

# EXPERIMENTALLY INDUCED LIFE-HISTORY EVOLUTION IN A KILLIFISH IN RESPONSE TO THE INTRODUCTION OF GUPPIES

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Received April 8, 2010

Accepted October 26, 2010

Life-history theory predicts that increased predation on juvenile age/size-classes favors delayed maturation and decreased reproductive investment. Although this theory has received correlative support, experimental tests in nature are rare. In 1976 and 1981, guppies (*Poecilia reticulata*) were transplanted into localities that previously only contained a killifish, *Rivulus hartii*. This situation presents an opportunity to experimentally test this life-history prediction because guppies prey upon young *Rivulus*. We evaluated the response to selection in *Rivulus* by measuring phenotypic and genotypic divergence between introduction and upstream “control” localities that lack guppies. Contrary to expectations, *Rivulus* from the introduction sites evolved earlier maturation and increased reproductive investment within 25 years. Such evolutionary changes parallel previous investigations on natural communities of *Rivulus*, but do not comply with predictions of age/size-specific theory. Guppies also caused reduced densities and increased growth rates of *Rivulus*, which are hypothesized indirect effects of predation. Additional life-history theories show that changes in density and growth can interact with predator-induced mortality to alter the predicted trajectory of evolution. We discuss how these latter frameworks improve the fit between theory and evolution in *Rivulus*.

**KEY WORDS:** Indirect effects, life-history evolution, predator-prey, *Rivulus*.

Ecological factors, such as predation, that alter mortality rates can exert strong selection on the evolution of life histories (Roff 1992; Stearns 1992). Many treatments of life-history theory evaluate how changes in rates of extrinsic mortality alter the costs of reproduction and thereby the timing of maturation and reproductive effort. Age- and size-specific life-history theory predicts that increased predation on adults or large individuals favors the evolution of earlier maturation and increased reproductive effort, whereas the opposite predictions are made when mortality is increased in young or small individuals (Gadgil and Bossert 1970; Law 1979; Michod 1979; Charlesworth 1980; Taylor and Gabriel 1992; Ernande et al. 2004; Gardmark and Dieckmann 2006). Although these life-history theories have received much correlative empirical support (Reznick and Endler 1982; Roff 1992; Wellborn 1994; Martin and Clobert 1996; Sparkes 1996; Johnson

2001; Johnson and Belk 2001; Jennions and Telford 2002; Walsh and Reznick 2009), experimental tests of this theory, particularly in a natural setting, are far less common (see Reznick et al. 1990, 1997).

In 1976 and 1981, guppies (*Poecilia reticulata*) were experimentally transplanted from sites in which they co-occurred with large predators to localities upstream, above barrier waterfalls, which only contained a killifish, *Rivulus hartii* (Reznick et al. 1990, 1997). Waterfalls further upstream in each introduction site limited the upstream dispersal of guppies and hence created an experimental *Rivulus* population that was exposed to introduced guppies (“guppy-introduction” sites) and an additional population upstream, above a second barrier waterfall, from which guppies were excluded (“guppy-absent control” sites). These introduction and control sites are located within tens of meters of one another.

We have previously shown, in a series of paired comparisons of naturally occurring sites with and without guppies, that the presence or absence of guppies is not associated with any correlated differences in physical habitat or environmental variables such as water temperature, pH, or dissolved oxygen (Walsh and Reznick 2009).

The introduction of guppies has the potential to impose selection on *Rivulus* because guppies prey upon small *Rivulus* (<15 mm) (Gilliam et al. 1993; D. F. Fraser et al., unpubl. ms.). Theory predicts that such predation should select for the evolution of delayed maturity and reduced reproductive effort (e.g., Law 1979; Charlesworth 1980). However, the presence of guppies is correlated with changes to the population biology of *Rivulus*. Comparisons between guppy-introduction and guppy-absent control sites in one experimental stream show that guppy introductions are associated with a 42% decline in *Rivulus* density (Mean number of fish/m<sup>2</sup>: Introduction =  $1.33 \pm 0.37$ , Control =  $2.31 \pm 0.37$ ) and a threefold increase in rates of growth (Mean instantaneous growth in grams per day: Introduction =  $0.0029 \pm 0.0003$ , Control =  $0.0009 \pm 0.0003$ ; Walsh et al., in press). Such differences in density and growth between sites with and without guppies mimic those observed in naturally occurring populations of *Rivulus* (Gilliam et al. 1993; Walsh et al., in press).

The differences in *Rivulus* densities and individual growth rates have the potential to modify how *Rivulus* evolves in response to the introduction of guppies. Age/size-specific life-history theory, in its most basic form, evaluates the impacts of increased mortality independently of the ecological consequences of predation. Yet, the indirect consequences of predators, which can include reduced prey population densities, increased per capita resource availability, and increased individual growth rates (Wootton 1994), may modify the evolutionary consequences of increased juvenile mortality rates. Predictions from theoretical frameworks that consider changes in mortality in conjunction with such added ecological complexity can yield evolutionary predictions that are different from theory that models the consequences of changes in mortality rate alone (Gadgil and Bossert 1970; Michod 1979; Charlesworth 1980; Stearns and Koella 1986; Kozłowski and Wiegert 1987; Kozłowski and Uchmanski 1987; Hutchings 1993; Mylius and Diekmann 1995; Abrams and Rowe 1996; Gardmark and Dieckmann 2006).

An alternative body of theory partitions the life history into discrete stages, rather than being based on age or size. Stage-structured life-history theory evaluates the selective impacts of changes in mortality rates in organisms that undergo ontogenetic niche shifts between juvenile and adult stages, such as amphibians and insects (Roff 1981; Kusano 1982; Werner and Gilliam 1984). These models also predict that increased predation causes the evolution of changes to the timing of developmental thresholds, although the specific predictions can differ between age- and

size-structured models (Roff 1992). For example, stage-structured models predict that increased predation on juveniles favor more rapid development (i.e., earlier metamorphosis/emergence) because metamorphosis enables the organism to escape to a different environment where risk of predation is assumed to be diminished. Theory that considers the impact of predation on specific age or size-classes, as opposed to specific stages (juvenile vs. adult), is the most appropriate body of theory for *Rivulus* because *Rivulus* does not undergo a habitat shift with metamorphosis or the attainment of maturity.

Here, we measure phenotypic divergence between wild-caught *Rivulus* from the guppy-introduction and guppy-absent sites as well as genetically based differences after two generations of common garden rearing. In our common garden experiment, we evaluated the expression of life-history traits across two levels of food availability that we have previously shown match the differences in growth rate between introduction and control localities (Walsh and Reznick 2010). Age/size-specific theory predicts that guppy predation on young *Rivulus* favors the evolution of an older age at maturation and a decreased reproductive effort in *Rivulus* from the guppy-introduction sites compared with *Rivulus* from the guppy-free control localities. A failure to observe such patterns of divergence would indicate that the interaction between guppies and *Rivulus* is more complex and that a consideration of theories that incorporate variation in population density and growth rates may be needed to better explain evolution in *Rivulus*.

## Materials and Methods

### GUPPY INTRODUCTION

In 1976, 200 guppies were introduced from an area of the Aripo River in which they co-occur with several large predators into a tributary that previously contained just *Rivulus*. A similar introduction occurred in the El Cedro River in 1981, although only 100 guppies were transplanted (Reznick et al. 1990, 1997). The introduction sites are always downstream of the guppy-absent control sites, by necessity, because guppies would naturally colonize all sites downstream of their introduction.

### PHENOTYPIC TRAIT EVALUATION

*Rivulus* were collected from the guppy-introduction and upstream guppy-absent, control localities in the Aripo and El Cedro Rivers in January 2007. At each site, small seines were used to collect *Rivulus* and all *Rivulus* were euthanized shortly after collection and preserved in 5% formalin.

We quantified the number of eggs per female, egg size, and “reproductive allotment” (total reproductive tissue wt./somatic body wt.) for fish from the introduction and control sites. As a proxy for age/size at maturation, we estimated the size at which

50% of females attained maturation ( $L_{\text{mat}}$ ) by fitting a logistic regression curve through a plot of the proportion of fish mature versus size for each population. We compared the size at which 50% of females attained maturation among communities via a Kruskal–Wallis test. For the remaining traits, general linear models were used with river entered as a fixed blocking factor and fish community entered as a fixed effect. We first analyzed data with a multivariate analysis of variance and subsequently analyzed each dependent variable separately (Scheiner 1993). In the multivariate analysis of variance (MANOVA), we included river and fish community as fixed effects and included data for egg number, egg size, and reproductive allotment. Normality was evaluated with a Kolmogorov–Smirnov test, whereas homogeneity of variances was examined with Levene’s test. The data for fecundity and egg size were log transformed to improve fits with normality, whereas reproductive allotment was log transformed to improve homogeneity of variances. Female size was included as a covariate for egg size and fecundity.

