

Experimental Evidence for Density-Dependent Regulation and Selection on Trinidadian Guppy Life Histories

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ABSTRACT: Recent study of feedbacks between ecological and evolutionary processes has renewed interest in population regulation and density-dependent selection because they represent black-box descriptions of these feedbacks. The roles of population regulation and density-dependent selection in life-history evolution have received a significant amount of theoretical attention, but there are few empirical examples demonstrating their importance. We address this challenge in natural populations of the Trinidadian guppy (*Poecilia reticulata*) that differ in their predation regimes. First, we tested whether natural populations of guppies are regulated by density dependence and quantified in which phases of the life cycle the effects of density are important. We found that guppies from low-predation (LP) environments are tightly regulated and that the density-dependent responses disproportionately affected some size classes. Second, we tested whether there are differences in density-dependent selection between guppies from LP or high-predation (HP) environments. We found that the fitness of HP guppies is more sensitive to the depressant effects of density than the fitness of LP guppies. Finally, we used an evolutionary invasion analysis to show that, depending on the effect of density on survival of the HP phenotype, this greater sensitivity of the HP phenotype to density can partially explain the evolution of the LP phenotype. We discuss the relevance of these findings to the study of feedbacks between ecology and evolution.

Keywords: ecoevolutionary feedbacks, life-history evolution, density regulation, density-dependent selection, integral projection models, life table response experiment.

Introduction

The role of population regulation in determining the abundance of organisms is a well-studied topic in the ecological literature (reviewed in Cappuccino and Price 1995). Density-dependent natural selection, a distinct yet related concept in evolutionary biology, was studied extensively from the 1960s through the 1980s, mostly as it

related to redefining the measure of fitness (MacArthur 1962; MacArthur and Wilson 1967; Pianka 1970; Charlesworth 1971). Recently, density-dependent selection, along with frequency-dependent selection, has reemerged in a resurgent literature on the role of feedbacks between ecological and evolutionary processes in determining the outcome of natural selection and ecological dynamics (Metz et al. 1992, 2008; Heino et al. 1997, 1998; Ferriere et al. 2004; Schoener 2011). Under this framework, the nature of intra- and interspecific density and frequency dependence determines whether or not feedbacks between ecological and evolutionary processes represent a critical component in deriving predicted evolutionary and ecological outcomes (Pimentel 1961, 1968; Roughgarden 1976; Levin and Udovic 1977; Heino et al. 1997, 1998). However, many empirical studies of life-history evolution continue to test predictions from theory that is density independent despite the potential importance of ecoevolutionary feedbacks and the inclusion of density dependence in numerous other models (Charlesworth 1971, 1994; Abrams 1993; Metz et al. 1996; Gardmark et al. 2003).

The Trinidadian guppy has been a well-popularized test of demographic theories of life-history evolution. Trinidadian guppies inhabit mountain streams on the Caribbean island of Trinidad that can be characterized by different predatory fish communities. In low-predation (LP) stream reaches, guppies coexist with Hart's killifish (*Rivulus hartii*). In high-predation (HP) reaches, guppies coexist with a variety of piscivorous fish predators including the pike cichlid (*Crenicichla* spp.) and the wolf-fish (*Hoplias malabaricus*). Studies of wild-caught guppies (Reznick and Endler 1982) and guppies raised under common garden conditions under two food levels (Reznick 1982) demonstrate that guppies from HP environments mature earlier, have more offspring, and devote more resources to reproduction than guppies from LP environments. When guppies are transplanted from HP to previously guppy-free LP environments, the LP phenotype

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evolves rapidly (Reznick and Bryga 1987; Reznick et al. 1990).

Life-history theory that does not incorporate density dependence and hence no feedback between ecology and evolution predicts such life-history evolution only if guppies from high-predation environments experience higher adult mortality rates but perhaps no difference in juvenile mortality rates or even lower juvenile mortality rates than those from LP environments. Detailed mark-recapture experiments in LP and HP sites have shown that guppies from HP environments have uniformly higher mortality rates across all size classes (Reznick et al. 1996, 2002). This same life-history theory predicts that in such circumstances, the life histories of guppies from HP and LP environments should be the same. Thus the question remains: Why does the LP phenotype evolve?

The alternative is that either intra- or interspecific density dependence is an important selective agent in guppy populations. Guppy population densities and biomasses are higher in LP compared to HP communities (Reznick and Endler 1982; Rodd and Reznick 1997; Reznick et al. 2001) as a consequence of being released from predation. Thus, it is possible that part of adapting to the LP environment involves adapting to higher densities and potentially lower per capita resources. Here we test the hypothesis that guppy populations in low-predation stream reaches evolve as a consequence of intraspecific density-dependent selection. We first test whether guppy populations are regulated via density by conducting in situ density manipulation experiments on 10 natural guppy populations. These experiments are important because they evaluate whether or not density is an important feature of the LP environment. We then modeled the size-specific demographic changes and calculated population growth estimates using an integral projection model (IPM) approach (Easterling et al. 2000). If intraspecific density is important in regulating the population size of guppies, then decreasing or increasing guppy densities should cause demographic changes that in turn cause population growth rate to respond in such a way as to return the population size toward the premanipulation population size. Moreover, unmanipulated populations should exhibit demographic trait values that result in no change in population size. Second, we tested whether LP and HP phenotypes are affected differently by the same density gradient using a factorial experiment executed in artificial streams (mesocosms) wherein we crossed guppy phenotype (LP vs. HP) with guppy density (12 vs. 24 fish). We measured the size-specific demographic responses in the experimental cohorts and calculated the fitness of each phenotype under the different density conditions for guppies derived from two different river drainages, each represented by an HP and LP population. If guppy fitness is density dependent,

then we expect to see an interaction between the phenotypes and density. Finally, we tested whether an observed interaction between the phenotypes and density can result in the evolution of the LP guppies from HP ancestors. Specifically, we combined the results of the field density manipulations and the mesocosm experiments to parameterize an evolutionary invasibility model that evaluates the ability of the LP phenotype to evolve under a spectrum of density-dependent survival regimes. We interpret positive results for these tests as supporting evidence that density-dependent regulation and selection are important in the evolution of the guppy phenotype.

