

# PHENOTYPIC DIVERSIFICATION ACROSS AN ENVIRONMENTAL GRADIENT: A ROLE FOR PREDATORS AND RESOURCE AVAILABILITY ON THE EVOLUTION OF LIFE HISTORIES

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Changes in age/size-specific mortality, due to such factors as predation, have potent evolutionary consequences. However, interactions with predators commonly impact prey growth rates and food availability and such indirect effects may also influence evolutionary change. We evaluated life-history differences in Trinidadian killifish, *Rivulus hartii*, across a gradient in predation. *Rivulus* are located in (1) "high predation" sites with large piscivores, (2) "*Rivulus/guppy*" sites with guppies, and (3) "*Rivulus-only*" sites with just *Rivulus*. *Rivulus* suffer higher mortality with large predators, and guppies may prey upon small/young *Rivulus* in *Rivulus/guppy* environments. In turn, population densities decline while growth rates increase in both localities compared to *Rivulus-only* sites. To explore how the direct and indirect effects of predators and guppies influence trait diversification in *Rivulus*, we examined life-history phenotypes across five rivers. High predation phenotypes exhibited a smaller size at reproduction, a greater number of eggs that were smaller, and increased reproductive allotment. Such changes are consistent with a direct response to predation. *Rivulus* from *Rivulus/guppy* sites were intermediate; they exhibited a smaller size at reproduction, increased fecundity, smaller eggs, and larger reproductive allotment than *Rivulus-only* fish. These changes are consistent with models that incorporate the impacts of growth and resources.

**KEY WORDS:** Growth rate, guppy, indirect effects, killifish, *Rivulus*.

A central goal of evolutionary biology is to understand the ecological factors that determine patterns of life-history diversification (Roff 1992; Stearns 1992). Age- or size-specific mortality, due to factors such as predators, has been shown to be an important factor that shapes the evolution of life histories (Lynch 1980; Reznick 1982; Martin and Clobert 1996; Bronikowski and Arnold 1999; Johnson 2001; Hilton et al. 2002; Jennions and Telford 2002). Early theories of life histories evolution predict that increased rates of predation on old or large individuals favor the evolution

of earlier maturation and higher reproductive effort, whereas the opposite trends are predicted when mortality during very young age-classes or small individuals is increased (Gadgil and Bossert 1970; Law 1979; Michod 1979; Charlesworth 1980; Taylor and Gabriel 1992; Ernande et al. 2004; Gardmark and Dieckmann 2006). However, these frameworks only consider the impacts of differential mortality and thereby implicitly ignore the biology and ecology of target organisms. Indirect effects of predators and competitors alter rates of growth and levels of food availability. For instance, predators often reduce prey density, which increases per-capita resource availability to surviving individuals (Wootton 1994), whereas predators and competitors commonly induce

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morphological, behavioral, or physiological changes that have both positive and negative impacts on rates of growth (reviewed in Werner and Peacor 2003; Schmitz et al. 2004; Preisser et al. 2005). This is important because other theories that incorporate these ecological interactions yield predictions that can mimic or contradict those of theory that only model the consequences of mortality (Gadgil and Bossert 1970; Kozłowski and Uchmanski 1987; Kozłowski and Wiegert 1987; Hutchings 1993; Mylius and Diekmann 1995; Abrams and Rowe 1996; Gardmark and Dieckmann 2006). In addition, some recent work has indicated that effects due to growth or resource availability may have contributed to observed trait diversification in nature (Hutchings 1993; Bronikowski and Arnold 1999; Bronikowski et al. 2002; Jennions and Telford 2002). Thus, changes in age/size-specific mortality, due to predation, as well as indirect changes in rates of growth and levels of food availability likely have evolutionary consequences.

Trinidadian killifish, *Rivulus hartii*, are located across a series of fish communities that differ in the impacts of ecological selective pressures such as predator-induced mortality, competition, and resource availability (Fraser and Gilliam 1992; Gilliam et al. 1993; Fraser et al. 1995, 1999, 2006; Walsh and Reznick 2008; see also Reznick and Endler 1982; Reznick and Bryga 1996). Progressing from lowland rivers to small headwater tributaries, *Rivulus* are commonly located in three fish communities: (1) “high predation” sites in which *Rivulus* coexist with numerous large piscivores, (2) “*Rivulus/guppy*” sites with guppies (*Poecilia reticulata*), and (3) “*Rivulus*-only” sites in which *Rivulus* are the

only fish species present. These communities are found within a close proximity to each other but are distinct because barrier waterfalls truncate the distribution of some species but not others. As a result, these communities contain similar physical habitats and do not differ in environmental variables such as water temperature or dissolved oxygen (Table 1). More importantly, there is evidence for direct and indirect effects of interactions with other species in both high predation and *Rivulus/guppy* communities. *Rivulus* suffer a 2× higher mortality rate per 60 days in high predation sites compared with *Rivulus*-only localities (Gilliam & Fraser, *personal communication*). In addition, *Rivulus* abundance dramatically decreases (7×) and rates of growth increase (2×) in high predation localities (Gilliam et al. 1993; Fraser et al. 1999). Because fish from these localities do not differ in growth rates when reared in the laboratory on controlled rations (see Walsh and Reznick 2008), faster growth in high predation sites are most likely due to higher levels of food availability that are possibly an indirect consequence of predation (Reznick et al. 2001).

In *Rivulus/guppy* sites, guppies alter the ecology of *Rivulus* and thereby the possibility of selection on the evolution of life histories. Field data show that the population density of *Rivulus* in *Rivulus/guppy* sites is only a half to a third of what it is in *Rivulus*-only localities (Gilliam et al. 1993). These differences in density are present even when guppies are included in the comparison of fish biomass in *Rivulus/guppy* and *Rivulus*-only localities (Gilliam et al. 1993). Because comparative mark–recapture studies demonstrate that adult *Rivulus* survival probabilities per 12 days differ little between sites with and without guppies

**Table 1.** Environmental variables among *Rivulus* communities. No significant differences (NS) ( $P < 0.05$ ) between community types were observed. Width and depth measurements are mean values.