#### PHENOTYPIC COMPARISONS BETWEEN EXPERIMENTAL STREAMS AND NATURAL POPULATIONS

Because we did not examine the life histories of *Rivulus* prior to the introduction of guppies, any differences that are found between *Rivulus* from introduction and control localities do not define which population may have evolved after the initiation of the perturbation experiments; that is, it is unclear if the guppy-introduction sites diverge from the guppy-absent control sites or vice versa. To evaluate the extent to which *Rivulus* from the guppy-introduction sites have evolved in response to the introduction of guppies, we compared phenotypic trait values of guppy-introduction and guppy-absent control sites with those of a series of natural populations where *Rivulus* were either found by themselves (*Rivulus*-only) or with guppies (*Rivulus*/guppy). These comparative data were derived from a recent study in which wild-caught fish were collected from localities with and without guppies across five rivers (Walsh and Reznick 2009). In some instances, multiple populations were located within the same drainage and overall six *Rivulus*/guppy and eight *Rivulus*-only populations were included in this analysis. We used two complementary approaches: First, we performed a discriminant function analysis using the data from the natural populations of *Rivulus* with fish community as a grouping variable. We then classified *Rivulus* from the introduction sites using this axis created from the natural populations. For this approach, we included data on fecundity, egg size, and reproductive allotment. All variables were log-transformed and we used size-corrected residuals for fecundity and egg size. If the differences between sites with and without guppies are similar for both naturally occurring populations as well as the introduction sites, then we expect that the

natural population discriminant function should be equally effective at classifying *Rivulus* from the guppy-introduction streams.

Second, we compared the trait values for fecundity, egg size, and reproductive allotment between naturally occurring populations and guppy-introduction localities using a series of one-way ANOVAs that included all sites described above. We compared the least square means among populations generated by the general linear model procedure. We then applied Bonferroni corrections for multiple comparisons. For each trait we compared: (1) differences between guppy-introduction sites and naturally occurring sites that contain *Rivulus* and guppies, and (2) differences between guppy-introduction sites with naturally occurring sites that contained just *Rivulus*. The same procedure was performed for the guppy-absent control communities. Four tests were performed for each trait and any difference with a  $P$ -value  $< 0.0125$  was considered significant.

#### COMMON GARDEN EXPERIMENT

A common garden experiment on second-generation, laboratory-born fish was performed to evaluate genetic divergence between guppy-introduction and guppy-absent control sites. All procedures follow prior published methodology (Walsh and Reznick 2008, 2010). Laboratory stocks were established from approximately 20 wild-caught males and females from each locality. Fish from the El Cedro River were collected in July 2005, whereas the Aripo River fish were collected in January 2007. Both experiments were performed in the same laboratory under the same conditions and all feedings and maintenance were performed by MRW to minimize temporally based differences. Each wild-caught female was placed in a 9-L aquarium and paired with a male from the same locality. In both the wild-caught parental generation and the first laboratory-reared generation, all pairings produced viable offspring. Eggs were collected from each pairing for approximately 20 days and reared in Petri dishes containing a methylene blue solution to reduce the prevalence of fungal infection. Upon hatching, larvae from each pairing were placed in 9-L aquaria at a density of 8–10 fish per tank and were reared on an ad libitum diet of Brine shrimp nauplii and liver paste. At an age of approximately 50 days, the sexes can be differentiated, and the density of each tank was reduced to two to four fish per tank (usually an equal number of males and females). These fish remained in these conditions until maturation.

The second generation of fish was generated by randomly arranging six to seven pairings between males and females within each of our four populations. For each pairing, we mated one female with a male from a different lineage to avoid sib matings (see Supplemental Figure in Walsh and Reznick 2010, for mating design schematic). Most of these pairings were unique as each of the crosses from the parental generation was represented by one female and one male in the subsequent generation. However,

there were three exceptions [Aripo guppy-introduction (1), Aripo guppy absent control (2)] where we used a female (or male) from a given lineage in an additional pairing because we only had males (or females) from the remaining pedigrees. For instance, in the Aripo River guppy-introduction population, females from one lineage were used in pairings with males from two different lineages. In all cases, pairings between males and females were randomly chosen. We designed the crosses to use and maintain as much genetic diversity from the founding stocks as possible; the wild-caught founders were, for the most part, equally represented in the subsequent generations. Eggs were collected from these second-generation pairings for 10–20 days and the eggs were reared in the same manner as described above. Upon hatching, 8–12 larvae from each pairing were reared in 9-L aquaria and fed ad libitum. At an age of 20 days, eight fish per pairing were randomly selected to enter the formal life-history experiment. Each of these fish was placed individually in a 9-L aquarium and reared until maturity. At random, four fish from each pairing were chosen to receive a high level of food availability, whereas the other four received a low ration level. A “high food” treatment sustained a growth rate typical of the guppy-introduction sites and a “low food” treatment approximated growth in the control sites (Walsh and Reznick 2010). To help control for the initial variation in fish density, we used initial fish size as a covariate in our analyses (see below).

Age and size at maturation was quantified by mating each individual with a mature conspecific for a period of 12–16 h as they approached maturity. The mating period constituted an overnight period that allowed ample opportunity for mating during the early morning hours, a period of time in which *Rivulus* frequently mate in the laboratory (M. R. Walsh, pers. obs.). An individual was classified as mature if a viable embryo(s) was found on the spawning substrate after a mating trial. When a mating trial failed to produce an egg, the individual was isolated and remated every three days thereafter. The mating assays began prior to maturation because all fish were mated at least once preceding the first successful pairing. When an individual was classified as mature, it was briefly removed from the aquarium and measured for total length and wet weight for estimates of size at maturation. After maturation, females were mated daily (using the same protocol as described above) for 20 days to quantify fecundity. A random sample of five eggs was preserved in formalin and weighed for estimates of egg size. The remaining eggs were placed in Petri dishes to measure rates of egg development and subsequent larval size at hatching. Upon hatching, a sample of larvae was preserved in formalin and weighed for estimates of offspring size. The average number of days between egg laying and egg hatching was used as an estimate of egg development rate. An estimate of the per-day allocation toward reproduction (RA) was subsequently calculated as: [(mean per day egg production  $\times$  mean egg size)/size of maturity]  $\times$  100.

Dependent variables were analyzed using general linear models with river entered as a fixed blocking factor (Aripo, El Cedro), and fish community (guppy-introduction, guppy-absent control) and food level (high, low) as fixed effects. The data for male and female traits (age at maturation, size at maturation, fecundity, egg size, reproductive allotment) were examined with a multivariate analysis of variance and each dependent variable was subsequently analyzed separately thereafter. The presence of normality and homogeneity of variance was evaluated for all traits. Data for male size at maturation were log transformed to improve normality; data for fecundity were log transformed to improve among population homogeneity of variances. Female size at maturation was included as a covariate in the analysis of fecundity and egg size, whereas the initial size of each fish was included as a covariate in the analysis of male and female age and size at maturation. Homogeneity of slopes was evaluated and confirmed for all analyses that included a covariate.

#### INTRINSIC RATES OF INCREASE ( $r$ )

We combined our estimates of age of maturation, fecundity, and egg development rate to calculate the intrinsic rate of increase ( $r$ ) of each fish community at each level of controlled food availability (high and low) across a range of juvenile mortality rates using standard life table formula (Gotelli 1998, pp. 52). We did so because  $r$  represents a commonly used index of fitness that is a composite of all of our independent variables. It thus allows us to visualize their combined influence on the life history of *Rivulus*. We calculated  $r$  using the formula:  $r = \ln(R_0)/G$  where  $R_0$  is the net reproductive rate (summation of fecundity schedule  $\times$  survivorship schedule) and  $G$  is generation time (defined as the average age of the parents of all offspring produced by a single cohort). We modeled our juvenile mortality estimates after a recent selection experiment on biomass compensation in a species of live-bearing fish (see Schroder et al. 2009) and we specifically imposed per capita mortality rates of 0.00, 0.005, 0.01, 0.017, 0.025, and 0.032 day<sup>-1</sup>. Each mortality regime was imposed for 30 days after hatching. *Rivulus* can grow to a size ( $\sim$ 20 mm) that is free from predation by guppies within 30 days after hatching.