Material and Methods

Density Manipulations in Natural Streams

Experimental Design. Between 1993 and 2009, we manipulated the density of guppies in 10 LP streams in the Northern Mountain Range in Trinidad, West Indies, during the dry season (February to June). We selected three pools in each stream that were roughly equal in morphology and canopy cover. All guppies were removed from the pools using butterfly nets and placed in buckets. Each pool was fished until no guppies were seen in the pool after 20 min of active search. Fish were sexed, measured for standard length, and marked using subcutaneous colored elastomer implants (Northwest Marine Technologies). We used two types of marking schemes, which reflect an increase in our ability to mark individual fish with unique colored marks through time. In early replicates (Arima, Aripo, Quare 2, Quare 6, Quare 7), fish were marked in size-specific cohorts corresponding to the nearest millimeter length. In later replicates (Campo, El Cedro, Endler, Tripp Trace, Turure), fish were marked so that individuals were identifiable based on the combination of mark location and colors. Guppies smaller than 10 mm were marked using Alizarin or Calcein (Leips et al. 2001). Pools were randomly assigned to one of four density treatments. “Decreased” density treatments received half the number of fish that were removed from the pool. “Control” pools received the same number of fish removed from the pool. The increased density treatment differed between earlier and later experiments. In the earlier series of experiments (Arima, Aripo, Quare 2, Quare 6, Quare 7), the “increased” pools received 2.0 times the number of fish removed from the pool. In the latter series of replicates (Campo, El Cedro, Endler, Tripp Trace, Turure), the increased pools received 1.5 times the number of fish removed from the pool. Analyses from part of design 1 have been previously reported in Reznick et al. (2012).

How guppies were assigned to treatment pools differed between early and late replicates. In early replicates, all

guppies removed from the three pools were grouped together after enumeration, sexing, measuring, and marking. Guppies were then drawn at random from these combined populations for reintroduction to the three pools. This design removed pool-specific carryover effects into the density treatment but also meant that most subjects were introduced into a new pool. In later replicates, all guppies were returned to their home pools with the exception that one-third of the guppies in the increased density treatment came from other pools. This design was meant to minimize any effects due to returning to a non-home pool but was unable to control for any pool-specific effects on the treatments. In the later design, guppies in increased density treatments that were not native were not used in the analysis of demographic rates. They served only to provide a density treatment for native guppies. In each design, numbers of males and females were equally manipulated in each pool, but only the demographic data from females were used in the analysis of treatment effects. In addition, in design 1 (early replicates), guppies were placed into pools with size distributions that represented the average size distribution across the three treatment pools in each stream. In design 2 (later replicates), guppies were placed in the pools with size distributions that reflected the original size distribution (see figs. S1 and S2, available online, for pretreatment, experimental, and estimated stable size distributions).

Approximately 25 days after reintroduction, guppies were again removed from each pool using the same techniques as the original capture. Guppies were euthanized using an overdose of MS-222, sexed, measured for standard length and body mass, and preserved in 5% formalin. Areas outside of the pools were searched for guppies that may have escaped the treatment pools. These emigrants were used to correct the survival estimates. Later, each female fish was dissected to determine number, size, and developmental stage of embryos using standard techniques (Reznick 1982; Reznick and Endler 1982).

Analyses. We used linear mixed and generalized linear mixed models to estimate size-specific demographic parameters. Growth and offspring size were modeled using linear mixed models that assume the unexplained variance has a normal distribution. All other demographic rates were modeled with generalized linear mixed models that allow a variety of error distributions. Offspring number was modeled as a normal distribution of the log-transformed data. Survival and reproduction probabilities were modeled as binomial distributions with logit link functions (table S1, available online). Density treatment was entered as a fixed effect with four levels: decreased ($0.5 \times$), control ($1 \times$), increased1 ($1.5 \times$), and increased2 ($2 \times$). Because the $1.5 \times$ density increase was performed only in the later

experiments and the $2.0 \times$ only in the earlier design, we nested density and all its interactions within experiment number (early or late). The square of initial length was also included as a covariate in the somatic growth and survival analyses. Interactions between experimental treatments and covariates were initially included in the models. We also modeled the effect of stream, the interaction between stream and density treatment, and their interactions with the covariates as random effects. We decided on the most parsimonious combination of fixed effects using the Akaike Information Criterion (AIC_c) or quasi AIC_c , and when no single model fit the data better than any other, we used model averaging techniques to calculate model parameters (Burnham and Anderson 1998). Details of the model selection procedure can be found in the online supplementary information, and the results of the selection procedures can be found in tables S2–S6, available online.

We tested the effects of the density treatments using linear combinations of the parameter and error estimates from the averaged models (i.e., planned comparisons). We constructed contrasts that compared the decreased and increased density treatments with the control densities. We constructed separate contrasts for early and later experiments for the field density manipulations. Contrasts were evaluated at a fish length of 18 mm. When interactions between the treatment effects and initial length were included in the averaged models, we tested treatment differences at three locations along the initial length covariate by centering the covariate at small (12 mm), medium (18 mm), and large (24 mm) sizes. These sizes correspond to fish that are juveniles (prereproductive), reproducing for the first time, and larger females that are reproducing for at least the second time. All contrasts were evaluated using one-tailed *t*-tests reflecting the directional predictions about how density should affect population or phenotypic growth rates.

We used an integral projection model (IPM; Easterling et al. 2000) framework to calculate population growth projections for each density treatment in each stream. Our projection models are based on guppy standard length. In principle, we could have created projection models that were functions of age and length. However, in wild guppies, length and age are highly correlated ($r^2 = 0.72$; Reznick et al. 1996). Moreover, because of the high covariation between age and size, our statistical models used to parameterize the projection models would not be able to reliably assign effects to age or size. So for simplicity we used only standard length. We present results from an analysis of the asymptotic dynamics assuming that the populations were in stable size distributions and results from an analysis of the transient dynamics produced when this assumption is relaxed. Details of the IPM construction and calculation of population growth rates and treatment

effects assuming each of these dynamics can be found in the online supplementary information.

