

Direct and Indirect Ecosystem Effects of Evolutionary Adaptation in the Trinidadian Guppy (*Poecilia reticulata*)

Ronald D. Bassar,^{1,*†} Regis Ferriere,^{2,3,*} Andrés López-Sepulcre,^{1,2} Michael C. Marshall,⁴ Joseph Travis,⁵ Catherine M. Pringle,⁴ and David N. Reznick¹

1. Department of Biology, University of California, Riverside, California 92521; 2. Laboratoire Ecologie et Evolution, CNRS Unité Mixte de Recherche 7625, École Normale Supérieure, Paris, France; 3. Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721; 4. Odum School of Ecology, University of Georgia, Athens, Georgia 30602; 5. Department of Biological Sciences, Florida State University, Tallahassee, Florida 32306

Submitted April 14, 2011; Accepted April 1, 2012; Electronically published June 22, 2012

Online enhancements: supplementary information PDF. Dryad data: <http://dx.doi.org/10.5061/dryad.pj846h4f>.

ABSTRACT: Ecological and evolutionary processes may interact on the same timescale, but we are just beginning to understand how. Several studies have examined the net effects of adaptive evolution on ecosystem properties. However, we do not know whether these effects are confined to direct interactions or whether they propagate further through indirect ecological pathways. Even less well understood is how the combination of direct and indirect ecological effects of the phenotype promotes or inhibits evolutionary change. We coupled mesocosm experiments and ecosystem modeling to evaluate the ecological effects of local adaptation in Trinidadian guppies (*Poecilia reticulata*). The experiments show that guppies adapted to life with and without predators alter the ecosystem directly through differences in diet. The ecosystem model reveals that the small total indirect effect of the phenotype observed in the experiments is likely a combination of several large indirect effects that act in opposing directions. The model further suggests that these indirect effects can reverse the direction of selection that direct effects alone exert back on phenotypic variation. We conclude that phenotypic divergence can have major effects deep in the web of indirect ecological interactions and that even small total indirect effects can radically change the dynamics of adaptation.

Keywords: food webs, evolution, eco-evolutionary feedback, indirect effects.

Introduction

There is growing evidence that evolutionary responses to ecological change can be measured over observable time (Hendry and Kinnison 1999; Fussmann et al. 2007). A well-known example is found in work on the Galapagos

ground finch *Geospiza fortis* evolving in response to fluctuating rainfall and correlated changes in available food resources (Grant and Grant 2002). Reznick and Ghalambor's (2001) review summarizes 47 studies demonstrating or implying rapid evolution in morphology, physiology, phenology, behavior, and life-history traits.

The realization that evolution can be rapid enough to happen in ecological time opens the door to the possibility that ecology and evolution are processes interlocked with one another in a dynamic feedback loop (Roughgarden 1971; Metz et al. 1992; Heino et al. 1998; Sinervo et al. 2000; Dieckmann and Ferriere 2004; Dercole and Rinaldi 2008), a notion tracing back to Chitty's (1960) and Pimentel's (1961) ideas of genetic feedback and cycling selection. Theory and laboratory experiments on simple ecological systems suggest that the interplay of ecology and evolution on a common timescale generates ecological patterns that are not predicted when the evolutionary dynamics are ignored (Loeuille et al. 2002; Yoshida et al. 2003; Dieckmann and Ferriere 2004; Dercole and Rinaldi 2008). While understanding the selective action of ecological factors has long been a focus of evolutionary biology, there is now a pressing need for integrative studies to quantify the effect of adaptive trait evolution on community and ecosystem dynamics, understand the paths through which these effects are manifested, and explore the subsequent feedback to a new selective milieu (Fussmann et al. 2007; Schoener 2011).

Several experimental studies in the field or using in situ mesocosms have shown that adaptation in a target species can have demonstrable ecological effects on its surrounding community or ecosystem (Post et al. 2008; Harmon et al. 2009; Palkovacs et al. 2009; Bassar et al. 2010). None of these studies have evaluated the individual ecological

* These authors contributed equally to this work.

† Corresponding author; e-mail: rdbassar@yahoo.com.