#### RATES OF EVOLUTIONARY CHANGE

Rates of evolutionary change were estimated in both Darwins and Haldanes for age and size at maturation in males and females. Rates of change between introduction and control sites were examined from the high food treatments only because this food level was designed to approximate the growing environment in the guppy-introduction localities. Darwins were calculated as  $(\ln X_2 - \ln X_1)/\Delta t$ , where  $X_1$  and  $X_2$  are the mean trait values at the beginning and end of the time interval and  $\Delta t$  is the length of the time interval in years. The values for  $\Delta t$  were estimated as 25 and 32 years for the El Cedro and Aripo River, respectively. Haldanes

were calculated by  $[(X1/s) - (X2/s)]/g$ , where X1 and X2 are the mean trait values at the beginning and end of the time interval,  $s$  is the pooled standard deviation, and  $g$  is the generation time in age. For both parameters, X1 and X2 were estimated using the control and introduction populations, respectively. The mean age at first reproduction was used as a proxy for generation time ( $g$ ). Because the real generation time is generally longer, using age at first reproduction as a proxy will tend to yield underestimates of the true rate of evolution.

### EFFECT SIZE COMPARISONS

We were interested in contrasting the results of the common garden experiment from the present study with a previous common garden experiment performed on naturally occurring *Rivulus* communities that differ in the presence and absence of guppies (see Walsh and Reznick 2010). As in the present study, our prior experiment consisted of two populations of *Rivulus* that inhabit portions of streams with guppies and two populations from sites in which *Rivulus* are alone. The difference between these two experiments is that in the present study we are dealing with the response to an experimental introduction whereas in the past study we were dealing with a natural distribution. We compared the results of the two experiments by measuring the effect sizes of three contrasts for all life-history traits. We evaluated the effect size due to (1) guppy introduction and/or guppy presence (i.e., compare guppy-introduction high food, guppy-introduction low food mean vs. control high food, control low food mean), (2) food level (guppy-introduction high food, control high food vs. guppy-introduction low food, control low food), and (3) the interaction between fish community and food level (guppy-introduction high food, control low food vs. guppy-introduction low food, control high food). We used the log response ratio as our measure of effect size of each contrast ( $\ln[\text{treatment mean}/\text{control mean}]$ ). The analysis of treatment responses relative to that of the control is more informative than standardized differences between means because the log response ratio does not incorporate sample variability and does not confound effect size with differences in standard deviation (Hedges et al. 1999).

## Results

The multivariate analyses of variance revealed significant phenotypic differences between wild-caught *Rivulus* from the guppy-introduction and guppy-absent control sites ( $F_{3,122} = 14.5$ ,  $P < 0.001$ ). For the common garden experiment, the MANOVA revealed significant effects of fish community (Female:  $F_{2,65} = 2.51$ ,  $P = 0.039$ ; Male:  $F_{2,128} = 9.3$ ,  $P < 0.001$ ), river (Female:  $F_{5,65} = 23.37$ ,  $P < 0.001$ ; Male:  $F_{2,128} = 45.7$ ,  $P < 0.001$ ), food level (Female:  $F_{5,65} = 24.77$ ,  $P < 0.001$ ; Male:  $F_{2,128} = 146.6$ ,  $P < 0.001$ ) as well as significant fish community-by-food level

interactions (Female:  $F_{5,65} = 3.97$ ,  $P = 0.003$ ; Male:  $F_{2,128} = 4.6$ ,  $P = 0.012$ ). In the presentation that follows, all data were evaluated with univariate analyses to further characterize the nature of the differences among treatments.

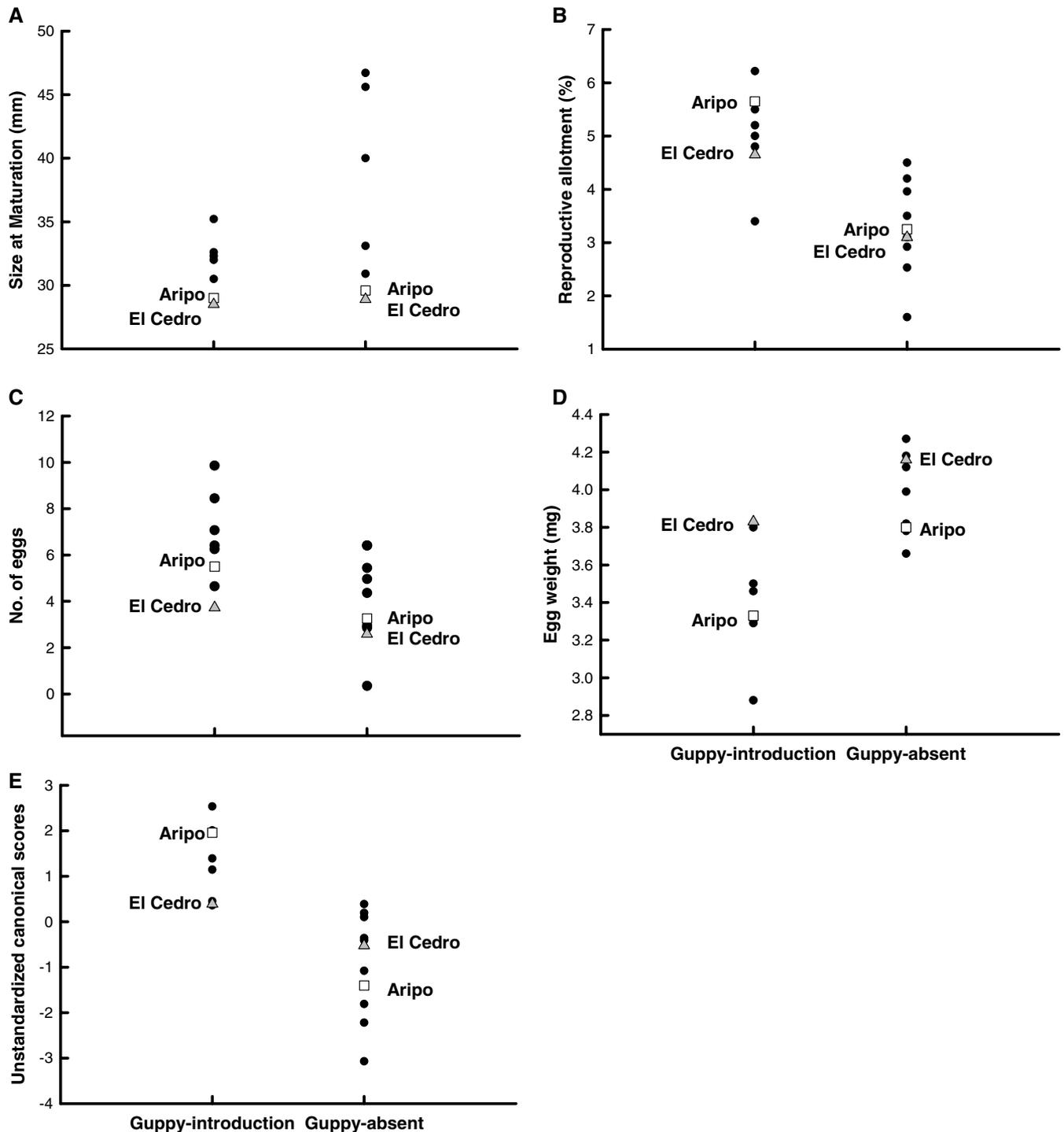
### PHENOTYPIC EFFECTS

Wild-caught *Rivulus* from the guppy-introduction sites exhibited a significantly higher number of eggs, a smaller egg size, and a larger investment in reproduction than those from the control sites (Fig. 1). This divergence was pronounced as the reproductive allotment and fecundity were 37% higher while egg size was reduced by 10% in the guppy-introduction sites relative to the guppy-absent control sites (Fig. 1). There was little difference among populations in the size at which 50% of the females attained maturation (see Fig. 1,  $P > 0.05$ ).

### PHENOTYPIC COMPARISONS BETWEEN EXPERIMENTAL STREAMS AND NATURAL POPULATIONS

We performed a discriminant function analysis to determine how well *Rivulus* from the guppy-introduction streams can be classified using the axis created from data on naturally occurring population of *Rivulus* that differ in the presence and absence of guppies. Using fish community as a grouping variable (*Rivulus*/guppy vs. *Rivulus*-only sites), the first component effectively distinguished between phenotypes as 71% of individuals were correctly classified. The total structure coefficients distinguished between individuals with a high fecundity, small egg size, and large reproductive investment versus those with a low fecundity, large egg size, and small reproductive investment (Total canonical structure: Fecundity = 0.75, Egg size = -0.67, Reproductive allotment = 0.81). More importantly, this discriminant function was equally effective at classifying *Rivulus* from the guppy-introduction streams as the correct classification rate was 68%. The classification frequencies were similar between natural and experimental populations for each fish community (Classification frequency for natural populations vs. introduction sites: *Rivulus* with guppies = 72% vs. 63%, *Rivulus* alone = 69% vs. 72%; see Fig. 1E).