Assessment of Density-Dependent Fitness

Experimental Design. We evaluated the role of density in the evolution of the LP phenotype from HP ancestors in artificial streams (mesocosms) using a factorial design in which we crossed fish density (low and high) with phenotype (LP and HP). These mesocosms are eight $\sim 3 \times 1$ -m cinder block flow-through structures located along a natural stream in the Arima Valley, Trinidad. These structures were longitudinally divided to yield 16 independent mesocosms. We added a commercially available mixture of sand and gravel to a depth of 5 cm as substrate. We then allowed water to fill and run through the channels for 1 week to allow the accumulation of biofilm and detritus. We added invertebrates and benthic organic material to the mesocosms from collections from the nearby stream. We allowed the invertebrates to acclimatize to the mesocosm environments for 4 days, after which we added guppies to the mesocosms. All guppies were measured for standard length and individually marked using elastomer implants (Northwest Marine Technologies) before being placed in the mesocosms. The full experiment also included four mesocosms that received no fish as part of another study examining the effect of the phenotype on ecosystem properties (Bassar et al. 2010b). Density treatments levels were chosen to represent the natural densities of guppies observed in HP and LP habitats (Reznick and Endler 1982; Reznick et al. 1996; Rodd and Reznick 1997; Bassar et al. 2010b). Sex ratios and size distributions in each treatment were the average between the two habitat types, not including individuals smaller than 10 mm (newborn babies; Reznick and Endler 1982; Reznick et al. 1996, 2001; Rodd and Reznick 1997). After 28 days, the guppies were removed from the mesocosms, identified, measured for standard length and mass, euthanized with an overdose of MS-222, and preserved in 5% formalin. The females were later dissected in the lab to determine number, size, and developmental stage of embryos using standard techniques (Reznick 1982; Reznick and Endler 1982). We ran the experiment twice, using guppies from LP and HP habitats on the Aripo and Guanapo Rivers in order to make some inference about the generality of the results. Complete details of the mesocosm construction and preparation can be found in Bassar et al (2010b).

Analyses. We used linear mixed and generalized linear mixed models to statistically evaluate whether the individual demographic rates of LP and HP guppies differed in their response to density. We collapsed the factorial design (predation \times density) into a single fixed treatment

effect with four levels. Initial standard length was entered as a covariate when appropriate. We modeled the effect of the two independent drainages and their interactions with other variables as fixed effects. Mesocosm number was included as a random effect on the model intercept to remove intramesocosm variance. Random effects were processed by subjects (mesocosms), and we used between-within degrees of freedom to calculate the appropriate degrees of freedom for tests of fixed effects. Model selection was identical to the procedure performed for the field manipulations (see online supplementary information and tables S7–S11 for model selection results). As in the analysis of the field density manipulations, all final averaged models that included length as a covariate were evaluated at a length of 18 mm by centering the covariate on 18 mm before analysis. If significant interactions between the main effects and the length covariate were present, then the models were rerun to obtain main effect tests at 12 and 24 mm. As in the field density manipulations, we used linear combinations of effects to construct tests of the effects of phenotype, density, and their interaction on the demographic variables. When drainage by treatment interactions were present in the mesocosm analyses, we conducted separate contrasts for each drainage using partial interaction contrasts (treatment \times drainage; offspring size only).

We tested the combined effect of density-dependent variation in the demographic rates of LP and HP guppies in the mesocosms by combining the demographic rates in a population projection model. We constructed a separate IPM (Easterling et al. 2000) for each phenotype \times density \times drainage replicate by using the predicted fixed and random effects (BLUPs) parameters from the model-averaged statistical models. From these, we calculated the asymptotic phenotypic growth rates (λ), stable size distributions, and reproductive values for each replicate.

Because the experimental design included removing the effects of size distribution between the phenotype treatments, the asymptotic phenotypic growth rates derived here are different from those that would actually be observed in the mesocosms had we allowed them to continue for more than 28 days. However, the intention of the experiment was to generate demographic rates that were functions of number or biomass of guppies. Additionally, survival in the mesocosms represents an artificial survival rate. We addressed this issue by constructing separate projection models that included either the observed size-specific survival in the mesocosms or the size-specific survival that was observed in the field density manipulations for the control and decreased densities because these are proportionally the same as the density manipulations between the high- and low-density (low mesocosm density is 0.5 times high mesocosm density, where high mesocosm den-

Table 1: Effects of density manipulation for the two field experimental designs and effects of phenotype, density, and their interaction for mesocosms on the estimated λ values for the Guanapo and Aripo drainages

	Assuming stable size distribution				Using observed initial size distribution			
	Effect	SE	Lower CI	Upper CI	Effect	SE	Lower CI	Upper CI
Field experiment (LP):								
Control (1) – 1	-.04	.025	-.094	-.006	-.15	.016	-.187	-.122
Control (2) – 1	.05	.031	-.001	.122	.10	.086	-.038	.289
Decrease (1) – control (1)	.10	.012	.070	.116	.10	.024	.041	.139
Decrease (2) – control (2)	.15	.013	.124	.178	.19	.140	-.082	.472
Increase (1) – control (1)	-.18	.022	-.218	-.140	-.16	.007	-.170	-.140
Increase (2) – control (2)	-.18	.008	-.200	-.169	-.21	.084	-.497	-.110
Mesocosm:								
Guanapo:								
Phenotype	.28	.022	.225	.305	.30	.025	.233	.328
Density	-.51	.030	-.589	-.475	-.51	.024	-.568	-.477
Phenotype \times density	-.19	.073	-.346	-.092	-.14	.055	-.258	-.067
Aripo:								
Phenotype	.06	.042	-.026	.122	.22	.033	.157	.271
Density	-.46	.028	-.527	-.426	-.49	.022	-.547	-.458
Phenotype \times density	-.19	.038	-.274	-.138	-.18	.035	-.252	-.127

Note: Standard errors were created using 1,000 bootstrap replicates, and 95% confidence intervals (CIs) were created using bias-corrected SEs.

sity is the natural density of LP sites) treatments in the mesocosms. These were calculated by averaging the estimates for the two density designs. This assumes that there are no differences between LP and HP survival as a function of density. Phenotype growth rates and tests of effects are shown for both survival conditions. Reproductive values and stable size structures are shown in figures S5 and S6.

Evolutionary Invasion Analysis. We tested whether the observed interactions between phenotype and density are important in determining whether the LP phenotype can evolve using an evolutionary invasion analysis. Evolutionary invasion analysis asks this question: can one phenotype invade a population of another phenotype and be used to determine the evolutionary stable strategy (ESS)? Briefly, we used the model-averaged parameter estimates from the mesocosm experiment to construct density-dependent IPMs for each phenotype \times drainage. Because we did not have enough density treatments to determine the functional form of the density-dependent response curves, we assumed that the functional form could be described by either the exponentially decreasing Ricker function or a linear decrease. We then determined the equilibrium population state for pure and intermediate phenotypes using numerical iteration techniques. Once the population equi-

libria for each resident type was found, we then tested whether each of the other pure and intermediate types could grow (have positive population growth rates) in the context of the resident type. Details of this analysis can be found in the online supplementary information.