Am. Nat. 2012. Vol. 180, pp. 167–185. © 2012 by The University of Chicago. 0003-0147/2012/18002-5297\$15.00. All rights reserved.
DOI: 10.1086/666611

pathways through which these net effects were generated. The ecological effects of adaptation may be mediated by direct pathways that involve the physical interaction of the target species with another species, as in predation, parasitism, or interference competition. The ecological effects of adaptation may also be mediated by indirect pathways and thus represent second- or higher-order effects through which the target species interact with other community members via a shared resource, as in exploitative competition, a common predator or mutualist that influences all of them, or through an induced change in the physical environment, such as changes in the amount of available limiting nutrients (Wootton 1994; Werner and Peacor 2003).

This is a pressing issue for understanding both ecology and evolution. If ecological effects of evolutionary change in a target species are largely restricted to direct pathways, those effects will have a limited scope on community structure and ecosystem processes. Alternatively, if they propagate through the web of indirect ecological interactions, they can have more profound and global effects on community dynamics and ecosystem function.

We know that indirect effects triggered by ecological (nonevolutionary) factors can have considerable impact on community organization and ecosystem processes (Strauss 1991; Wootton 1994; Schmitz 2009) and that multiple indirect effects can swamp the influence of direct ecological effects (Stone and Roberts 1991; Miller 1994). We know little about the efficacy of either indirect ecological effects as agents of selection or the extent to which adaptive evolution will feed back into ecology via indirect effects. Miller and Travis (1996) predicted that evolution would proceed differently under different combinations of direct and indirect ecological effects, a prediction supported by terHorst's (2010) experiments. If Miller and Travis's logic were taken further, applying it to a case in which the direct and indirect effects of a species on its ecosystem changes as it evolves and adapts to its environment, then it suggests that the associated direct and indirect effects would also change, which in turn would change the selection on and subsequent evolution of the target species. Thus, the logic suggests that indirect effects could ultimately alter the results expected solely from considering direct effects alone.

The goal of this study is to integrate mesocosm experiments and mathematical modeling in order to decouple and evaluate the direct and indirect ecological effects of adaptive phenotypic divergence in a focal species, the Trinidadian guppy (*Poecilia reticulata*). Guppies function as primary and secondary consumers in the ecosystem. We employ different phenotypes of guppies that represent the outcome of genetic adaptation to different predatory environments. While direct ecological effects can be mea-

sured relatively easily, experimentally documenting indirect effects in complex webs of species interactions is more challenging. Theoretical and empirical studies indicate that predicting the sign and magnitude of indirect effects in an ecosystem from knowledge of direct effects alone is non-trivial (Leroux and Loreau 2010). The problem is exacerbated in communities with a significant number of omnivores that exploit several trophic levels (Polis and Strong 1996). Because indirect effects represent, by definition, two or more links (second- or higher-order effects), they are often not quantifiable through simple observational studies. When there are two or more possible indirect pathways between two ecosystem components, experimental studies that document indirect effects measure the total effect of all possible indirect links in the system. If two or more separate indirect links have an opposing influence, then this may show up as a small and possibly nonsignificant total indirect effect, even though the effect of each individual pathway may be very large.

Our study overcomes these difficulties by combining controlled experiments with the numerical analysis of a mechanistic model of ecosystem dynamics. Our approach involves a replicated, factorial experiment in mesocosms in which we manipulate guppy presence, phenotype, and densities. Our different phenotypes were guppies adapted to high- versus low-predation environments. Our different guppy population densities were representative of average densities observed in natural high-predation (low density) and low-predation (high density) environments. We used electric enclosures that excluded guppies and their direct effects to quantify the total indirect effect of guppies. We then used these results to parameterize the ecosystem model and hence evaluate the contributions of each indirect ecological pathway to the total indirect effect of guppies. This three-pronged approach allows us to estimate the direct ecological effect of phenotypic divergence while controlling for the effect mediated by the difference in population density between phenotypes and then compute the indirect ecological effects of phenotypic divergence and compare them with the direct ecological effect. We use these results to discuss the potential for direct and indirect ecological effects of the phenotype to feed back on guppy adaptive evolution and thus close the eco-evolutionary feedback loop.

