fish had deeper abdomens than RO/HP fish, but the opposite is true in 2011.

Table 3.2: Results of the MANCOVA for the Guanapo River Populations. F values were approximated using Wilks’ λ for the Community factor and its interactions. All others are exact F. Partial variance explained was calculated using Wilks’ partial η^2 .

Factor	<i>F</i>	<i>df</i>	<i>P</i>	Partial Variance Explained (%)
Centroid Size	31.0322	34, 141	<0.0001	88.2
Sex	12.1618	34, 141	<0.0001	74.6
Community	5.4424	170, 704.16	<0.0001	56.5
Sex x Centroid	1.9424	34, 141	0.0039	31.9
Community x Sex	1.5059	170, 704.16	0.0002	26.5
Community x Centroid	1.3453	170, 704.16	0.0054	24.3

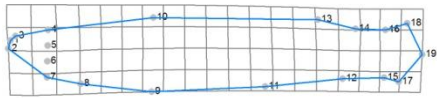


Figure 3.1a: Maximum Observed Positive CA1 Score

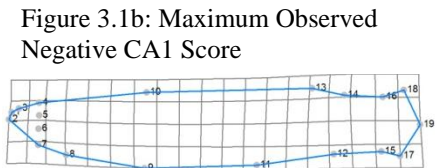


Figure 3.1b: Maximum Observed Negative CA1 Score

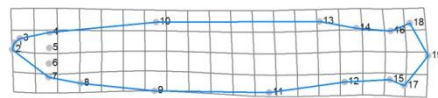
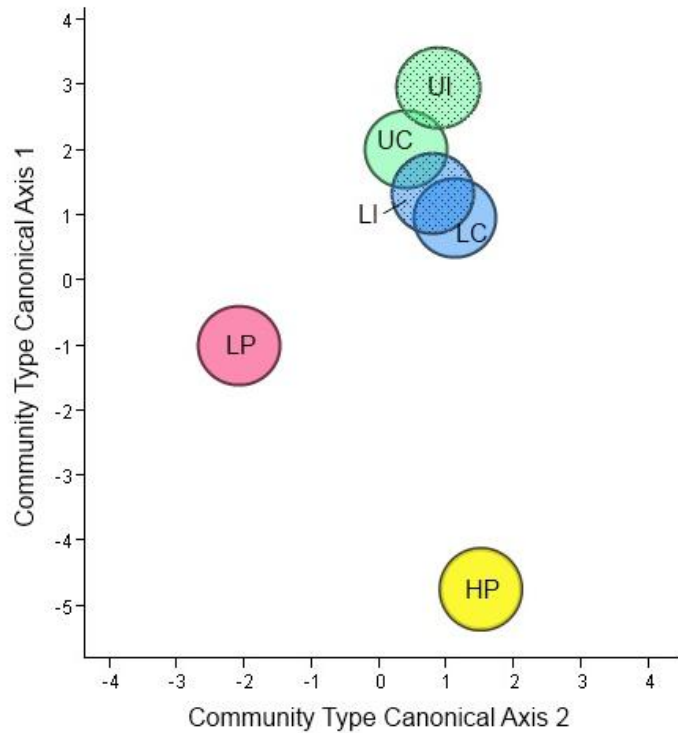


Figure 3.1c: Maximum Observed Negative CA2 Score

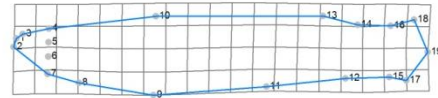


Figure 3.1d: Maximum Observed Positive CA2 Score

Figure 3.1: Canonical Centroid Plot of Morphological Variation Attributable to Community of Origin in the Guanapo River. The axes represent canonical variates from the MANCOVA optimized to reflect variation attributable to community of origin (i.e., High Predation, HP; Low Predation, LP; Upper La Laja Rivulus-Only Control, UC, Upper La Laja Guppy Introduction, UI; Lower La Laja Rivulus-Only Control, LC, and Lower La Laja Guppy Introduction, LI). Ellipses represent the 95% confidence limit for the mean of each community. Figures 3.1a-3.1d represent thin-plate spline grids showing the maximum observed positive and negative scores along each canonical axis.

Long-term Response: the Aripo River Sites

For the Aripo River data, the MANCOVA found significant effects for all factors except for the interaction term centroid size x sex which was then removed from the model (Table 3.3). Once again, centroid size (the size covariate) and sex accounted for the greatest portions of partial variance (accounting for 88.3% and 71.3% respectively) and again, there was no difference in response pattern between the sexes. In the Aripo River, community of origin accounted for 70.5% of the partial variance. Investigation of the canonical plot depicting morphological variation by community (Figure 3.2) showed that, like in the Guanapo, the Aripo guppy introduction site (EI) has shifted more positively on the first canonical axis, making it more “extreme RO-like” relative to its RO control population. The *Crenicichla* introduction site (CI) did not significantly diverge along the first canonical axis from its LP control.