Results

We found that wild LP guppy populations were strongly regulated by density. Assuming stable size distributions, λ values for control treatments were not different from 1 for experimental design 2 (table 1; fig. 1). Control treatments were significantly less than 1 for experimental design 1 (table 1; fig. 1). Decreasing population density by 50% caused λ to increase from 0.96 to 1.06 in experimental design 1 and from 1.05 to 1.20 in experimental design 2 (table 1; fig. 1). Increasing population density by 200% decreased λ from 0.96 to 0.78, and increasing the population density by 150% reduced λ from 1.05 to 0.87 (table 1; fig. 1). The qualitative results were not sensitive to the assumption that populations were in a stable size distribution because most were very close to the stable condition in both types of density manipulations (figs. S1 and S2).

Decomposition of the population growth responses into contributions from each demographic rate showed that the demographic rates governing density dependence were

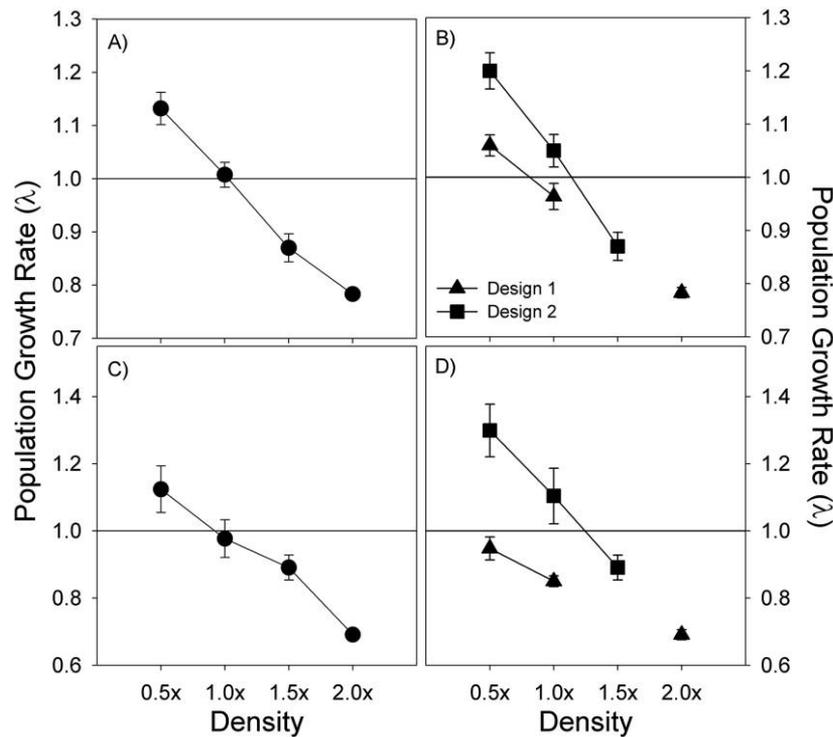


Figure 1: Projected population growth rates (λ) versus density treatments for field study. A and C show the λ values when the two experimental designs were combined. B and D show the λ values of each design separated. Upper panels assume stable size distribution. Lower panels calculated based on observed size distributions at the beginning of the experiment. Error bars are ± 1 SE.

different between increased and decreased density treatments. In general, the smallest, immature individuals had the largest total effect on population growth rates in the decreased density treatments (fig. 2). In contrast, reproductive size classes caused the largest decrease in population growth rates in the increased density treatments (fig. 2). Changes in survival usually had the largest effects, but somatic growth sometimes was comparable in magnitude (fig. 2).

Experiments in mesocosms with LP and HP populations of guppies at low and high densities show that at low density, HP phenotypes have higher λ than LP phenotypes (fig. 3). However, there was a strong interaction between phenotype and density (table 1; fig. 3). The nature of this interaction was such that at low densities, HP phenotypes had significantly higher rates of population growth than LP phenotypes, but this advantage evaporated at high densities. These results were not sensitive to whether we used survival estimates from the mesocosms or equal survival estimates among phenotypes taken from the field density manipulations (table 1). However, for the trial using guppies from the Aripo drainage, the nature of the interaction was more extreme when survival in the mesocosms was used (fig. 3). LP guppies live at higher densities compared

to HP guppies, so these results show that at least part of adapting to an LP environment also involves reducing the sensitivity of population growth rate to density.

The smallest guppies contributed the most to the interaction between phenotype and density for both stream replicates, but the effects of other size classes were non-trivial (figs. 4, S11). Somatic growth of the smallest size class contributed the most to the interaction, but no single demographic rate dominated the effect. This strong interaction between phenotype and density was evident even though we failed to find a significant interaction in any of the individual demographic variables at any size when we used standard inferential statistical techniques, with the exception of somatic growth of the smallest sizes (table 2). This indicates that it is the cumulative influence of many individual variables that drive the differential responses to density.

We examined the consequence of this interaction using an evolutionary invasion analysis. The analysis shows that the success of the LP phenotype in invading (or the ability of LP to invade) a population of HP guppies depends heavily on the density-dependent component of survival of HP guppies. When HP guppies have equal or slightly lower survival than LP guppies (fig. 5), incipient LP gup-