Locality	Dissolved oxygen (%)	Temperature (°C)	Salinity (ppt)	pH	Width (m)	Depth (m)
Arima R.						
High predation	82.5	25.5	0.1	7.8	5.9	0.33
<i>Rivulus/guppy</i>	79.8	24.7	0.1	7.8	2.1	0.19
<i>Rivulus</i> only	77.9	24.6	0.1	7.7	1.7	0.34
Guanapo R.						
High predation	96	25.1	0.1	7.8	14.7	0.3
<i>Rivulus/guppy</i>	89.7	24.9	0.1	7.8	3	0.27
<i>Rivulus</i> only	87.3	25.3	0.1	7.8	2.6	0.23
Aripo R.						
High predation	87	25.7	0.2	8.3	13.5	0.42
<i>Rivulus/guppy</i>	89.6	25	0.1	7.8	3.6	0.39
<i>Rivulus</i> only	81.5	25	0.1	7.8	1.7	0.25
Turrence R.						
High predation	87.7	25.2	0.1	7.8	5.9	0.42
<i>Rivulus/guppy</i>	87.9	25	0.1	7.7	9.7	0.25
<i>Rivulus</i> only	87.9	24.8	0.1	7.8	6.1	0.19
Kruskal–Wallis (H):	1.75 <sup>NS</sup>	5.7 <sup>NS</sup>	2.0 <sup>NS</sup>	2.45 <sup>NS</sup>	4.81 <sup>NS</sup>	4.43 <sup>NS</sup>

(apparent survival  $\pm$  SE: *Rivulus*/guppy =  $0.79 \pm 0.071$  vs. *Rivulus*-only =  $0.75 \pm 0.042$ ; M.R. Walsh et al., unpubl. ms.), one possible explanation for the decline in abundance in sites with guppies is that a bottleneck exists during the early life history of *Rivulus* in *Rivulus*/guppy sites that limits recruitment to the adult stage. This is because prior work has demonstrated a role for guppies as predators and also competitors with very small *Rivulus*. Guppies readily prey upon larval *Rivulus*, but not *Rivulus* eggs in aquaria (Gilliam et al. 1993) and guppies increased *Rivulus* larval mortality via predation in field mesocosms (Fraser et al. unpubl. ms.). Furthermore, in field experiments guppies reduced the growth rate of an equal-sized *Rivulus* (~25 mm) to the same degree as a *Rivulus* (Gilliam et al. 1993), and such competition could increase juvenile mortality rates (see Werner and Gilliam 1984; Mittelbach 1988; Wilbur 1988; Bystrom et al. 1998). Irrespective of the mechanism, any increase in the mortality of very young/small individuals is predicted to be an important selective pressure on life-history evolution (Gadgil and Bossert 1970; Law 1979; Michod 1979; Charlesworth 1980; Taylor and Gabriel 1992; Gardmark and Dieckmann 2006).

The presence of guppies is also correlated with large changes in rates of individual growth. *Rivulus* grow at least  $2\times$  faster in sites with guppies than sites without them, even when such sites are only a few meters away (M.R. Walsh et al., unpubl. ms.). These differences reflect environmental sources because *Rivulus* transplanted from a *Rivulus*-only locality into a *Rivulus*/guppy site greatly accelerated their rate of growth to match the high growth of *Rivulus* that are resident to such localities (M. R. Walsh, D. F. Fraser, and D. N. Reznick, unpubl. ms.). This variation in growth between sites with and without guppies does not appear to be explained by higher nutrient excretion rates in guppies as such fluxes do not enhance food availability for *Rivulus* (Palkovacs et al. 2009), nor are the differences in growth due to *Rivulus* predation on guppies because such an interaction likely occurs infrequently (Fraser et al. 1999). Because the addition of guppies to field mesocosms causes a net reduction in *Rivulus* growth (Gilliam et al. 1993), guppies do not appear to indirectly facilitate higher rates of growth (see Bronmark et al. 1991) or induce physiological or behavioral changes that enhance growth (see Werner and Peacor 2003; Schmitz et al. 2004; Preisser et al. 2005). For these reasons, we favor higher levels of food availability that are a density-mediated indirect effect of interactions with guppies as the explanation for the faster rates of growth in *Rivulus*/guppy sites (Grether et al. 2001; Reznick et al. 2001). At this stage, non-lethal, trait-mediated phenotypic responses to guppies cannot be eliminated as a contributing factor to the differences in growth. Most importantly, such large changes in growth and likely resources also provide an additional link to evolution in *Rivulus* from sites with guppies.

In general, the empirical study of adaptation in nature requires a multifaceted approach encompassing several steps, including both field and laboratory experiments, that clearly identify the environmental variables that impose selection, as well as the evolutionary consequences of these selective pressures (Reznick and Travis 1996). In this article, we begin to evaluate the role of the direct and indirect effects of predators on evolution in *Rivulus* by examining correlations between fish community and life-history phenotypes across much of the range of *Rivulus* in Trinidad. Such an approach is important because it establishes the consistency of the link between fish community and life history across a very wide geographic area. In other papers, we build upon this study by reporting on intensive mark-recapture work that provides a detailed understanding of the ecology of *Rivulus*, common garden experiments that measure the genetic basis of trait variation among *Rivulus* communities (see Walsh and Reznick 2008), and, finally, experimental transplants of guppies that examine the cause and dynamics of evolution in *Rivulus* (Reznick et al. 1990, 1997). However, laboratory and experimental constraints limit the geographic scope of these latter approaches. Thus, the large-scale survey of trait divergence performed in the present study represents an important and logical first step in the study of adaptation of *Rivulus*.

Here, we examine phenotypic life-history diversification among all three *Rivulus* communities from five independent river drainages. Because *Rivulus* suffer higher mortality rates in high predation sites, theory predicts that *Rivulus* from these communities will exhibit a smaller size at maturation and have higher levels of reproductive effort than their counterparts from *Rivulus*-only communities (Gadgil and Bossert 1970; Law 1979; Charlesworth 1980; Taylor and Gabriel 1992; Ernande et al. 2004; Gardmark and Dieckmann 2006). In *Rivulus*/guppy communities, *Rivulus* may suffer increased rates of mortality targeted at very small/young individuals. Age/size-specific theory predicts that *Rivulus*/guppy fish will thus begin reproduction at a larger size and exhibit a decreased investment in reproduction when compared with *Rivulus*-only communities (Gadgil and Bossert 1970; Law 1979; Charlesworth 1980; Taylor and Gabriel 1992). Yet, other theories that more fully consider the inherent complexity of natural systems often yield different predictions. Several models predict that faster rates of growth and higher levels of food availability can favor the evolution of earlier maturation and increased reproductive allotment (Gadgil and Bossert 1970; Stearns and Koella 1986; Hutchings 1993; Abrams and Rowe 1996). Consequently, if differences in growth and resource availability are important selective pressures, then *Rivulus* from *Rivulus*/guppy and *Rivulus*-only may mature earlier and allocate more toward reproduction than *Rivulus* from *Rivulus*-only sites.