We compared the life-history phenotypes of our guppy-introduction and guppy-absent control sites with many naturally occurring *Rivulus*/guppy and *Rivulus*-only populations. A posteriori comparisons from a general linear model show that the guppy-introduction sites did not differ significantly ( $P < 0.05$ ) from natural *Rivulus*/guppy sites for fecundity and reproductive allotment but differ significantly from all natural *Rivulus*-only sites for fecundity, egg size, and reproductive allotment (Fig. 1; Table 1). Likewise, the guppy-absent control streams did not differ significantly ( $P < 0.05$ ) from the natural *Rivulus*-only sites but differ significantly from all natural *Rivulus*/guppy communities



**Figure 1.** Phenotypic life-history differences between experimental sites as well as naturally occurring *Rivulus* communities. Closed circles = Natural populations, Open squares = Aripo River experimental populations, Gray triangles = El Cedro River experimental populations. RG = sites with *Rivulus* and guppies, Riv only = sites with just *Rivulus*. Sample sizes ( $n$  mature females/ $n$  dissected) for each site were: Aripo introduction 31/43, Aripo control 43/56, El Cedro introduction 32/50, El Cedro control 33/53. Error bars =  $\pm 1$  SE. Significant divergence was observed between the guppy-introduction and guppy-absent control sites for all traits except size at maturation: (A) Size at maturation (Kruskal–Wallis:  $H_{1,4} = 0.6$ ,  $P = 0.44$ ) (B) Reproductive allotment ( $F_{1,138} = 35.76$ ,  $P < 0.001$ ), (C) No. of mature oocytes ( $F_{1,142} = 20.9$ ,  $P < 0.001$ ), (D) Egg size ( $F_{1,124} = 10.87$ ,  $P = 0.0013$ ). Panel (E) displays the unstandardized canonical scores for each population from the discriminant function analysis (see Methods).

**Table 1.** Comparisons between experimental streams and natural populations. RG=sites with *Rivulus* plus guppies, RO=sites with *Rivulus* alone. The values represent the *F* statistics stemming from each post-hoc comparison. Each comparison contained one degree of freedom in the numerator and the denominator degrees of freedom (based upon the number of individuals) were as follows: fecundity=502, egg weight=449, reproductive allotment=503. We applied Bonferroni correction for multiple comparisons and a given comparison with a *P*-value less than 0.0125 was considered "significant."

Trait Comparison	Fecundity ( <i>F</i> )	Egg Weight ( <i>F</i> )	Reproductive Allotment ( <i>F</i> )
Introduction RG vs. Natural RG	5.85	13.07*	0.06
Introduction RG vs. Natural RO	17.33*	0.31	46.06*
Control RO vs. Natural RO	1.04	11.36*	0.001
Control RO vs. Natural RG	52.65*	58.72*	47.88*

\**P*<0.0125.

for egg number and reproductive allotment (Fig. 1; Table 1). In all comparisons, the qualitative differences between the introduction and control sites are the same as those between natural *Rivulus*/guppy and *Rivulus*-only localities. We conclude that, when the life history of *Rivulus* is characterized by the number of eggs, egg size, and reproductive allotment, the differences between localities with and without guppies are similar between naturally occurring and guppy-introduction communities.

**Table 2.** Analyses for life-history traits from the second-generation common garden experiment. General linear models were used with fish community and ration entered as fixed effects and river treated as a blocking factor. When covariates were nonsignificant (*P*>0.05), they were removed from the model and the data were reanalyzed without them.

	df	Male age at maturity <i>F</i>	Male Size at maturity <i>F</i>	Female age at maturity <i>F</i>	Female Size at maturity <i>F</i>	No. of eggs/day <i>F</i>	Egg size <i>F</i>	RA <i>F</i>	Size at hatch <i>F</i>
Covariates:									
Initial Size	1	0.11 <sup>NS</sup>	5.71*	1.37 <sup>NS</sup>	1.12 <sup>NS</sup>	....	....	....	....
Female size	1	....	....	....	....	4.38*	3.92 <sup>+</sup>	....	....
Main effects:									
River	1	48.83***	0.01 <sup>NS</sup>	113.4***	23.48***	0.74 <sup>NS</sup>	31.9***	0.99 <sup>NS</sup>	22.52***
Introduction	1	19.49***	12.51***	2.95 <sup>+</sup>	8.48**	0.49 <sup>NS</sup>	2.27 <sup>NS</sup>	0.57 <sup>NS</sup>	1.1 <sup>NS</sup>
Ration	1	32.26***	23.79***	32.46***	22.97***	9.09**	13.03***	5.46*	11.31**
Introd. × Ration	1	10.24**	5.58*	10.07**	8.09**	12.23***	4.5*	9.39**	5.12*
Error MS (df)		81.9 (133)	.005 (132)	131.1 (71)	0.012 (71)	.028 (68)	3.38E-9 (68)	.036 (69)	3.1E-9 (63)

Note: RA=Reproductive allotment.

<sup>NS</sup>*P*>0.1, <sup>+</sup>0.05<*P*<0.1. \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001.

**COMMON GARDEN EXPERIMENT**

*Introduction effects*

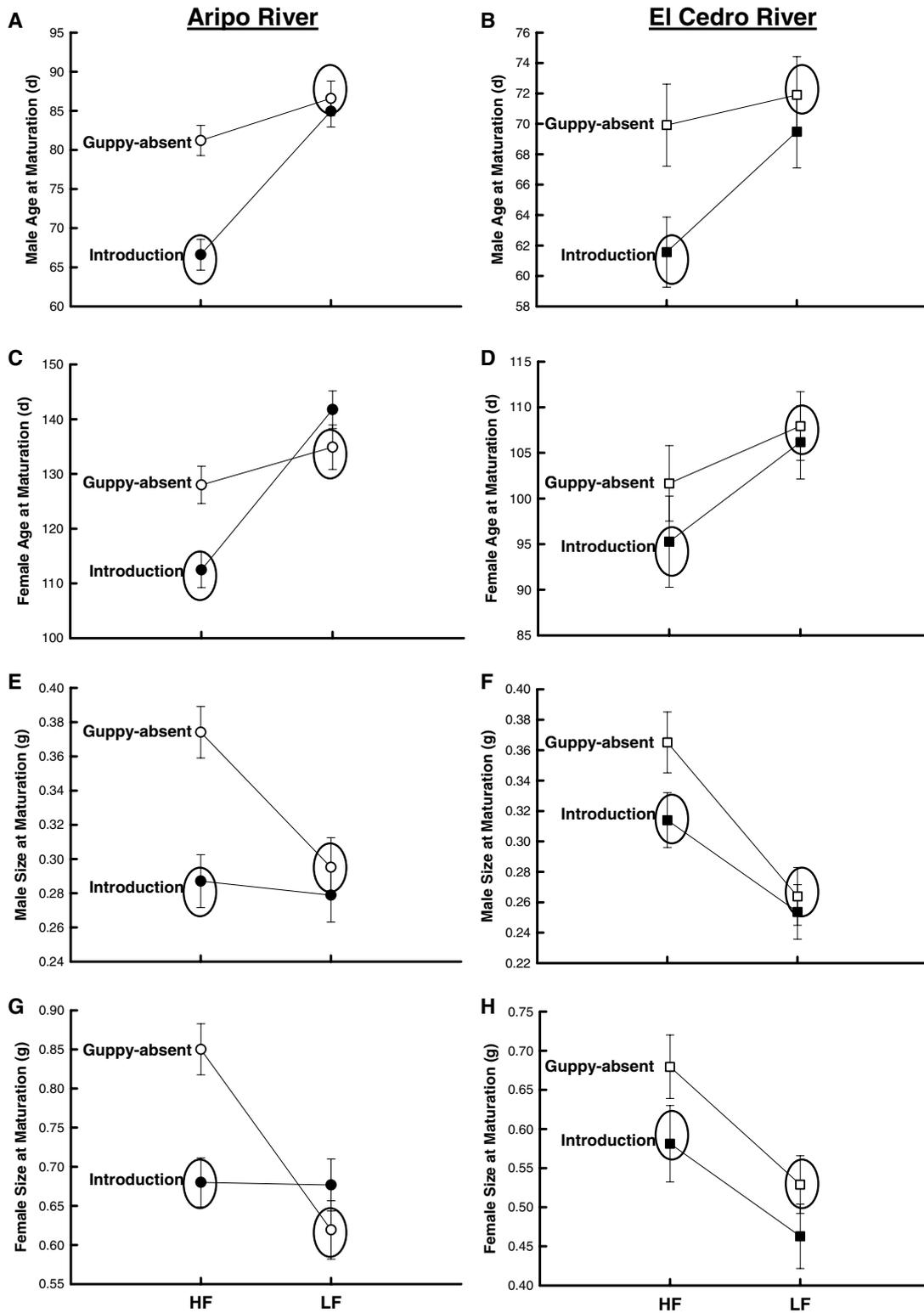
There were significant differences between introduction and control sites for male age and size at maturation, and female size at maturation (Table 2). Differences in female age at maturity were marginally not significant (*P* = 0.07). *Rivulus* from the introduction sites matured earlier and at a smaller size in both males and females (Fig. 2). Age at maturity in the introduction localities was reduced by 8.7% and 4.1% in males and females, and this corresponded with reductions in size at maturity of 13% and 12% in males and females (differences averaged across both food levels). Such differences are stronger when the comparisons are limited to high food levels; males and females from the control streams were 18% and 13% older at maturation than the individuals from the introduction sites whereas male and female size at maturation was increased by 24% and 23% in the control sites compared with the introduction communities.