Methods

In guppies, phenotypic divergence is associated with environments that differ in fish community composition and risk of predation. Guppies from low-predation (LP) communities coexist with the killifish (*Rivulus hartii*), which may prey on smaller size classes of guppies (Haskins et al. 1961). Guppies from high-predation (HP) communities

coexist with a variety of predatory fish species, including the pike cichlid (*Crenichla alta*) and the wolf fish (*Hoplias malabaricus*), that prey on adult size classes of guppies (Haskins et al. 1961; Reznick and Endler 1982).

LP and HP guppies differ genetically in their life-history adaptations to these divergent predation regimes (Reznick 1982). Guppies from HP communities mature at a younger age and a smaller size than their LP counterparts (Reznick 1982; Reznick and Endler 1982). HP guppies also have more, smaller offspring than LP fish and overall invest more resources in reproduction (Reznick 1982; Reznick and Endler 1982). The higher death and birth rates in HP environments combine to cause lower overall population densities and populations dominated by small young fish in comparison to LP environments (Reznick et al. 1996, 2001; Rodd and Reznick 1997). Moreover, many of the adaptive genetic differences between HP and LP guppies will evolve on ecologically relevant timescales. Reznick and Bryga (1987) and Reznick et al. (1997, 1990) report significant changes in time intervals of 4–11 years. Guppies in HP versus LP environments can differ in their impact on the ecosystem because of their differences in population density or size structure, their evolved differences in how they utilize their environment, such as diet, or other differences in phenotype, such as the excretion of nutrients. In a replicated common garden mesocosm experiment, mesocosms stocked with LP guppies had lower standing stocks of algae and more invertebrates compared with mesocosms stocked with HP guppies after only 28 days (Bassar et al. 2010). Gut content analysis from guppies in the mesocosms and from the wild showed that LP guppies consume more algae and fewer invertebrates than HP guppies (Bassar et al. 2010). Not surprisingly, given their respective diets, LP guppies had lower nitrogen excretion rates than HP guppies.

These differences between LP and HP phenotypes in ecosystem effects could simply be the result of differences in dietary preference (direct, first-order effect). However, because HP guppies also consume more invertebrates than LP guppies, they may strengthen a trophic cascade that leads to increased algal stocks (second-order, consumptive indirect effect). Also, increased algal stocks could result from the increased nutrient recycling rates seen in HP guppies (second-order nonconsumptive indirect effect). Higher-order indirect effects of guppy phenotype could also control ecosystem processes. This would be the case if higher invertebrate consumption by HP guppies would weaken the nutrient recycling pathway of invertebrate excretion, a third-order effect resulting from the combination of a direct consumptive effect and an indirect nonconsumptive effect.

Experimental Setting

We used 16 flow-through mesocosms ($\sim 3 \text{ m} \times 0.5 \text{ m}$) near a natural stream in the Arima Valley on the island of Trinidad. Details of their construction can be found in the study by Bassar et al. (2010), which describes a different set of results from this experiment. Here we summarize their key features. The benthic substrate consisted of a mixture of sand and gravel set to a depth of $\sim 5 \text{ cm}$. Flowing water came from a nearby fish-free spring and was set to an average depth of 16 cm. We allowed the mesocosms to sit with running water for 8 days to allow the buildup of epilithon and detritus. After this period, we inoculated the mesocosms with samples of the stream invertebrate community collected from low flow rate sections of a nearby midpredation stream. We collected invertebrates from an area comparable to the total benthic area of all 16 mesocosms. We removed large predatory taxa (primarily odonates) from these collections to avoid artifacts due to unevenly distributing rare predators that could have unpredictable effects on the individual mesocosms.