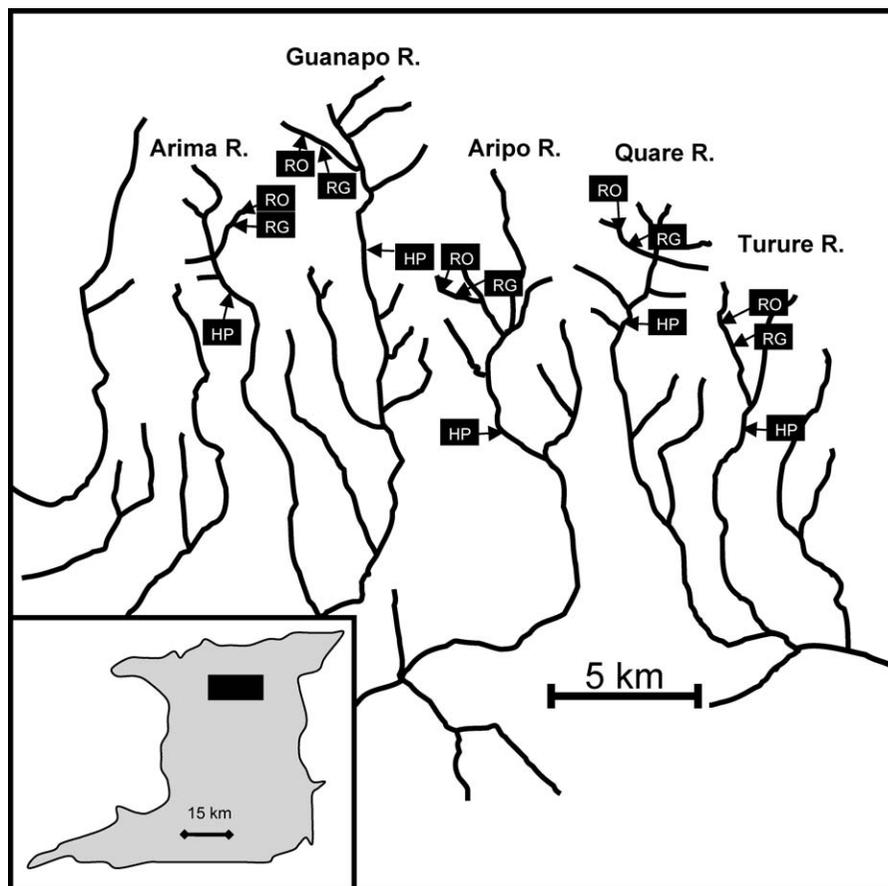
## Materials and Methods

*Rivulus hartii* are commonly found throughout the streams of Trinidad and adjacent islands. *Rivulus hartii* is an egg-laying species that reproduces via external fertilization (Fraser and Gilliam 1992; Gilliam et al. 1993). Reproduction in *Rivulus* is initiated by a male courtship dance. *Rivulus* will actively reproduce throughout the year in the field (Fraser and Gilliam 1992) and can often lay eggs every day in the laboratory (M. R. Walsh, pers. obs.). Males and females both exhibit indeterminate growth and can approach an asymptotic size of 90–100 mm.

### COLLECTION SITES

*Rivulus* were collected from five rivers along the South slope of the Northern Range Mountains of Trinidad (Fig. 1). These collections were made during January and early February 2007. The rivers sampled were the Arima, Guanapo, Aripo, Quare, and Turure. One of each *Rivulus* community type was collected from every river (high predation, *Rivulus*/guppy, *Rivulus*-only). Each high predation locality specifically contained a collection of potential predators including *Crenicichla alta*, *Hoplias malabaricus*,

*Aequidens pulcher*, *Astyanax bimaculatus*, and *Hemibrycon dentatum* (Reznick 1982; Gilliam et al. 1993; Fraser et al. 1999). Guppies and *Rivulus* were the only fish species present in *Rivulus*/guppy communities. Although, it is important to note that the catfish *Rhamdia sebae* was previously present near the area of collection of the *Rivulus*/guppy site in the Guanapo river (Gilliam et al. 1993), although they were not observed during the period of collection for the present study. The ecological role of *Rhamdia* is unknown, although an evaluation of stomach contents did not reveal any evidence for piscivory by *Rhamdia* on guppies or *Rivulus* (Gilliam et al. 1993). More importantly, *Rivulus* life-history phenotypes from this locality do not differ significantly from comparable *Rivulus*/guppy sites that lack *Rhamdia* in the Guanapo watershed (M.R. Walsh, unpubl. ms.). For each focal river, the *Rivulus*/guppy and *Rivulus*-only sites were located in tributaries to the main river (containing the high predation community) above barrier waterfalls that prevented the upstream movement of large predatory species. Distances between the collection of high predation and the sites further upstream generally exceeded 1 km, whereas the distances between *Rivulus*/guppy and *Rivulus*-only sites were generally less than 300 m (Fig. 1). We evaluated



**Figure 1.** Map of study sites in the Northern Range Mountains of Trinidad. HP, high predation; RG, *Rivulus*/guppy; RO, *Rivulus*-only. Locations of sampling sites are approximations.

potential differences in physical habitat and environmental variables among all sites except those from the Quare river and found no differences in stream width or depth, nor water temperature, pH, dissolved oxygen, or salinity among *Rivulus* communities (Table 1).

**CHARACTERIZATION OF LIFE-HISTORY PHENOTYPE**

At each site, small seines (<5 feet) were used to collect *Rivulus*. Immediately after collection, all *Rivulus* were euthanized and preserved in 5% formalin. The number of identifiable females in each collection ranged from 26 to 65 and all females from each sample were dissected (Table 2).

The following data were collected from each female: (1) total length, (2) number of mature oocytes, (3) wet weight of mature oocytes, (4) somatic dry weight (females total weight minus reproductive tissue and gastrointestinal tract), (5) total dry weight of reproductive tissue, and (6) “reproductive allotment” was measured as the percent of total dry weight that consists of eggs. Previous laboratory work has demonstrated that eggs greater than 1.65 mm in diameter are viable (Walsh and Reznick 2008) and therefore eggs greater than this size were considered mature. Generally, there was a very clear difference between immature and mature oocytes.

We evaluated the minimum size of reproduction in females using two similar approaches. We sorted all females into 2-mm size classes and determined the minimum size class in which the majority of females contained at least one egg. This criterion generally formed a clear division between smaller fish that did and did not contain mature oocytes and we then compared the minimum size class of gravid females among communities. For the second approach, we estimated the size at which 50% of females attained maturation ( $L_{mat}$ ) by fitting a logistic regression curve through a plot of the proportion of fish mature versus size for each population and subsequently compared the size in which 50% of females attained maturation among communities.