Table 3.3: Results of the MANCOVA for the Aripo River Populations. F values were approximated using Wilks’ λ for the Community factor and its interactions. All others are exact F. Partial variance explained was calculated using Wilks’ partial η^2 .

Factor	F	df	P	Partial Variance Explained (%)
Centroid Size	17.3856	34, 78	<0.0001	88.3
Sex	5.6962	34, 78	<0.0001	71.3
Community	5.5584	136, 313.15	<0.0001	70.5
Community x Centroid	1.7899	136, 313.15	<0.0001	43.6
Community x Sex	1.4951	136, 313.15	0.0022	39.2

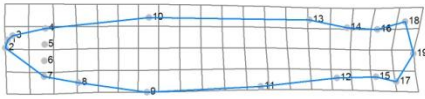


Figure 3.2a: Maximum Observed Positive CA1 Score

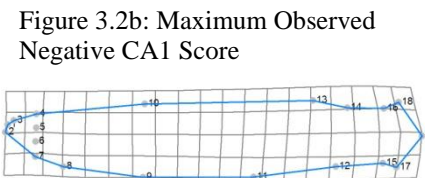


Figure 3.2b: Maximum Observed Negative CA1 Score

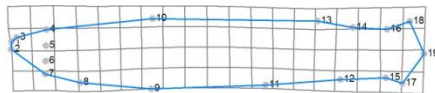


Figure 3.2c: Maximum Observed Negative CA2 Score

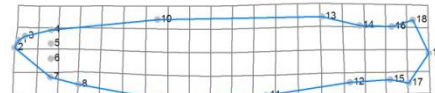


Figure 3.2d: Maximum Observed Positive CA2 Score

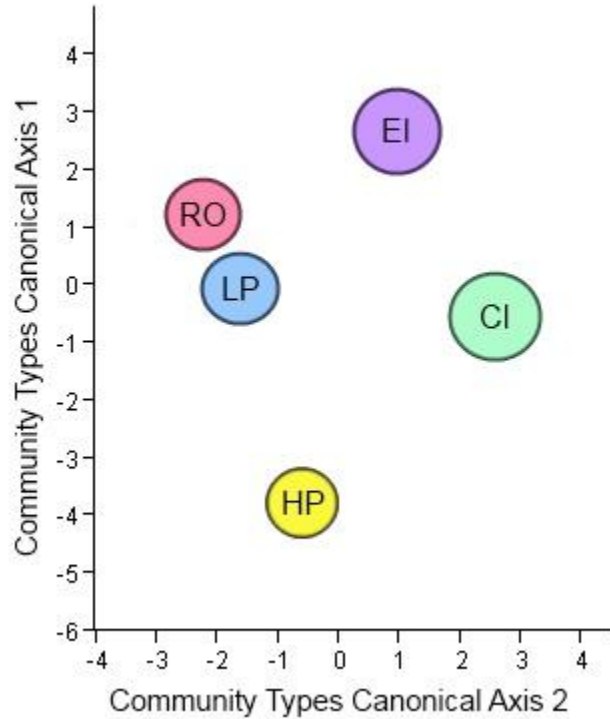


Figure 3.2: Canonical Centroid Plot of Morphological Variation Attributable to Community of Origin in the Aripo River. The axes represent canonical variates from the MANCOVA optimized to reflect variation attributable to community of origin (i.e., High Predation, HP; Low Predation, LP; *Rivulus*-only, RO; *Crenicichla* Introduction, CI; Ender’s Introduction, EI). Ellipses represent the 95% confidence limit for the mean of each community. Figures 3.2a-3.2d represent thin-plate spline grids showing the maximum observed positive and negative scores along each canonical axis.

The second canonical axis for this data set shows an apparent divergence between natural and introduction populations. Populations from introduction sites appear to have deeper abdomens than natural populations. However, this contrast needs to be interpreted in the context of the unintended confounding collection date (RO, LP, and HP in January 2011; EI and CI in March 2011) with unknown but possibly differing resource levels affecting abdominal depth.