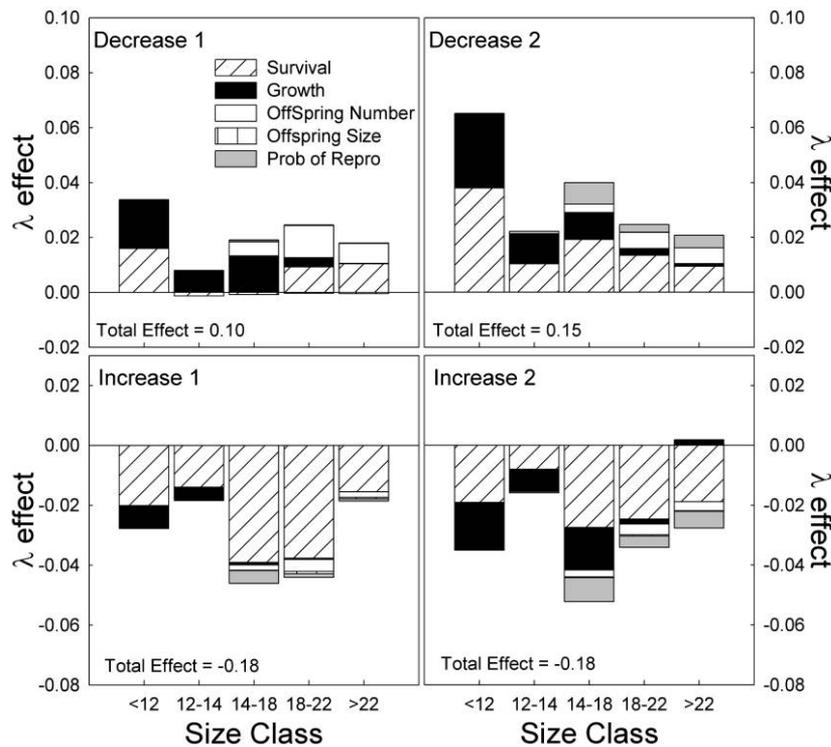


Figure 2: Size- and parameter-specific effects of density treatments on population growth rate for field study. Size classes correspond to immature individuals (<12 mm), juveniles (12–14 mm), individuals reproducing for the first time (14–18 mm), and those reproducing for the second or third time (18–22 mm, >22 mm). All are based on analysis with stable size distribution.

pies cannot invade the HP phenotype. However, further decreases in the density-dependent component of survival, such that LP guppies have higher survival rates at higher densities than HP guppies, allow incipient LP phenotypes to invade populations of HP guppies (lower two rows in fig. 5). Whether the pure LP phenotype is able to invade a population of HP phenotypes depends on the form of the density-dependent effects. If the density response is described by the Ricker function, then a 60% decrease in the density-dependent component of survival approaches but does not allow the evolution of the pure LP phenotype. If the density response is described by a linear function, then a 60% decrease in the density-dependent component of survival does not allow the pure LP phenotype to invade in the Guanapo drainage but is very close for the Aripo drainage.

Discussion

Previous studies of life-history evolution in Trinidadian guppies show that LP and HP populations display genetically based differences in life-history traits and that the divergence between population types is ultimately caused by the difference in predator abundance (Reznick 1982;

Reznick and Endler 1982). However, the mechanistic link between predators and guppies that had been assumed to drive this adaptive divergence—size-specific differences in mortality rates—cannot, by itself, explain these patterns (Reznick et al. 1996). Here we show that inclusion of the effects of population regulation and density-dependent selection is required to explain adaptive life-history evolution among guppy populations. Hence, our past investigations of life-history evolution got the correct answer about life-history diversification but for the wrong reasons. How these results generalize to other natural systems is unknown, but given that substantial effects of density on vital rates appear frequently in natural populations (Capuccino and Price 1995; Bassar et al. 2010a), it is likely that our result will be common.

By extension, if intraspecific density dependence is important in determining the evolutionary life-history optimum, then this demonstrates a role for feedbacks between ecological and evolutionary processes. This is because phenomena like density-dependent natural selection and frequency-dependent natural selection are black-box descriptions for these feedbacks. In many cases, we do not know the internal links that make the connections between the phenotypes and their ecological effects and

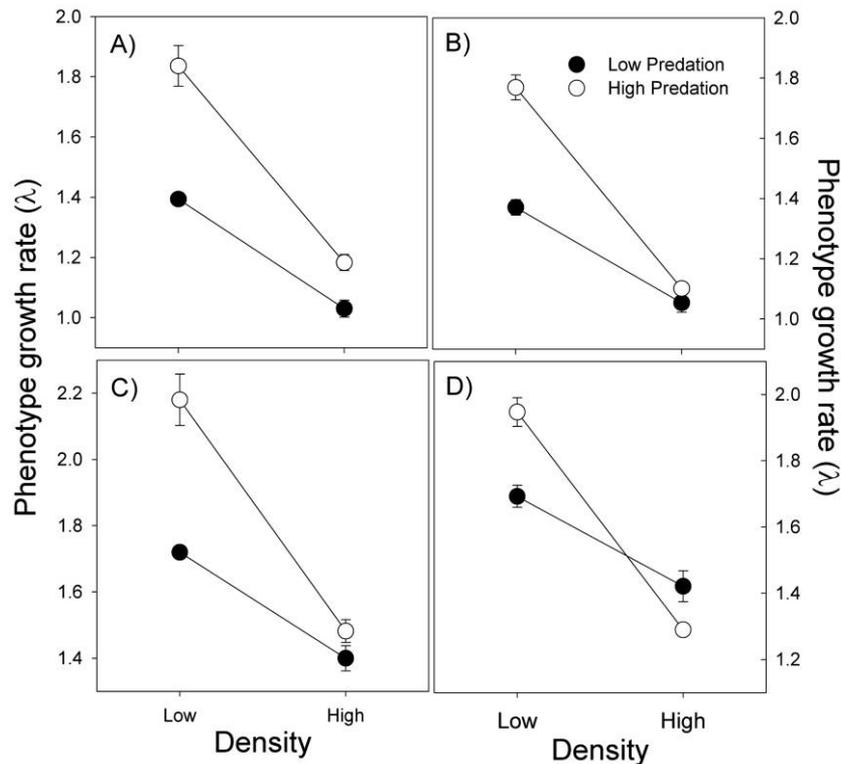


Figure 3: Population growth rates of each phenotype under low- and high-density conditions in the mesocosms. Upper panels are calculated using the survival estimates from the density manipulations with equal survival between phenotypes. Lower panels are based on survival estimated from the mesocosm experiment. *A* and *C* are for Guanapo, and *B* and *D* are for Aripo. Error bars are ± 1 SE.

how these effects then feed back to influence fitness. In this study, we have shown that the black box is important in the evolution of the guppies. Through comparative studies in the wild and experiments in mesocosms, we have begun to illuminate the inner workings by showing that changes in density and exchanging the two guppy phenotypes alter the stream environment in independent ways (Bassar et al. 2010*b*; Zandonà et al. 2011). HP phenotypes consume more, higher-quality invertebrates and less algae compared to the LP phenotypes. Lower consumption of invertebrates combined with the observation that LP guppies live at higher population densities than HP guppies suggest that LP guppies have evolved to be more generalist foragers, possibly as a function of decreased per capita resource availability. We have recently shown that these dietary preferences likely have evolved through complex ecosystem feedbacks that involve far-reaching direct and indirect ecosystem effects of guppy foraging (Bassar et al. 2012).