**STATISTICAL ANALYSES: UNIVARIATE APPROACHES**

The dependent variables that were evaluated included the minimum size of gravid females (and  $L_{mat}$ ), number of eggs per female, egg size, and two estimates of reproductive investment (described below). Because we only have one value per population for female size at maturation, we used a nonparametric Kruskal–Wallis test to compare differences across *Rivulus* communities. To evaluate differences in the remaining variables, we used general linear models with river (Arima, Guanapo, Aripo, Quare, and Turure) and *Rivulus* community (high predation, *Rivulus/guppy*,

**Table 2.** Least-square means (SE) for minimum size of gravid females, number of mature oocytes per female, egg weight, reproductive allotment, and reproductive tissue weight. Data for egg size are wet weight, while reproductive allotment and reproductive tissue weight are dry weights. These differences likely explain why multiplying the number of eggs with egg weight does not yield reproductive tissue weight.

Community (n females/n dissected)	Minimum size of gravid females (mm)	No. of eggs	Egg weight (mg)	Reproductive allotment (%)	Reproductive tissue weight (mg)
<b>Arima R.</b>					
High predation (13/31)	31	7.65 (1.2)	2.65 (0.17)	5.1 (0.6)	10.4 (1.4)
<i>Rivulus/guppy</i> (23/38)	35	4.65 (0.91)	3.31 (0.14)	3.4 (0.5)	6.6 (1.0)
<i>Rivulus</i> -only (10/50)	41	0.35 (1.39)	4.12 (0.21)	1.6 (0.7)	1.5 (1.6)
<b>Guanapo R.</b>					
High Predation (19/33)	31	9.39 (1.0)	2.6 (0.14)	6.7 (0.5)	11.3 (1.1)
<i>Rivulus/guppy</i> (41/64)	33	8.44 (0.68)	3.5 (0.12)	5.5 (0.4)	11.0 (0.8)
<i>Rivulus</i> -only (40/65)	37	6.41 (0.7)	3.81 (0.13)	4.5 (0.4)	9.8 (0.8)
<b>Aripo R.</b>					
High Predation (18/41)	33	8.84 (1.03)	2.42 (0.15)	5.0 (0.5)	9.2 (1.2)
<i>Rivulus/guppy</i> (26/33)	33	6.41 (0.86)	2.88 (0.13)	4.8 (0.5)	8.8 (1.0)
<i>Rivulus</i> -only (24/32)	35	6.41 (0.87)	3.66 (0.16)	4.2 (0.5)	8.1 (1.0)
<b>Quare R.</b>					
High Predation (9/37)	33	11.26 (1.37)	2.57 (0.21)	6.9 (0.8)	12.6 (1.6)
<i>Rivulus/guppy</i> (25/37)	31	7.07 (0.87)	3.8 (0.13)	5.2 (0.5)	8.9 (1.0)
<i>Rivulus</i> -only (23/43)	41	4.36 (0.93)	4.27 (0.13)	3.1 (0.5)	4.9 (1.1)
<b>Turure R.</b>					
High Predation (14/26)	35	4.41 (1.09)	3.39 (0.18)	3.8 (0.6)	9.5 (1.3)
<i>Rivulus/guppy</i> (22/36)	33	6.25 (0.88)	3.46 (0.14)	5.0 (0.5)	8.5 (1.1)
<i>Rivulus</i> -only (26/34)	33	5.44 (0.79)	3.78 (0.13)	3.5 (0.4)	7.4 (0.9)

or *Rivulus*-only) entered as fixed effects. We classified all as “fixed” effects because Sokal and Rohlf (1995) state that a fixed-effect analysis of variance (ANOVA) tests for differences among group means due to an added treatment component, although the treatment does need to be understood or manipulated by the experimenter, as long as it is repeatable (see Sokal and Rohlf 1995). The influence of different rivers and *Rivulus* communities is similar to this scenario because the nature of the fish community and the rivers from which our fish were sampled are repeatable features of our sampling design (see Walsh and Reznick 2008). Yet, given the ambiguity that exists in the designation between fixed and random effects, we also examined the degree to which our results change when river is entered as a random effect. Both designs yield very similar results (see Results: River effects).

We evaluated fish’s investment into reproduction in two ways. Using the statistical design described above, we first examined differences in “reproductive allotment.” However, such an analysis assumes that the relationship between body mass and reproductive allotment is isometric (Tomkins and Simmons 2002). Therefore, as recommended by Tomkins and Simmons (2002), we also used reproductive tissue dry mass as a dependent variable with female body size entered as a covariate in a second analysis. Both analyses yielded similar results (Table 3). We display the results for both analyses (see Table 3), yet we mostly report (in

the text and figures) on the differences in reproductive allotment because this parameter directly accounts for variation in female body size.

For the analyses of fecundity, reproductive allotment, and reproductive tissue weight, we only included fish that contained at least one egg. This eliminates potential biases due to differences among populations in the number of females from mature size classes that were actively reproducing. Furthermore, in all cases in which we obtained significant differences, we used a post hoc Fisher LSD test to evaluate differences among community types. Female size was included as a covariate for the egg size, fecundity, and reproductive dry mass analyses. All variables were examined for homogeneity of variances and homogeneity of slopes when a covariate was used. Logarithmic transformations were required for the analyses of reproductive dry weight and fecundity.

### MULTIVARIATE ANALYSES

We used a discriminant function analysis to determine how well populations can be separated and classified based upon fish community. The patterns of separation for female size at reproduction, number of eggs, egg size, and reproductive allotment were evaluated. Significant differences among *Rivulus* communities were examined by quantifying Mahalanobis distances between group centroids.

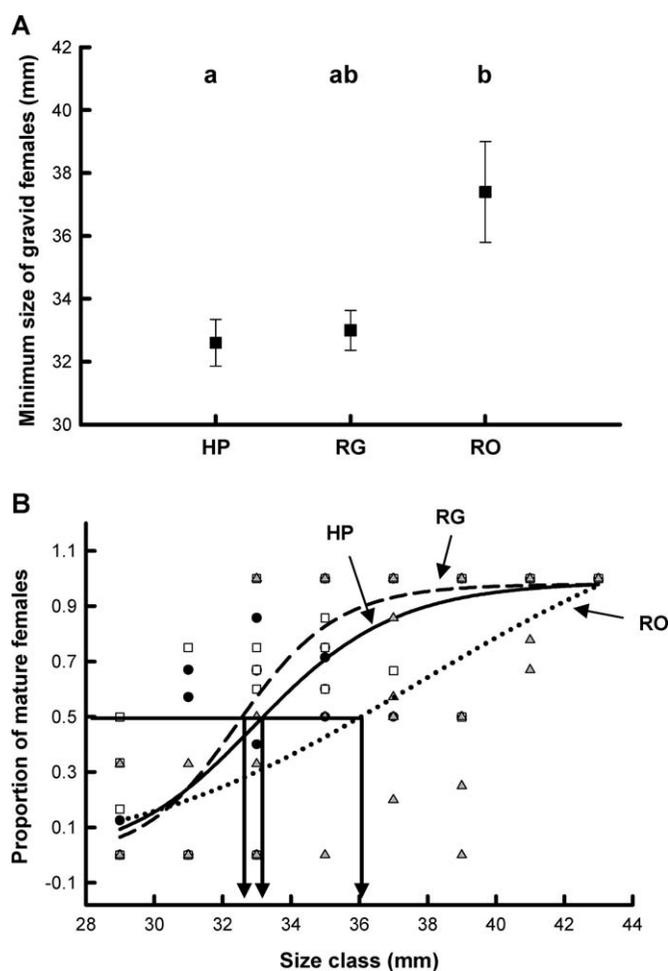
**Table 3.** Analyses of life-history traits for *Rivulus* communities. (A) Includes all populations from each of the five rivers. (B) Includes populations from all rivers except the Turure River (see Results for details). Female size at maturation was analyzed with a Kruskal-Wallis test, whereas all other traits used general linear models. Also, the egg size differences among fish communities are similar when body size is not included as a covariate ( $F_{2,282}=53.17$ ,  $P<0.001$ ; Mean Egg size $\pm$ 1 standard error: high predation= $2.73\text{ mg}\pm 0.09$ , *Rivulus/guppy*= $3.31\text{ mg}\pm 0.07$ , *Rivulus-only*= $3.98\text{ mg}\pm 0.08$ ).