*Food effects*

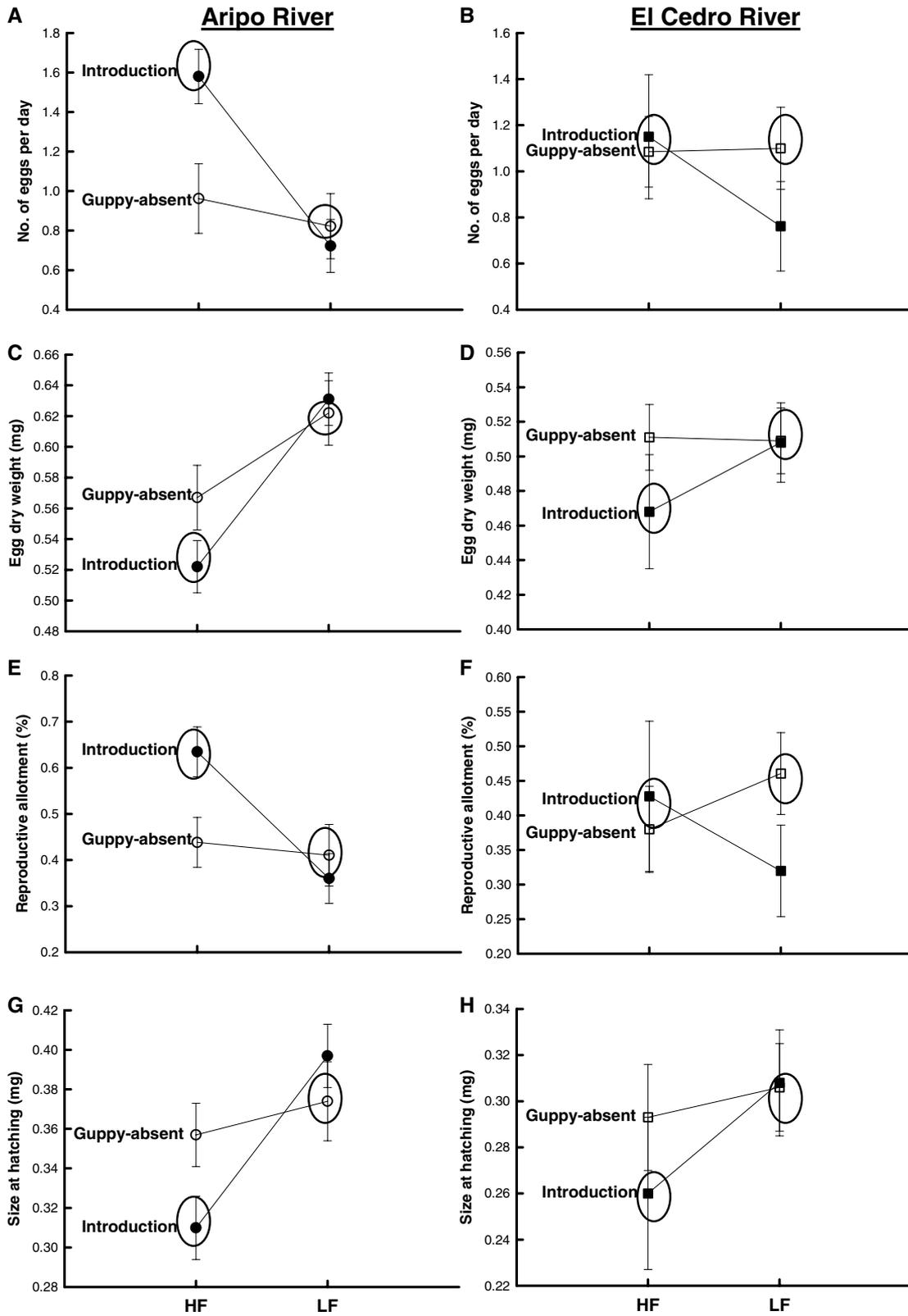
Reduced food levels had a significant impact on all traits (Table 2; Figs. 2 and 3). *Rivulus* delayed maturation and matured at a smaller size in response to less food. Lower food resulted in delays in the timing of maturation of 13% in males and 14% in females, whereas male and female size at maturity was reduced by 19% and 17% on a lower ration, respectively. Female *Rivulus* on low food rations also produced fewer eggs (34% decline) that were larger in size (13% increase) and yielded larger hatchlings (16% increase).

*Population × Food Interaction*

The expression of life-history traits depended strongly upon controlled level of food in the laboratory as all fish



**Figure 2.** Results from the common garden experiment. (A, B) male age at maturation, (C, D) female age at maturation, (D, E) male size at maturation, and (G, H) female size at maturation. Closed circles = Aripo River guppy-introduction, Open circles = Aripo River guppy-absent control, Closed squares = El Cedro River guppy-introduction, Open squares = El Cedro River guppy-absent control. HF = high food, LF = low food. Error bars =  $\pm 1$  SE. Significant differences ( $P < 0.05$ ) between populations were observed for male age at maturation, male size at maturation, and female size at maturation. Significant introduction-by-food interactions were observed for male and female age and size at maturation. Circled values are the guppy-introduction and guppy-absent control sites at their natural levels of food availability.



**Figure 3.** Differences in reproductive traits from the common garden experiments. (A,B) fecundity, (C,D) egg size, (E,F) reproductive allotment, and (G,H) size at hatching. Closed circles = Aripo River guppy-introduction, Open circles = Aripo River guppy-absent control, Closed squares = El Cedro River guppy-introduction, Open squares = El Cedro River guppy-absent control. HF = high food, LF = low food. Error bars =  $\pm 1$  SE. Significant introduction-by-food level interactions were observed for all four traits. Circled values are the guppy-introduction and control sites at their natural levels of food availability.

community-by-food level interactions were significant ( $P < 0.05$ ; Table 2; Figs. 2 and 3). Second-generation laboratory born male and female *Rivulus* from the guppy-introduction sites matured earlier and at a smaller size than *Rivulus* from the guppy-absent control populations (Figs. 2A–H), but only on high food rations. Female *Rivulus* from the guppy-introduction localities also produced a greater number of eggs (Figs. 3A,B), smaller eggs (Figs. 3C,D) and hatchlings (Figs. 3G,H), and their total reproductive investment was greater than the controls (Figs. 3E,F), but again, only when they were reared in high food rations. For most traits, there were no differences between fish communities on low food levels (age at maturation, size at maturation, egg size, size at hatching) and for a few traits, these trends were reversed on low food rations (reproductive allotment, fecundity). *Rivulus* from the guppy-absent control sites actually exhibited greater rates of egg production and allocated more toward reproduction than the guppy-introduction sites when all fish were reared on a low ration that sustains a low growth rate typical of the control sites.