Understanding the demography and numerical dynamics of populations is important for the study of selection because demography and dynamics determine the most appropriate measure of fitness for generating evolutionary

predictions. If populations are growing exponentially, then the Malthusian growth parameter (m) is appropriate. If populations are at equilibrium, then the net reproductive rate (R_0) should be used but only when regulation is achieved by equivalent adjustment of vital rates across all sizes or ages. The results from our in situ density manipulations show that LP guppy populations are regulated. The results from the mesocosm experiments show that fitness in guppy populations is density dependent. In the in situ density experiments, the mechanism of regulation depends on the direction of change in population size; decreasing population density caused increased growth rates primarily by altering demographic rates of the smallest individuals, and increasing population density decreased population growth rates through effects on larger, reproducing individuals. Likewise, it was not the case that regulation was achieved by altering a single vital rate. The life table response (LTRE) analyses instead show that multiple life-history traits make substantial contributions to the changes in population growth rate in response to either increased or decreased population density.

When simple measures of fitness are insufficient, measuring fitness requires identifying, for each of the alter-

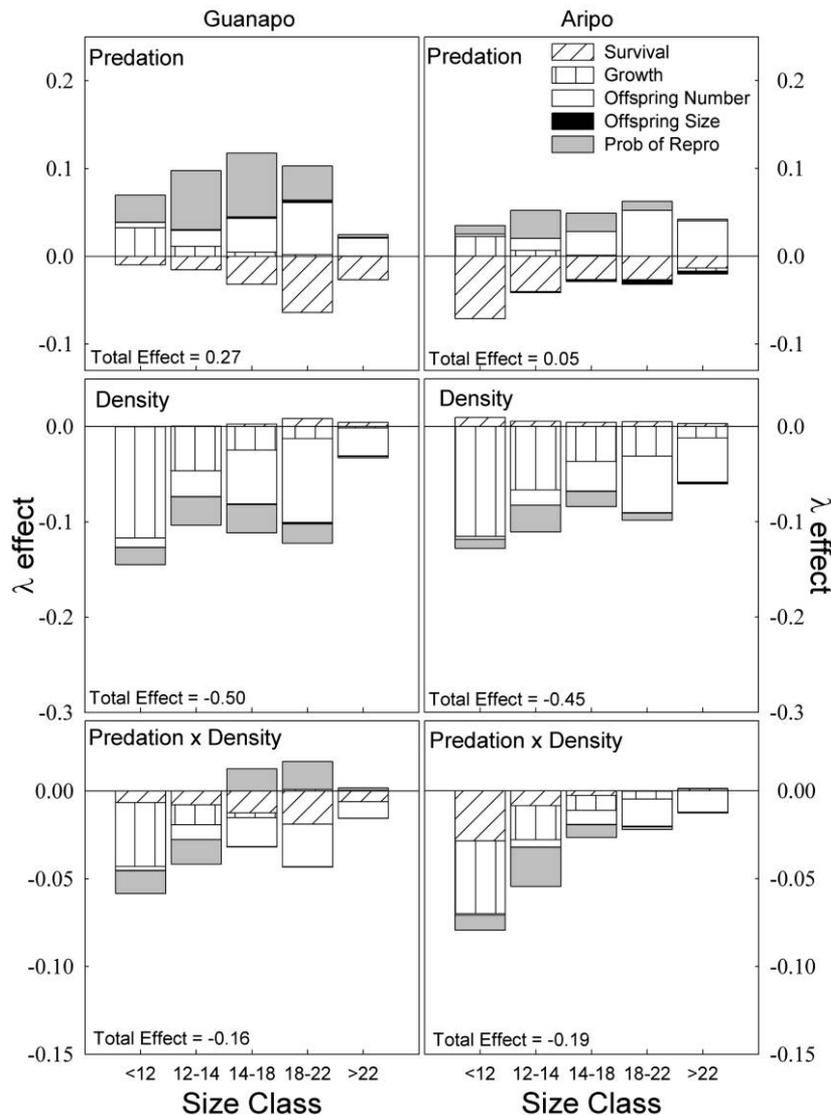


Figure 4: Size- and parameter-specific effects of predation, density, and predation \times density interaction on phenotype growth rate when survival is estimated from the mesocosm study. Size classes correspond to immature individuals (<12 mm), juveniles (12–14 mm), individuals reproducing for the first time (14–18 mm), and those reproducing for the second or third time (18–22 mm, >22 mm). Phenotype contrasts are based on HP-LP, and density contrasts are based on high-low density.

native phenotypes under consideration, how each stage's vital rates are affected by density regulation and how each phenotype fares in combination with the others (Mylius and Diekmann 1995; Metz et al. 1996). This is the framework of invasibility analysis, where fitness is defined as the ability of a rare genotype to invade in the demographic context determined by the numerically dominant genotype (Metz et al. 1992; Ferriere and Gatto 1995). In our mesocosm experiment, high and low densities were chosen to represent the demographic environments of low- and high-predation guppies, respectively.

The invisibility framework is particularly relevant for considering the evolution of LP populations. Molecular evidence suggests that HP guppies, in downstream reaches, are ancestral to LP populations farther upstream (Alexander et al. 2006). Thus, LP populations are established when HP phenotypes make it over a barrier waterfall and evolve in situ in the previously guppy-free stream reaches where the lack of predators allows guppy populations to grow to higher population densities than in the downstream reaches. Our results suggest that this increase in density (or biomass) changes the environment and that it

Table 2: Test statistic (*t* value) and df (subscripts) from contrasts of fish treatment from linear mixed models of demographic parameters from field study