Discussion

While our previous work has shown that *Rivulus* in the absence of prawns do follow the ecomorphological paradigm of unsteady-swimming morphology (i.e., small anterior region and deeper caudal peduncle) in high-predation environments and steady-swimming morphology (i.e., deeper anterior region and narrower caudal peduncle) in low-predation environments, the paradigm did not predict how *Rivulus* would respond morphologically to changes in their community gradient in the present study. While the suggested trajectory of the CI site's morphology is consistent with predictions, it failed to significantly diverge from the LP control site despite 30 years of increased predation pressure. This may be due to the fact that *Crenicichla* are likely rather weak predators for *Rivulus* since they are most active during the day whereas *Rivulus* are most active at night (Endler 1987). Natural HP sites also contain *Hoplias malabaricus*, a nocturnal predator known to induce changes in movement behavior, growth, and other aspects of *Rivulus* biology (Fraser and Gilliam 1992; Gilliam et al. 2001). Unfortunately, since this predator-introduction involved only *Crenicichla*, it is not

possible to know whether a different predator would induce a stronger shift in morphology in the same period of time.

Despite the strong pattern of morphological divergence observed between the natural populations of three different rivers in the Caroni Drainage (see Figure 1.3), both the populations representing very recent guppy range expansion (UI and LI) and our more established range expansion (EI) failed to shift from RO towards LP morphology. The absence of a rapid shift was less surprising than the presence of a shift in the opposite direction from predicted. Both the UI and EI populations developed significantly more fusiform body morphologies than their RO control populations. While the LI population is not yet significantly divergent from its control population, it too is suggesting a shift to a more fusiform morphology. This suggests that the invasion of guppies in these populations exerts stronger competition pressure than experienced in a natural RO population. This difference in presumed competition level is especially remarkable in the Upper La Laja site since the introduction occurred only 34 months prior to sampling and the introduction site is only separated from its control by a man-made barrier and thus the divergence occurred despite possibly high levels of gene flow. This shift towards more ideal steady-swimming morphology in guppy-introduction populations is surprising considering that we believe the pattern observed in the Caroni Drainage populations in the 2008 and 2011 data sets actually reflects recent morphological divergence after being released from prawns (see Chapter 2). Prawns were extirpated from the Caroni Drainage sometime around the 1960s (Guillozet 1999), and therefore fish in the Caroni Drainage have likely adapted morphologically to their fish communities in the last 50 years. We would therefore expect that at least the EI

population, which has been established for over 30 years, would more closely reflect natural LP populations, yet this is not the case.

A recent mesocosm experiment by Palkovacs et al. (2009) suggests that our observed differences between *Rivulus* morphology in the guppy-introduction sites and natural LP sites may be due to the phenotype of the introduced guppies. Palkovacs et al. compared algal biomass and accrual, aquatic invertebrate biomass, and detrital decomposition in mesocosms containing RO *Rivulus* alone, RO *Rivulus* with HP guppies, RO *Rivulus* with LP guppies, and sympatric LP *Rivulus* and guppies. Mesocosms with HP guppies had higher nitrogen excretion rates leading to an increase in primary production compared to mesocosms with LP guppies. HP guppies also fed on algae at lower rates than LP fish. Combined, this resulted in a significantly higher algae biomass and accrual rate in the mesocosms containing HP guppies. Palkovacs et al. also found that coevolved *Rivulus*-guppy treatments (i.e., both fish populations from LP origin) had a lower aquatic invertebrate biomass than the non-coevolved treatments (i.e., RO *Rivulus* and HP/LP guppies) and that algal biomass and accrual rates in these mesocosms were similar to the RO state. These results indicate that the phenotype of the interacting populations has a significant effect on the ecosystem's function. Since all of our introduction sites used guppies from HP sites, their effect on RO *Rivulus* morphology is likely different from the effect of natural LP guppies on natural LP *Rivulus* to which they were being compared. If the results of the mesocosm experiments hold true in natural settings, the introduction of HP guppies into these sites likely resulted in an increased algal biomass and accrual rate and a higher aquatic invertebrate biomass than a natural LP site. Therefore, the observed difference in morphology may reflect a transition period as the HP

guppies and RO *Rivulus* coevolve to reflect their new LP status and the environment shifts in response.

Recent work has shown that this coevolution is already occurring in the EI site. Walsh and Reznick (2011) found that *Rivulus* from the EI site had significantly higher reproductive allotment and fecundity, and produced smaller eggs than *Rivulus* from a control RO site. These results are consistent with life-history differences between natural LP and RO populations (Walsh and Reznick 2009). These differences also persisted in common garden experiments and therefore reflect a genetic divergence in the EI population from its historical RO status (Walsh and Reznick 2011). Similarly, a recent study by Palkovacs et al. (2011) showed that guppies in the EI site have diverged in feeding performance and morphology from their original HP status to reflect their new LP status. While *Rivulus* morphology has not yet shifted to resemble a natural LP population as the guppy population has, it may just be a matter of time.

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