#### River effects

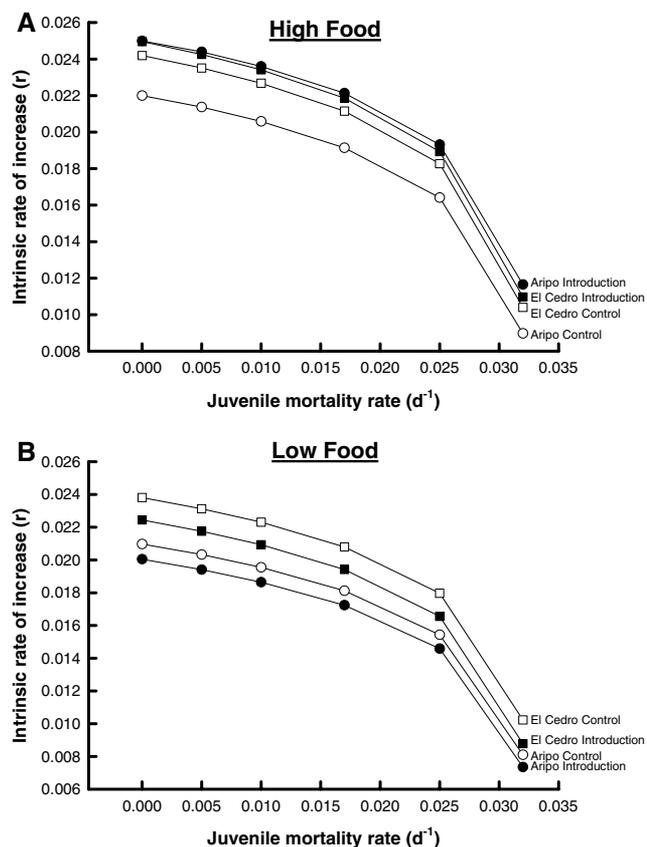
There were significant differences between rivers for male and female age at maturation, female size at maturation, and egg and larval size (Table 2; Figs. 2 and 3). Males and females from the Aripo River matured 11 and 27 days later than fish from the El Cedro River, whereas females from the El Cedro were 20% smaller at maturation compared with females from the Aripo River. Egg and larval size was 18% and 20% larger in the Aripo River, respectively.

#### INTRINSIC RATES OF INCREASE ( $r$ )

Differences in intrinsic rates of increase between guppy-introduction and control localities depended upon food level (Fig. 4). *Rivulus* from the guppy-introduction sites exhibited 10% higher rates of intrinsic increase than the control populations on high food levels but these trends were reversed on low food levels (introduction  $r$  was 6% lower than the controls), which facilitated a significant fish community  $\times$  food level interaction (ANCOVA:  $F_{1,39} = 6.15$ ,  $P = 0.015$ ). These differences in  $r$  were apparent across all per capita juvenile mortality rates (Fig. 4) and hence are independent of any assumptions concerning the extent of guppy predation on juvenile *Rivulus*.

#### RATES OF EVOLUTION

We examined the rate of evolutionary change for age and size at maturation between guppy-introduction and guppy-absent control localities. Rates of evolution ranged from 1000 to 8500 Darwins and 0.002–0.02 Haldanes. Estimates of evolutionary divergence were similar in both rivers (Table 3).



**Figure 4.** Intrinsic rates of increase ( $r$ ) as a function of resource availability between guppy-introduction and guppy-free control communities. (A) High food, (B) Low food. Closed circles = Aripo River guppy-introduction, Open circles = Aripo River guppy-absent control, Closed squares = El Cedro River guppy-introduction, Open squares = El Cedro River guppy-absent control.

#### EFFECT SIZE COMPARISONS

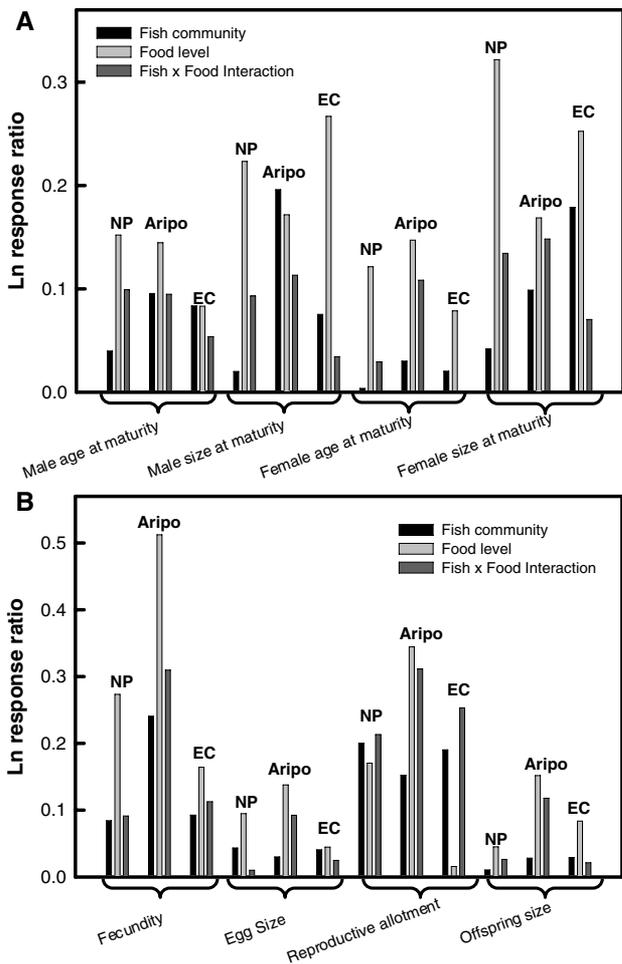
The comparison of effect sizes between the experiments performed on naturally occurring populations of *Rivulus* versus the guppy-introduction streams revealed qualitative differences between them (Fig. 5). For male and female age and size at maturation, the guppy-introduction streams yielded a larger fish community effect size in all four comparisons (average effect size: Introduction = 0.097, Natural population = 0.026). There were no consistent differences in the food level or fish community  $\times$  food level interaction effect size between guppy-introduction versus natural populations for these traits. For reproductive traits (i.e., fecundity, egg and offspring size, and reproductive allotment), females from the Aripo River introduction locality exhibited a larger food response and larger fish  $\times$  food interaction effect size for all four traits, indicating that the fish from this introduction stream were more sensitive to differences in food availability than were fish from natural *Rivulus*/guppy and *Rivulus*-only communities. In contrast, females from the El Cedro River introduction site exhibited a smaller food effect size than the natural populations for

**Table 3.** Rates of evolutionary change in Darwins and Haldanes for age and size at maturation in males and females.

	Male age at maturity (d)		Male size at maturity (g)		Female age at maturity (d)		Female size at maturity (g)	
	Rate		Rate		Rate		Rate	
	(10 <sup>3</sup> darwins)	(haldanes)	(10 <sup>3</sup> darwins)	(haldanes)	(10 <sup>3</sup> darwins)	(haldanes)	(10 <sup>3</sup> darwins)	(haldanes)
Aripo River	6.17	0.02	8.46	0.017	4.96	0.013	7.44	0.012
El Cedro River	5.09	0.013	6.02	0.011	1.06	0.002	6.69	0.007

three-fourth traits and no consistent differences for the interaction. Overall, the effect sizes for fish community, food level, and the fish × food interaction were frequently, but not universally,

larger in the guppy-introduction populations than the natural populations, especially in the Aripo River populations. There were also some differences in effect sizes between introduction experiments; the Aripo River populations exhibited a larger fish community versus food-level interaction for all eight traits and a larger food response for all four reproductive traits than the El Cedro River introduction localities.



**Figure 5.** Effect sizes (log response ratio) from the common garden experiments on the guppy introduction sites (present study) and naturally occurring populations of *Rivulus* that differ in the presence and absence of guppies (Walsh and Reznick 2010). (A) Male and female age and size at maturation. (B) Female reproductive traits. NP = Natural *Rivulus*/guppy and *Rivulus*-only sites, Aripo = guppy-introduction populations from the Aripo River, EC = guppy-introduction populations from the El Cedro River. Effect sizes were calculated for “fish community,” “food level,” and the “fish community × food level interaction” terms in both experiments (see Methods for details).

### Discussion

The introduction of guppies in two independent rivers caused significant, parallel evolutionary changes in *Rivulus* within 25 years (Figs. 1–3). *Rivulus* phenotypes from the guppy-introduction sites had significantly higher reproductive allotment and fecundity, and produced significantly smaller eggs than *Rivulus* from the guppy-absent control sites (Fig. 1). Our common garden experiment revealed that such differences persist in fish reared for two generations in a common environment and hence have a genetic basis. Laboratory-reared male and female *Rivulus* from both guppy-introduction sites matured earlier and at a smaller size than their counterparts from the controls (Fig. 2). Females from guppy-introduction sites produced more eggs that were smaller, yielded smaller hatchlings, and exhibited a greater total investment in eggs than *Rivulus* from the controls (Fig. 3); however, the expression of all traits in the laboratory experiment depended strongly upon controlled levels of food availability. All fish community-by-food level interactions were significant (Table 2) and the differences between guppy-introduction and control sites either disappeared or the trends reversed when compared on low food rations (Figure 2 and 3). These results, based on two introduction experiments, beg two questions: (1) how general are the results? and (2) can we interpret the differences between the introduction and control populations as evolution caused by the introduction of guppies?