	Growth	Survival	Offspring number	Offspring size	Probability of reproduction		
Small (12 mm):							
Decrease1	2.3 ₁₄	1.8 ₁₃	.5 ₁₅	-.2 ₁₄	...		
Decrease2	2.2 ₁₄	-.4 ₁₃	1.8 ₁₅	.6 ₁₄	...		
Increase1	- 2.7 ₁₄	-.8 ₁₃	-1.1 ₁₅	- 2.1 ₁₄	...		
Increase2	-1.1 ₁₄	-1.6 ₁₃	-1.0 ₁₅	-.2 ₁₄	...		
Centered (18 mm):							
Decrease1	.4 ₁₄	-.2 ₁₃	.9 ₁₅	.2 ₁₄	1.4 ₁₄		
Decrease2	2.7 ₁₄	-.1 ₁₃	2.0 ₁₅	.1 ₁₄	.3 ₁₄		
Increase1	- 3.2 ₁₄	-1.6 ₁₃	-1.1 ₁₅	- 2.6 ₁₄	- 2.1 ₁₄		
Increase2	-.4 ₁₄	- 2.0 ₁₃	-.9 ₁₅	-1.4 ₁₄	-.8 ₁₄		
Large (24 mm):							
Decrease1	-1.0 ₁₄	-.3 ₁₃	1.0 ₁₅	.4 ₁₄	...		
Decrease2	1.3 ₁₄	.6 ₁₃	1.8 ₁₅	-1.6 ₁₄	...		
Increase1	-1.6 ₁₄	-1.5 ₁₃	-.8 ₁₅	-1.3 ₁₄	...		
Increase2	.5 ₁₄	- 1.7 ₁₃	-.5 ₁₅	- 2.6 ₁₄	...		
		<u>Guanapo</u>	<u>Aripo</u>		<u>Guanapo</u>	<u>Aripo</u>	
Small (12 mm):							
Phenotype	1.4 _{19,7}	- 1.9 _{271,9}	- 2.0 _{271,9}	3.4 _{19,8}
Density	- 9.3 _{19,7}	.5 _{271,9}	.5 _{271,9}	- 2.2 _{19,8}
Phenotype × density	- 2.4 _{19,7}	-.4 _{271,9}	-.6 _{271,9}	-.9 _{19,8}
Centered (18 mm):							
Phenotype	.6 _{19,7}	- 2.1 _{271,9}	- 2.1 _{271,9}	3.6 ₂₀	12.6 ₁₆	- 17.0 ₁₆	3.3 _{19,8}
Density	- 7.6 _{19,7}	.5 _{271,9}	.5 _{271,9}	- 4.8 ₂₀	- 2.0 ₁₆	-.8 ₁₆	- 2.0 _{19,8}
Phenotype × density	-1.0 _{19,7}	-.5 _{271,9}	-.5 _{271,9}	- 4.2 ₂₀	-.3 ₁₆	-.4 ₁₆	-.8 _{19,8}
Large (24 mm):							
Phenotype	-.4 _{19,7}	- 1.9 _{271,9}	- 1.8 _{271,9}	2.8 _{19,8}
Density	- 2.5 _{19,7}	.5 _{271,9}	.5 _{271,9}	-1.4 _{19,8}
Phenotype × density	.7 _{19,7}	-.6 _{271,9}	-.4 _{271,9}	-.6

Note: Small, Centered, and Large indicate values of the covariate where treatment effects are evaluated. When there was no interaction between size and the main effects, only the contrasts centered at medium size (18 mm) are shown. Contrasts for the field study were constructed such that positive *t* values indicate increases over controls and negative values indicate decreases over controls. Contrasts for the mesocosm experiment were constructed such that positive *t* values for the predation and density effects indicate increased values in the high-predation and high-density treatments. Negative values indicate decreased values for high predation and high density. The interaction was constructed such that negative *t* values indicate smaller differences between the phenotypes at high density compared to the low-density treatments. When interactions between drainage and treatment were included in the average model for the mesocosms experiment, separate contrasts are shown for each drainage. Values with $P < .05$ are shown in bold.

is this guppy-modified environment that selects for the evolution of the LP life history.

Despite clear evidence in the mesocosm experiment for an interaction between density and LP versus HP phenotype in relative fitness, we failed to find many significant interactions between phenotype and density in individual life-history traits (table 2). Previous experiments with lab-reared LP and HP guppies at two food levels showed that life-history traits are plastic to resource levels but also failed to find significant interactions between the genotype and food level for all traits except somatic growth rates (Reznick 1982; Reznick et al. 2004; Arendt and Reznick 2005). The results presented here indicate that even small

differences in individual traits, which may not be statistically different using standard inferential statistics (e.g., ANOVAs), can be important when the effects of those differences jointly contribute to measures of fitness indexed on population growth rate. They also indicate that using values of life-history traits like fecundity as surrogates for genuine measures of fitness can be grossly misleading (Travis and Henrich 1986). For understanding life-history evolution in guppies, these results suggest that life-history traits themselves cannot fully account for adaptation to living at high or low densities of other guppies. Additional targets of density-dependent selection may be features that affect activity patterns, foraging rates, feeding