	(df)	Female size at maturity (H)	No. of eggs (F)	Egg size (F)	Reproductive allotment (F)	Reproductive weight (F)
(A) All populations						
Covariate:						
Total Length	1		138.1***	77.1***		325.9***
Main effects:						
River	4	0.35 <sup>NS</sup>	10.43***	6.5***	9.38***	8.12***
Predator	2	6.3*	21.6***	60.5***	21.28***	24.13***
River $\times$ Predator	8		2.96**	2.74**	2.39*	2.49*
Error MS (df)			0.081 (318)	0.44 (281)	4.67 (319)	0.045 (318)
(B) Turure river removed						
Covariate:						
Total Length	1		98.17***	55.89***		236.57***
Main effects:						
River	3	1.03 <sup>NS</sup>	9.93***	7.09***	9.35***	9.46***
Predator	2	7.77*	19.82***	72.33***	19.92***	19.53***
River $\times$ Predator	6		1.98 <sup>NS</sup>	1.62 <sup>NS</sup>	1.58 <sup>NS</sup>	2.98**
Error MS (df)			.088 (258)	.392 (222)	5.26 (259)	.048 (258)

NS = not significant ( $P>0.05$ ); \*\*\* $P<0.001$ ; \*\* $P<0.01$ ; \*  $P<0.05$ .

## Results

*Rivulus* exhibit strong phenotypic differences among fish communities (Figs. 2 and 3). This generally includes extensive divergence in both *Rivulus/guppy* and high predation communities when compared with *Rivulus*-only sites. However, our results for one population were unusual. Differences in the high predation site from the Turure River contradicted with the patterns observed in our other focal rivers (Table 2), although the divergence between *Rivulus/guppy* and *Rivulus*-only from this same river was consistent with the trends observed across all other rivers (Table 2). This one, atypical population, out of a total of



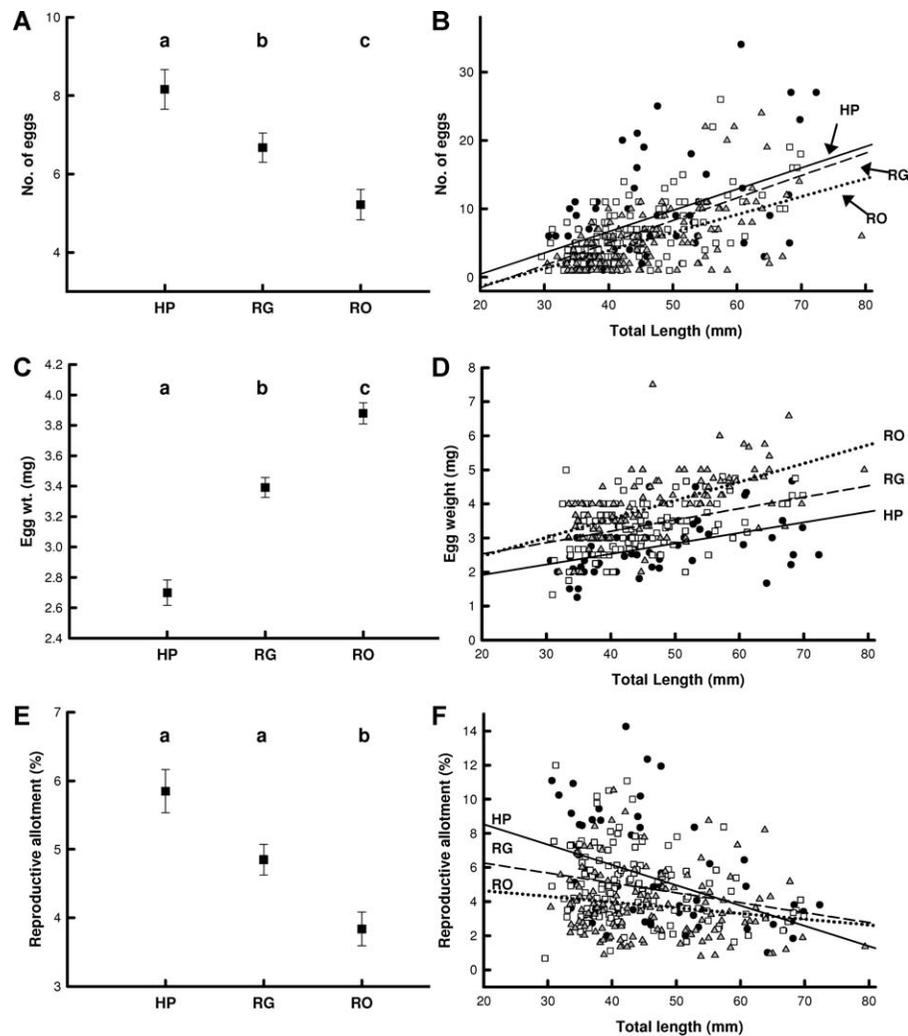
**Figure 2.** Differences in maturation size between fish communities. (A) Minimum size of gravid females, (B) Size in which 50% of the females attained maturation ( $L_{mat}$ ). In each graph, HP, high predation; RG, *Rivulus/guppy*; RO, *Rivulus*-only. Closed circles, solid regression line – HP; open squares, dashed regression line – RG; gray triangles, dotted regression line – RO. Error bars =  $\pm 1$  SE. Significant differences ( $P < 0.05$ ) were found in (A), but not (B). See the text (Results: Predator effects) about differences between these results. The letters across the top of Figure 2A correspond to the results of the post hoc Fisher LSD tests.