Our first argument for the generality of our results is that our introduction experiment was duplicated and that each duplicate yielded the same results. Our second argument derives from prior comparative studies of natural communities. The life-history responses in *Rivulus* to the introduction of guppies mimics the results of previous investigations performed on naturally occurring populations (see Walsh and Reznick 2009, 2010). In our prior research, we used pairwise comparisons of *Rivulus* populations

naturally separated by waterfalls that excluded guppies but not *Rivulus*. These barrier waterfalls create communities that are just tens of meters apart but differ in the presence and absence of guppies. Walsh and Reznick (2009) compared phenotypic divergence between *Rivulus*-only localities versus downstream *Rivulus*/guppy sites across five rivers. *Rivulus* from *Rivulus*-only sites all shared a very similar life history and were always distinct from *Rivulus* collected from *Rivulus*/guppy localities regardless of any other feature of the habitat. In each stream, *Rivulus* from *Rivulus*/guppy sites were smaller at maturation, produced more eggs, smaller eggs, and invested more heavily in reproduction than *Rivulus* from *Rivulus*-only localities. Walsh and Reznick (2010) performed laboratory genetics studies on a subset of these streams using fish collected from just above and below the barriers that excluded guppies. We found that there is a genetic basis to these traits and also, as observed in the introduction experiments, the life-history differences between sites with and without guppies were often reduced or reversed when they were reared on low rather than high rations. The high rations sustained growth rates typical of *Rivulus*/guppy localities whereas the low food rations sustained growth rates typical of sites with *Rivulus* alone. This work on natural populations in conjunction with the changes observed in the guppy-introduction experiments thus demonstrates that there is a consistent association between *Rivulus* life histories and the presence or absence of guppies.

Our second question addresses one potential shortcoming of our introduction experiments—we did not assess the life histories of *Rivulus* before guppies were introduced, so a comparison of *Rivulus* from the guppy-introduction and control sites alone does not define which of these populations may have evolved after the introduction of guppies. To evaluate the nature of evolution in response to the introduction, we first performed a discriminant function analysis based upon *Rivulus* phenotypes from the naturally occurring populations (see Walsh and Reznick 2009) and then classified *Rivulus* from the guppy-introduction streams according to this natural population function. This discriminant function was equally effective at classifying *Rivulus* from natural and guppy-introduction localities into the correct fish community (~70% correct classification rates for natural and introduction sites). We also statistically compared the phenotypic trait values of our introduction and control sites with those of the natural populations of *Rivulus*. Such comparisons show that the phenotypes from the guppy-introduction sites generally did not differ significantly ( $P < 0.05$ ) from five natural *Rivulus*/guppy sites but differ significantly from natural *Rivulus*-only sites (Fig. 1; Table 1). The opposite was true for fish from the guppy-absent, control sites (Fig. 1; Table 1). Such consistent patterns of divergence between our replicated introduction experiments and natural populations of *Rivulus* support two conclusions. First, our comparative studies show that the life-history differences between *Rivulus* from *Rivulus*-only

and *Rivulus*/guppy habitats are consistent regardless of differences among sites in stream morphology—the only predictor of life-history attributes is the presence or absence of guppies. From this pattern, we infer that the life histories of *Rivulus* from the control and guppy-introduction sites would have been the same prior to the introduction of guppies, because they shared the attribute of being *Rivulus*-only localities. Second, if the *Rivulus* from these sites were typical of those found in *Rivulus*-only localities prior to the introduction of guppies, then it follows that differences between *Rivulus* from the guppy-introduction and control sites represents the evolution of *Rivulus* in response to the addition of guppies whereas those from the control sites retained a life-history typical of *Rivulus*-only habitats.

### MECHANISM OF LIFE-HISTORY EVOLUTION

The results of our guppy introduction experiments (and studies on natural populations of *Rivulus*) do not comply with the predictions of age- and size-specific life-history theory that considers the impacts due to predation on juveniles alone. Such theory predicts that predation on small or young individuals favors delayed maturation and reduced reproductive investment (e.g., Law 1979; Charlesworth 1980). Yet, the introduction of guppies has driven *Rivulus* life histories in the opposite trajectory of evolutionary change (Figs. 1–3). We can thus reject these versions of life-history theory as an explanation for our results and ask “what is the cause of life-history evolution in *R. hartii*?”

The ecological correlates of guppy predation on small *Rivulus*, which include reduced densities and increased growth rates of *Rivulus* in the introduction localities, raise possible alternative mechanisms of life-history evolution in *Rivulus*. Our previous studies show that these differences in growth almost certainly reflect environmental sources (Walsh et al. in press). *Rivulus* grow at the same rate in the laboratory on equal rations, although a transplant experiment, in which we moved *Rivulus* from a *Rivulus*-only locality into a downstream locality with *Rivulus* plus guppies, caused a rapid increase in the growth rate of the transplanted fish (Walsh et al., in press). Covariation between some unmeasured ecological factor, such as environmental productivity, is unlikely to explain the differences in the growth between localities with and without guppies because these sites are virtually identical in habitat and environment and often in a close proximity to one another. There is little evidence that guppies facilitate physiological or behavioral responses in *Rivulus* that enhance growth (e.g., McPeck 2004; Schmitz et al. 2004) because guppies suppress the growth of *Rivulus* to the same degree as an equal-sized *Rivulus* in field enclosures (Gilliam et al. 1993). Predation by *Rivulus* on guppies also cannot account for the differences in growth because all measured size classes of *Rivulus* (25–90 mm total length) exhibit faster growth in sites with guppies; the smallest size classes of *Rivulus* are too small to consume guppies (Walsh et al., in

press). The simplest explanation is that the faster rates of growth in sites with guppies are instead mediated by the lower abundances of *Rivulus* in these sites. We specifically favor increased per capita resource availability that is a hypothesized indirect consequence of guppy predation as the cause of increased growth rates of *Rivulus* from sites with guppies (Walsh et al., in press). This proposal is supported by contrasting diet preferences between *Rivulus* and guppies. Both guppies and *Rivulus* consume benthic invertebrates although a large component of the diets of *Rivulus*, but not guppies, is terrestrial organisms, such as ants (Gilliam et al. 1993; Fraser et al. 1999). The lower densities of *Rivulus* in sites with guppies may relax the intensity of intraspecific competition for this food resource that, in turn, facilitates faster rates of growth.

Changes in density, growth, and resources are important because they can interact with age/size-specific mortality to alter the predicted trajectory of evolution. For example, several models show that life-history predictions are highly sensitive to the presence and form of density regulation in a population (e.g., Michod 1979; Charlesworth 1980; Gardmark and Dieckmann 2006). Gardmark and Dieckmann (2006) examined the influence of size-specific mortality on the evolution of size at maturation and found 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 (an assumption of the model). All of these biological requirements are common in fish and such a prediction may therefore be consistent with our results. Impacts due to density- and growth-related effects could also impose selection on the evolution of life histories irrespective of age- or size-specific mortality. Several models predict that increased resource availability or faster rates of growth can favor the evolution of earlier maturation (Gadgil and Bossert 1970; Stearns 1992) and increased investment in reproduction (Gadgil and Bossert 1970; Stearns 1992). All of these frameworks yield predictions that are consistent with our results but only if impacts due to population density and individual growth rates are explicitly considered.

A similar manner in which to reconcile our results with evolutionary theory is to consider the relative importance of the direct versus indirect effects of predation on resultant evolutionary change. Abrams and Rowe (1996) evaluated interactions between the direct effects of predator-induced mortality and the indirect effects of this mortality mediated through increases in food availability on the evolution of age at maturation. Although this approach yields many predictions that depend upon specific mortality rate functions, it shows that evolutionary responses to indirect effects on prey food supply will often oppose and be greater than the direct effect of predators. This model, at least qualitatively, yields predictions that may be applicable to, and consistent with, our results.