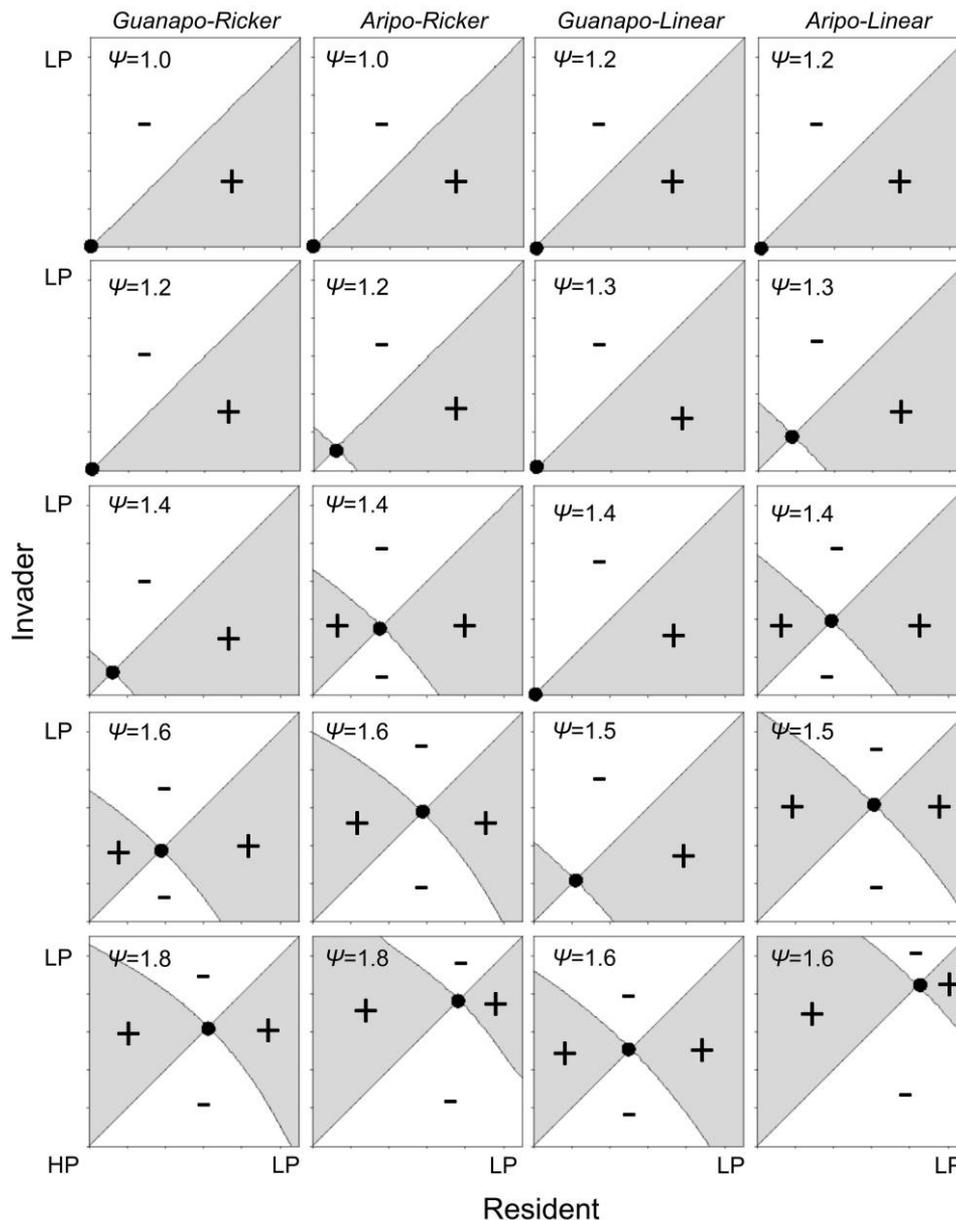


Figure 5: Pairwise invasibility plots (PIPs) from the evolutionary invasion analysis. Intersection of the two isoclines indicates the evolutionary stable strategy (ESS). Gray areas in each graph represent invader-resident combinations where the invader can exhibit positive population growth. White areas represent invader-resident combinations where the invader exhibits negative population growth. Dots at intersection of isoclines represent equilibrium values. Two left columns are the results when the exponentially decreasing Ricker density-dependent function is assumed. Right two columns are when the linear function is assumed. Within each, the left column is for the Guanapo and right column is for the Aripo drainages. Rows represent increased sensitivity of the density-dependent component of survival for the HP phenotype. ψ values are indicated in the upper left of the left-most panels. $\psi = 1.0$ is the case where density-dependent survival of HP and LP are equal.

habits and efficiency, or the ability to cope with the stress of crowding at high densities or the stress of predator avoidance at low densities (Wingfield and Sapolsky 2003; McCormack 2006; Walsh and Reznick 2011).

Despite the results presented here, there are still some

outstanding questions. First, the results of the evolutionary invasion analysis show that the incipient LP phenotypes can invade the ancestral HP resident phenotypes, but whether or not they fully evolve to the observed LP phenotype depends on the density-dependent survival of the

HP individuals (fig. 5). Gordon et al. (2009) showed that the evolved LP phenotypes have higher survival rates than newly transplanted ancestral HP fish following an experimental introduction. They do not know whether these changes in survival are related to density dependence or other differences in the LP community. They also do not report the size-specific survival rates, so their data cannot address whether or not the newly evolved LP phenotype could displace the HP phenotype.

Second, we have characterized how the life histories of LP and HP guppies respond to density, but life histories may not be the only difference between the LP and HP phenotypes that govern their fitness in these alternative environments. The analyses presented here assume that density dependence is really something more like biological demand dependence. This is because the density effects are manifested through the effect of allometry and metabolic demands. We know that the growth allometry of the two phenotypes is different—LP guppies and HP guppies have different shapes (Langerhans and DeWitt 2004). We do not know whether other features such as resting or maximum metabolic rates are different between the two phenotypes. Comparative studies along life-history gradients in other taxa have shown that life history tends to covary with physiological attributes, including metabolic rates and immunocompetence (Ricklefs and Wikelski 2002). Under this hypothesis, HP guppies should have higher metabolic rates to facilitate higher reproductive output. If so, then they may require more food and/or higher-quality food, such that they have a higher per capita influence on the environment. Such a difference in demand would yield a further advantage to the LP phenotype under higher-density conditions.

Finally, we have considered only the effects of intraspecific density dependence. LP streams also contain killifish that may simultaneously compete with and consume some size classes of guppies. When guppies invade the upstream reaches, killifish decrease in number and biomass and also evolve different life histories (Walsh and Reznick 2008, 2010, 2011). If resource overlap between the two species is significant, then this would increase the effective densities of each species leading to interspecific density dependence. If these relationships are altered as a function of the phenotypes of the two fish species, then this would incorporate interspecific frequency dependence (*sensu* Roughgarden 1976). In particular, if resource partitioning is minimal when HP guppies first invade sites with only killifish, then the effective densities of both species would increase, and then selection should favor the evolution of increased partitioning among the species. If part of evolving the LP guppy phenotype involves decreasing resource overlap with killifish, then this could also increase the ability of the LP phenotype to evolve. This scenario seems

likely, since killifish feed almost exclusively on invertebrates and LP guppies have evolved to consume more algae and fewer invertebrates compared to HP phenotypes.

If intra- and interspecific density and frequency dependence are important, then it suggests that evolutionary studies may not be able to accurately predict the outcome of evolutionary dynamics without considering the broader ecological and coevolutionary context in which they occur. For guppies, it may be precisely these types of dynamic interactions that give the LP phenotype the extra fitness advantage to displace the HP phenotype. This study, combined with our previous assessment of the differential effects of the guppy phenotypes on the environment, argues for a critical role of ecoevolutionary feedbacks in determining the evolution of life history and other aspects of the phenotypes of guppies. Because it is so often true that the presence or absence of predators has similar impacts on the abundance of their prey, followed by cascading effects on ecosystem structure (e.g., Estes et al. 2011), we anticipate a similar, critical role of ecoevolutionary feedbacks in a diversity of ecosystems.

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