15, caused significant fish community by river interactions for fecundity, egg size, reproductive allotment, and reproductive tissue weight (Table 3A). This is because the removal of the Turure river populations from the overall analysis eliminates all of the significant interactions save one (see Table 3B), but does not alter our main conclusions as the significance of the main effects and the comparisons among communities and rivers were equivalent in both analyses (Table 3). The only other result that changed when the Turure river populations were removed was that the post hoc differences in reproductive allotment between high predation and *Rivulus/guppy* sites went from significant ( $P < 0.05$ ) to marginally nonsignificant ( $P = 0.06$ ). Thus, trends in the remaining four rivers, as well as all populations but one, were consistent. Below we examine the differences among fish communities and rivers based upon the entire dataset. We also explore the atypical results for the Turure river in a separate analysis (see Results: Turure River Effects) as well as in the discussion (see High Predation Anomaly).

### PREDATOR EFFECTS

*Rivulus* from high predation sites were characterized by having a smaller minimum size at reproduction, producing a greater number of eggs that are smaller in size, and having a greater reproductive allotment than *Rivulus* from *Rivulus*-only communities (Tables 2 and 3; Figs. 2 and 3). All of these differences were significant ( $P < 0.05$ ) and the magnitude of the divergence was particularly dramatic (note: the differences in size at maturation were not significant when the 50% maturation values from a logistic regression were used; see below). *Rivulus* from high predation localities exhibited a 13% reduction in the minimum size of gravid females and they had a reproductive allotment that was 38% greater than *Rivulus*-only fish (Figs. 2 and 3). This increase in reproductive allotment in high predation *Rivulus* was characterized by a 48% increase in the number of eggs per female and a 31% reduction in egg weight. The analyses of reproductive allotment and reproductive dry mass yielded similar significant trends (Table 3).

*Rivulus/guppy* phenotypes were intermediate between high predation and *Rivulus*-only females. Compared with *Rivulus*-only sites, *Rivulus/guppy* female phenotypes were characterized by a smaller minimum size of gravid females, increased reproductive allotment, a greater number of eggs, and a smaller egg size (Tables 2 and 3; Figs. 2 and 3). A posteriori comparisons revealed that all of these trait differences were significant ( $P < 0.05$ ) except for minimum size at reproduction. *Rivulus/guppy* fish exhibited a reproductive allotment that was 29% greater than *Rivulus*-only sites. This pattern of reproductive investment in *Rivulus/guppy* fish is characterized by a 32% increase in the number of eggs and a concomitant 13% reduction in egg size compared with fish from *Rivulus*-only sites (Fig. 3A–D).



**Figure 3.** Differences in reproductive traits among *Rivulus* communities. (A, B) – Fecundity, (C, D) – Egg weight, (E, F) – Reproductive allotment. In each graph, HP, high predation; RG, *Rivulus/guppy*; RO, *Rivulus* only. Closed circles, solid regression line – HP; open squares, dashed regression line – RG; gray triangles, dotted regression line – RO. Error bars =  $\pm 1$  SE. Significant differences among communities ( $P < 0.05$ ) were found for all traits. The figures in the left column (graphs A, C, E) represent least-square means from all five river drainages, while the right column (graphs B, D, F) show the raw data. The letters across the top of Figure 3A, C, and E correspond to the results of the post hoc comparisons.

Differences between high predation and *Rivulus/guppy* communities were less remarkable than either of the previous two comparisons. Females from high predation sites contained a greater number of eggs that were smaller and they exhibited a higher reproductive allotment (Fig. 3), although there was little difference in the initial size at first reproduction between high predation and *Rivulus/guppy* communities (Fig. 2). The differences in fecundity and egg size were significant as high predation females exhibited a 13% increase in reproductive allotment, 24% higher fecundity, and a 20% decrease in egg size compared with *Rivulus/guppy* phenotypes.

Our two methods for estimating maturation size yielded slightly different results (Fig. 2A, B). Using the minimum size class in which the majority of females were actively reproduc-

ing yielded significant differences between fish communities and high predation and *Rivulus/guppy* females commenced reproduction at a smaller size than *Rivulus*-only fish (only the differences between high predation and *Rivulus*-only fish were significant,  $P < 0.05$ ). Using the size in which 50% of females attained maturation as measured from a logistic regression curve, the divergence among fish communities was similar, yet the differences were reduced and therefore not significant ( $H_{2,15} = 1.46$ ,  $P = 0.48$ , see Fig. 2A,B).

#### RIVER EFFECTS

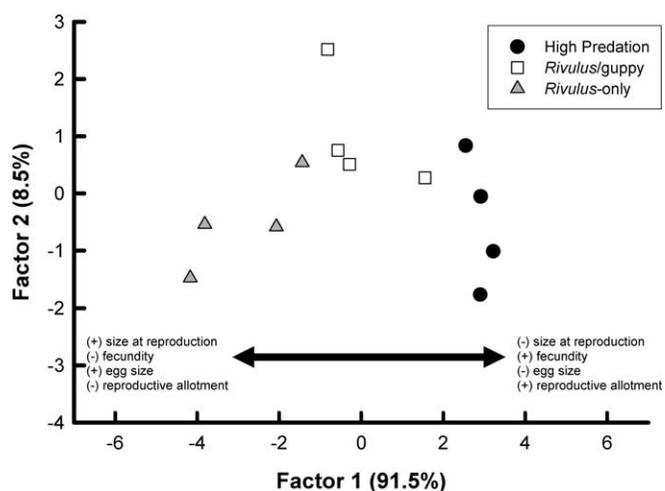
There were highly significant differences among rivers for all traits except the minimum size of reproduction (Table 4). These significant differences were due to the influence of specific rivers,

**Table 4.** Least-square means (SE) for life-history traits from each river.

River	Minimum size of gravid females (mm)	No. of mature oocytes	Egg weight (mg)	Reproductive allotment (%)	Reproductive tissue weight (mg)
Arima	35.67 (2.9)	3.69 (0.6)	3.27 (0.12)	3.17 (0.32)	5.3 (0.67)
Guanapo	33.67 (1.8)	7.68 (0.4)	3.39 (0.09)	5.37 (0.23)	10.5 (0.48)
Aripo	33.67 (0.7)	6.68 (0.5)	2.94 (0.11)	4.71 (0.28)	8.4 (0.59)
Quare	35 (3.1)	6.97 (0.6)	3.77 (0.11)	4.65 (0.3)	8.28 (0.64)
Turure	33.67 (0.7)	5.31 (0.5)	3.58 (0.1)	4.06 (0.29)	7.35 (0.6)

often the Arima River, which exhibited lower trait values (see Table 4). For example, *Rivulus* from the Arima River contained 43% fewer eggs and displayed a reproductive allotment that is 32% less than the average value across all rivers, and post hoc comparisons show that such patterns differed significantly from all other rivers ( $P < 0.05$ ). Differences in egg size among rivers were less remarkable. *Rivulus* from the Aripo River contained eggs that were 13% smaller than the other rivers (Table 4), whereas females from the Quare River produced eggs that were 12% larger than the grand mean. Both of these changes in egg size were significantly different from all other rivers ( $P < 0.05$ ).