An additional feature of our results that argues for an important role for growth and resource availability as selective forces is the presence of significant food  $\times$  locality interactions in our common garden experiment (see also Walsh and Reznick 2010). We observed large differences in all life-history traits between guppy-introduction and guppy-absent control sites on a high food level that sustained a growth rate typical of *Rivulus*/guppy sites. Such trends were either reduced or reversed when *Rivulus* were fed a ration that sustained the lower growth rates typical of *Rivulus*-only localities. As a result, *Rivulus* from the guppy-introduction sites exhibited higher intrinsic rates of population growth ( $r$ ) than the *Rivulus* from the controls when the two were compared on high food rations, but lower values of  $r$  when they were compared on low food rations (Fig. 4). The presence of these food  $\times$  locality interactions parallels the results of common garden experiments in which we compared *Rivulus* from natural *Rivulus*-only and *Rivulus*/guppy communities (Walsh and Reznick 2010). These interactions argue that introduction of guppies selected for *Rivulus* with an enhanced ability to use higher levels of food availability to produce offspring at the expense of their ability to convert resources into offspring when food is scarce (Figs. 2 and 3). Conversely, *Rivulus* from sites in which they are the only fish present appear adapted to environments characterized by high population densities, slow growth, and low resources and appear to be adapted to low food availability (see also Walsh and Reznick 2008). Our interpretations of the significant food  $\times$  locality interactions are similar to conclusions drawn from reciprocal transplant experiments (reviewed: Schluter 2000, pp. 95–104) and selection experiments performed on populations of *Drosophila* or mice that were reared under contrasting food or density treatments (Falconer and Latyszewski 1952; Mueller and Ayala 1981; Bierbaum et al. 1989; Hillesheim and Stearns 1991, 1992; Mueller et al. 1991), both of which argue that fitness trade-offs across divergent environments or selection regimes indicate local adaptation.

Our perturbation experiments provide evidence demonstrating that the introduction of guppies alters the selective landscape and thus drives evolution in *Rivulus*. Results from recent experimental work in Trinidad strengthen this argument. Bassar et al. (2010) evaluated the impact of the introduction of guppies into artificial stream channels on community and ecosystem variables. These authors showed that guppies substantially alter ecosystem processes with impacts on prey community structure, rates of primary production, and leaf decomposition rates. This article makes a strong case that the introduction of guppies has a rapid and significant impact on their ecosystem and may thereby alter selection on *Rivulus*. Using these same artificial streams, Palkovacs et al. (2009) explored ecological interaction between guppies and *Rivulus* and showed that they have coevolved in their patterns of resource utilization.

A common feature of all of the models of life-history evolution considered in this study is that they require specific information about the ecology and population biology of *Rivulus* that is often not yet available. For example, some of them require that we know not only whether natural populations are density regulated, but also that we know the precise demographic mechanisms (e.g., reduced fecundity, increased juvenile mortality rate, increased adult mortality rate) by which density regulation is attained (e.g., Charlesworth 1980). Because we do not have this knowledge, we cannot at this time define the exact causal mechanism for the observed pattern of life-history evolution.

### EFFECT SIZE COMPARISONS

Our qualitative comparisons of effect sizes from the present study and that performed on natural populations of *Rivulus* (see Walsh and Reznick 2010) revealed interesting differences between studies (Fig. 5). Most prominently, the differences between fish communities were often stronger in both guppy-introduction streams than for natural *Rivulus*/guppy communities for age and size at maturation. In addition, the food level and fish community  $\times$  food level interaction effect sizes for all female reproductive traits (i.e., fecundity, egg size, offspring size, reproductive allotment) were stronger in the Aripo River introduction stream (but not El Cedro River) than that observed in the naturally occurring *Rivulus* communities (Fig. 5). One potential cause for the differences in effect sizes between guppy-introduction and natural *Rivulus* communities is that the background effect of guppies and, potentially, the duration of exposure to guppies, differs between introduction and naturally occurring localities. In the guppy-introduction streams, guppies were derived (in 1976 and 1981) from sites with several large predatory fish species. Guppies from these high-predation sites exhibit genetically based differences in life-history, morphological, behavioral, and physiological traits when compared with guppies from sites with just *Rivulus* (Reznick et al. 1990; Houde 1997; Ghalambor et al. 2004; Magurran 2005). It is also known that guppies from communities with and without large predators differentially alter ecosystem processes (Bassar et al. 2010) and there is experimental evidence for coevolution between *Rivulus* and guppies (Palkovacs et al. 2009). These differences could alter the selective environment experienced by *Rivulus* and contribute to the effect size differences between introduction and natural communities.

Effect sizes also differed between guppy-introduction experiments. The response to reduced food for all female reproductive traits and the magnitude of the fish  $\times$  food interaction for all traits was stronger in *Rivulus* from the Aripo River versus the El Cedro River (Fig. 5). Such differences could be influenced by the longer duration of residence of guppies in the Aripo River (1976 vs. 1981). Alternatively, the stronger food-related effect sizes in the Aripo River could be impacted by variation in productivity

between rivers. The Aripo River is narrower and the canopy is less open than the El Cedro River (D. N. Reznick, pers. obs.). Such differences could alter ecosystem productivity (see Reznick et al. 2001) and, in turn, the strength of food-mediated selection between rivers.

### RATES OF EVOLUTION

We examined the rate of evolutionary change for age and size at maturation between guppy-introduction and guppy-absent control localities. Rates of evolution ranged from 1000 to 8500 darwins (0.002–0.02 haldanes) and the estimates were similar in both rivers (Table 3). Such rates are orders of magnitude faster than estimates from the fossil record, but slower than values from artificial selection experiments (Gingerich 1983), and comparable to rates of contemporary evolution in natural populations (Hendry and Kinnison 1999; Kinnison and Hendry 2001). The estimated rates of evolution of the guppies that were introduced to these sites were higher than the values observed in *Rivulus* (Reznick et al. 1997). This difference may simply be a byproduct of *Rivulus* having evolved to their local optimum before we sampled these populations, so that an interval of rapid evolution was averaged with a subsequent interval of little or no change. Alternatively, selection on *Rivulus* may be weaker and/or the heritability of these traits may be lower.

### CONCLUSIONS

Our results show that the experimental introduction of guppies has caused evolutionary changes throughout the life history of a killifish in Trinidad. The observed trajectory of evolution is inconsistent with a predicted response to the direct effect of predation by guppies on very small or young *Rivulus* (e.g., Law 1979; Charlesworth 1980). Our results are better explained by theoretical models that consider changes in juvenile mortality rates in conjunction with the ecological consequences of increased mortality rates. This includes important roles for changes in population density (Charlesworth 1980; Gardmark and Dieckmann 2006) and increased growth rates or resource availability (Gadgil and Bossert 1970; Abrams and Rowe 1996), which are both hypothesized indirect effects of guppy predation on small size-classes of *Rivulus*. The consistent presence of fish community  $\times$  food level interactions in our laboratory study further indicates a role for resource availability as an agent of selection. These interactions, whereby *Rivulus* from the introduction sites sustain higher population growth rates than those from the control sites when they are compared on high food rations, but lower population growth rates when compared on low food rations, provide evidence that *Rivulus* are adapted to differences in resource availability. Although it is well established that the direct effects of predation cause evolution (Reznick et al. 1990, 1997; Young et al. 2004), here we show that changes in density, growth rates, and

resource availability, likely indirect effects of predation, can cause evolutionary changes that are as rapid and pronounced as direct effects.

There has been a recent rise in studies that document rapid, or contemporary evolution (reviewed by Hendry and Kinnison 1999; Reznick and Ghalambor 2001). Most such studies are heavily weighted toward studies of selection, rather than evolution. This distinction is important because short-term estimates of selection do not show that evolution has occurred nor does it demonstrate that evolution will occur. Many factors, such as genetic correlations and competing selection on correlated traits, could potentially limit responses to selection (see Price and Grant 1984; Barrett et al. 2008). For these reasons, it is important to appreciate the distinction between the experimental study of evolution versus the study of selection. Experimental studies of selection quantify what might happen if certain assumptions are met whereas studies of evolution reveal what actually does happen. Most studies that do demonstrate contemporary evolutionary changes are often in association with the accidental introduction of species to new environments, which thus lack the precision of planned experiments because we often do not know where the introduced species came from, what it was like before the introduction, or when the introduction occurred (reviewed by Reznick and Ghalambor 2001). Experiments that manipulate ecological selective pressures and track evolutionary change (including the establishment of a genetic basis to any change), such as the present study, remain rare (see Endler 1980; Reznick et al. 1990, 1997; Losos et al. 1997, 2004). Despite the inherent difficulty in performing experimental studies of evolution in nature, these studies remain the best means to test hypotheses derived from evolutionary theory because they evaluate how evolution proceeds despite genetic constraints and environmental heterogeneity.

#### ACKNOWLEDGMENTS

We thank D. Fraser, M. Schrader, C. Oufiero, J. Ogren, E. Kam, and R. Sandhu for field or laboratory assistance. D. Roff, L. Nunney, the Reznick lab, and three anonymous reviewers provided helpful comments. This work was supported by a National Science Foundation Doctoral Dissertation Improvement Grant DEB0808039, and National Science Foundation Grants DEB0416085 and EF0623632 to DNR.

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Associate Editor: B. Sheldon