All of the above results treated river as a fixed effect. The results are similar when river is entered as a random effect. The main difference is that variation in egg size and reproductive tissue weight across rivers is no longer significant. All other results, including all of the differences across *Rivulus* communities, remained significantly different.



**Figure 4.** Bivariate comparison between discriminant functions 1 and 2 when populations are delineated by fish community. Closed circles, high predation; open squares, *Rivulus/guppy*; gray triangles, *Rivulus* only. Factor 1 separated fish characterized by a small size at maturation, a high fecundity, small egg size, and large reproductive allotment and those that have low fecundity, large eggs, low reproductive allotment, and a large size at reproduction. The Turure River populations were not included in this analysis.

**MULTIVARIATE ANALYSES**

The results of the discriminant function analysis bolstered the trends revealed by the univariate analyses. Using predator community as a grouping variable, the first component distinguished among fish communities (Fig. 4) as only two populations were misclassified into the incorrect community type (a 16.7% misclassification rate). The first discriminant function accounted for 91.5% of the variance and the factor structure coefficients demonstrate that this factor differentiates between fish characterized by a small size at maturation, a high fecundity, small egg size, and large reproductive allotment and those that have low fecundity, large eggs, low reproductive allotment, and a large size at reproduction (Table 5). The second discriminant function accounted for only 8.5% of the variance and generally separated fish characterized by a small size at first reproduction and large egg size versus those with a large size at first reproduction and small egg size (Table 5). An analysis of the distances among groups demonstrated that *Rivulus*-only populations were significantly different from high predation communities ( $F_{4,6} = 11.08, P = 0.006$ ). The differences between *Rivulus*-only and *Rivulus/guppy*

**Table 5.** Results of the discriminant function analysis with predator community as the grouping variable and minimum size at reproduction, fecundity, egg size, and reproductive allotment included as dependent variables. The results shown are the standardized (canonical) coefficients and the factor structure coefficients. Factor structure coefficients are the bivariate correlation between a population's value for a dependent variable and the population's discriminant function score and are thus important to characterizing the contribution of each dependent variable.

Trait	Standardized coefficients		Factor structure coefficients	
	DF1	DF2	DF1	DF2
Size at reproduction	-0.151	-1.30	-0.540	-0.727
Fecundity	-0.002	-0.364	0.410	-0.050
Egg size	-0.894	0.428	-0.970	0.131
Reproductive allotment	0.133	-0.348	0.394	0.056

communities ( $F_{4,6} = 3.47$ ,  $P = 0.085$ ), and *Rivulus*/guppy and high predation populations ( $F_{4,6} = 3.61$ ,  $P = 0.079$ ) were both marginally nonsignificant.

### TURURE RIVER EFFECTS

In the Turure river, there were no significant differences among the three populations in fecundity ( $F_{2,59} = 2.75$ ,  $P = 0.072$ ), egg size ( $F_{2,60} = 1.85$ ,  $P = 0.17$ ), and reproductive tissue weight ( $F_{2,59} = 0.89$ ,  $P = 0.41$ ), although differences in reproductive allotment were significant ( $F_{2,58} = 6.54$ ,  $P = 0.003$ ). The lack of significant differences between populations in this river was due to unusual trait values in the high predation site. *Rivulus*/guppy females exhibited the highest fecundity and reproductive allotment, whereas high predation females actually displayed the lowest fecundity and reproductive allotment in this river (Table 2). This is in stark contrast to the very consistent pattern observed in the other four drainages. In the other four rivers, high predation females exhibited the highest fecundity, smallest egg size, largest reproductive allotment, and reproductive tissue weight (Table 2). *Rivulus*-only females were located at the opposite end of the spectrum and *Rivulus*/guppy fish were intermediate. Such trends were qualitatively apparent for all female traits across all rivers save the lack of fecundity differences between *Rivulus*/guppy and *Rivulus*-only communities in the Aripo river (Table 2). More importantly, the pattern and magnitude of divergence between the *Rivulus*/guppy and *Rivulus*-only communities from the Turure river were qualitatively similar to those witnessed in our other focal drainages. Thus, it is the only high predation community from the Turure river that deviates from the patterns seen in the other four drainages. We briefly explore this locality in the discussion (see Discussion: High Predation Anomaly).

## Discussion

*Rivulus* exhibit compelling life-history diversification across a gradient in fish communities that differ in predators, competitors, and likely food availability (Figs. 2 and 3). This includes extensive divergence in both high predation and *Rivulus*/guppy sites when compared with localities in which *Rivulus* are the only fish present. In agreement with the predictions of age- and size-specific life-history theory (Gadgil and Bossert 1970; Law 1979; Michod 1979; Charlesworth 1980; Taylor and Gabriel 1992; Ernande et al. 2004; Gardmark and Dieckmann 2006), much prior work on guppies in Trinidad (Reznick 1982; Reznick and Endler 1982; Reznick and Bryga 1996) and other species (Bronikowski and Arnold 1999; Johnson 2001; Johnson and Belk 2001; Jennions and Telford 2002), *Rivulus* from sites with large predators are characterized by a significantly smaller size at the onset of reproduction, a greater number of eggs that are smaller in size, and an increased allocation toward reproduction than sites with just *Rivulus* (Figs. 2 and 3, see also Fraser et al. 1999). Because *Rivulus*

suffer increased mortality in high predation sites (D.F. Fraser and J.F. Gilliam unpubl. ms.), such divergence is consistent with a response to the direct effect of predator-induced mortality. This notion is bolstered by the results of a common garden experiment that evaluated life-history differences between high predation and *Rivulus*-only communities across two rivers after two generations of laboratory rearing (Walsh and Reznick 2008). Such work demonstrated that the geographically widespread phenotypic differences measured in the present study have a strong genetic basis. However, Walsh and Reznick (2008) also showed that food may be an important factor in the evolution of *Rivulus*. By rearing all fish on multiple controlled levels of food availability that approximate the natural differences in growth between high predation and *Rivulus*-only environments, this work demonstrated that the magnitude of the trait divergence between these localities depended upon the ration the fish received; strong life-history differences were observed on a food level that approximated growth in a high predation environment, although such differences were reduced or disappeared entirely on a ration that sustained a growth rate typical of a *Rivulus*-only environment. Such fitness  $\times$  food level interactions suggest that *Rivulus* are adapted to contrasting levels of resource availability (Stearns and Koella 1986) and thus the higher levels of food availability observed in high predation sites, which are likely an indirect effect of predators, may also contribute to the phenotypic differences measured in the present study.

The comparisons between *Rivulus* from *Rivulus*/guppy versus *Rivulus*-only sites are incompatible with the predictions of age- and size-specific life-history theory. Evidence indicates that *Rivulus* experience a bottleneck during their early life history in *Rivulus*/guppy sites that is likely mediated by predatory and/or competitive interactions with guppies. Life-history theory predicts that increases in mortality on small or young individuals favor the evolution of delayed maturation at a larger size, and decreased reproductive effort (Gadgil and Bossert 1970; Schaffer 1974; Law 1979; Michod 1979; Charlesworth 1980). Yet, *Rivulus* from sites with guppies exhibit a smaller minimum size at reproduction, a greater fecundity, a smaller egg size, and increased reproductive allotment than *Rivulus*-only communities (Figs. 2 and 3). Because *Rivulus*/guppy sites exhibit lower densities and faster rates of growth than *Rivulus*-only sites (Gilliam et al. 1993; M.R. Walsh et al., unpubl. ms.), predictions from evolutionary theories that consider the ecology of target organisms may therefore help to explain the trends revealed between these fish communities. First, a few models predict that faster rates of growth and/or higher levels of food availability select for earlier maturation and increased investment in reproduction (Gadgil and Bossert 1970; Stearns and Koella 1986; Roff 1992; Stearns 1992; Hutchings 1993; but see Kozłowski and Uchmanski 1987; Kozłowski and Wiegert 1987). Second, Abrams and Rowe (1996) specifically

evaluated the relative importance of the direct and indirect effects of predators on the evolution of age at maturation. They found that if an indirect effect of predators is to reduce population density and increase per capita food availability, then this indirect effect can offset the direct impact of predators. Increases in food availability can select for earlier maturation even when the opposite trend is predicted in response to increases in mortality on small or young individuals. Finally, Gardmark and Dieckmann (2006) examined the influence of size-specific mortality on the evolution of size at maturation. This framework incorporated ecological processes as well as biological attributes and showed that a smaller size at maturation can evolve when mortality is targeted at small individuals, mortality decreases with size, fecundity increases with size, and density regulation is manifested during the juvenile stage. These biological attributes are common in fish, including *Rivulus*. Most importantly, all of these theories yield predictions that are, at least in part, consistent with the life-history differences observed in the present study and may therefore be applicable to the changes observed in *Rivulus/guppy* sites. Thus, the divergence between *Rivulus/guppy* and *Rivulus*-only sites is better explained by theories that consider common ecological factors such as differences in growth, population density, and food availability that are a hypothesized indirect effect of predation and/or competition.

We recently evaluated the genetic basis of life-history differences between *Rivulus* derived from localities with and without guppies by rearing multiple populations through two generations in a common environment (M.R. Walsh and D.N. Reznick in review). As in our previous study (Walsh and Reznick 2008), we reared all populations on two food levels that approximated the natural differences in growth between *Rivulus/guppy* and *Rivulus*-only localities and measured interactions between fitness and food treatment. When *Rivulus* were reared on a ration that sustained a growth rate typical of *Rivulus/guppy* communities, *Rivulus* from such sites displayed a similar pattern of divergence as observed in the present study (i.e., smaller maturation, higher fecundity, smaller egg size), which shows that the phenotypic divergence observed in the present study has a genetic basis. However, the differences between fish communities in the common garden experiment depended strongly upon the ration that the fish received; significant interactions between fitness and food level were observed for many life-history traits. These interactions, in conjunction with the patterns of divergence between *Rivulus/guppy* and *Rivulus*-only sites, argue that differences in growth and likely resource availability are important factors in the evolution of *Rivulus*.

Resource availability has long been assumed to be a potent ecological force on the evolution of life histories (Lack 1954, 1968). Yet, evidence for a connection between food availability and resultant evolutionary change in natural populations is rare

(but see Walsh and Reznick 2008). This is important because the phenotypic differences measured between *Rivulus/guppy* and *Rivulus*-only communities indicate that changes in growth and likely food availability, which appears to be an indirect consequence of interactions with guppies, as a cause of selection. The subsequent common garden experiment established the genetic basis of trait differences the different fish communities, although feasibility and physical constraints limit the number of drainages that can be evaluated to just two rivers. Thus, the virtue of the current study is that we were able to evaluate life-history phenotypes across a much larger number of rivers, the results of which help to guide subsequent experimental research.

#### HIGH PREDATION ANOMALY

Phenotypes of high predation females from the Turure river were inconsistent with the trends observed in the other rivers (Table 2). For instance, high predation females exhibited the smallest egg size as well as the lowest fecundity and reproductive allotment in this river. In contrast, divergence between high predation sites and the upstream communities in the Arima, Guanapo, Aripo, and Quare rivers were very consistent, as were the differences between *Rivulus/guppy* and *Rivulus*-only sites across all five focal rivers (see Table 2). Based upon the very consistent trends in 14 of 15 populations in the current study, and much previous work, spanning a diverse array of taxa, demonstrating a highly repeatable, almost universal evolutionary response to predator-induced mortality targeted at large/old individuals (Lynch 1980; Reznick 1982, 1990; Wellborn 1994; Martin and Clobert 1996; Sparkes 1996; Johnson 2001; Jennions and Telford 2002), the trait values of the high predation Turure river population are unusual and currently lack an explanation. A plausible explanation is that rates of gene flow from *Rivulus/guppy* to high predation localities are higher in this river. Future work needs to evaluate the causes and consistency of this atypical result.

### Conclusions

It has long been proposed that adaptation in nature represent responses to multiple agents of selection (Ashmole 1963; Reznick and Travis 1996). In support of this view, here we have demonstrated extensive phenotypic life-history diversification across a gradient in predation and competition that indicates an important role for predators and resource availability as selective pressures. Trait divergence in high predation sites is consistent with the predictions of theory that consider the direct effects of predation (Gadgil and Bossert 1970; Law 1979; Charlesworth 1980; Taylor and Gabriel 1992; Ermande et al. 2004; Gardmark and Dieckmann 2006), whereas the differences between *Rivulus/guppy* and *Rivulus*-only communities are consistent with a response to faster rates of growth and higher levels of food availability in *Rivulus/guppy* sites that are a hypothesized indirect consequence of interactions

with guppies (Abrams and Rowe 1996; see also Gardmark and Dieckmann 2006). These latter results are important because indirect effects have received extensive attention from an ecological perspective (Wootton 1994; Werner and Peacor 2003; Miner et al. 2005) and here we show that such effects may also be an important mechanism of evolutionary change in nature.

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