We ran this experiment twice, first using guppies from HP and LP localities from the Guanapo drainage and then from the Aripo drainage. In each trial, guppies were added 8 days after invertebrates to allow the latter to acclimate and disperse within the mesocosms. Each mesocosm received one of five guppy treatments. Four mesocosms received no guppies (no fish), and the remaining mesocosms received one of four crossed phenotype by density factors (LPLD, low predation–low density; LPHD, low predation–high density; HPLD, high predation–low density; HPHD, high predation–high density). Treatments were arranged in three spatial blocks with one treatment replicate per block except one block that received two no-fish treatments. High-density (24 individuals) and low-density (12 individuals) treatments were chosen to reflect the natural densities for HP and LP localities, on the basis of previous surveys of these communities (Reznick and Endler 1982; Rodd and Reznick 1997; Reznick et al. 2001); the sex ratios and size structure of the fish populations in each treatment were the mean of the size distributions across HP and LP sites (Reznick and Endler 1982; Reznick et al. 2001). Details of initial and final densities and biomass of guppies in each fish treatment are reported by Bassar et al. (2010). Twenty-eight days after the guppy introduction, we measured the responses of the ecosystem to the different guppy treatments.

We were able to partition the net effects of each fish effect on algae (fish presence, phenotype, and density) into first-order and higher-order components by excluding fish from a small portion (4%) of each mesocosm, using electrified enclosures (Pringle and Blake 1994; Connelly et al. 2008). Enclosure plots consisted of $12 \times 50\text{-cm}$ copper

frames attached to Speedrite Viper 3000 electric fence chargers delivering 3 J every 2 s (for details, see Pringle and Blake 1994; Connelly et al. 2008). Nonelectrified control plots consisted of an identical, nonelectrified copper wire frame. Weekly observations confirmed that while guppies frequented the nonelectrified plots, they never entered the electrified enclosures.

We measured chlorophyll *a* accrual in each enclosure by placing five ceramic tiles (5 cm × 5 cm) as substrate and collecting them at weekly intervals. On the final sampling (day 28), we collected two tiles per enclosure. Tiles were scrubbed and chlorophyll *a* measured using standard fluorometric techniques (Steinman et al. 2006). We measured leaf decomposition rate by placing five leaf packs in each plot, removing and drying one leaf pack weekly, and regressing the natural log–transformed percent remaining against time. The slope of this regression was used as a measure of the decay rate (*k*; Benfield 2006). Leaf packs were constructed by attaching 3.0 g of dried blackstick leaves (*Pachystachys coccinea*) by their petioles using binder clips. On the final day of the experiment (day 28), we sampled the benthic invertebrate community and benthic organic matter (BOM) by sealing off a circular area of the benthos with an 11.75-cm-diameter polyvinyl chloride pipe and collecting all the material inside it after sieving through a 63- μ m sieve. Samples were stained with rose bengal for 24 h, and invertebrates were separated from the BOM under a dissecting scope. Invertebrates were identified to the family or genus level, counted, and measured for length. Biomass estimates were obtained using known length–mass regression equations (Feller and Warwick 1988; Benke et al. 1999; Baumgärtner and Rothhaupt 2003). We present analyses of biomass estimates for non-predatory taxa. We also report analyses of the mean size and count for the most common of these taxa (Chironomidae) to examine which of these factors were responsible for changes in biomass. The remaining BOM was dried and ashed in a muffle furnace to obtain ash-free dry mass.

Statistical Analyses

We analyzed the final biomass of ecosystem compartments using linear mixed models. We included fish treatment type as a fixed effect with five levels (no fish, LPLD, LPHD, HPLD, HPHD) and plot type as a fixed effect with two levels (electrified and nonelectrified). We treated mesocosm identity as a random effect nested within fish treatment to estimate the proper error and degrees of freedom for the treatment effect (Kutner et al. 2005). We included drainage of origin and spatial block as fixed effects. Interactions between drainage and treatment and plot type were initially included in the model. We used separate

error variances for the enclosure or treatment effects if a likelihood ratio test indicated that they were different. We fit the complete fixed effects model with alternative error structures using restricted maximum likelihood. Once the most parsimonious error structure was chosen, we employed maximum likelihood techniques and used the Akaike information criterion (AIC) to decide the removal of fixed effects. Models were selected using an Δ AICc of 4. The best model was then rerun using restricted maximum likelihood to obtain unbiased fixed effects parameter estimates.

We used a series of planned, partial interaction contrasts to separate and test the direction and magnitude of ecosystem effects attributable to guppy presence, phenotype, and density. All contrasts included both the treatment main effect and the treatment by enclosure type interaction. We calculated three contrasts for each fish treatment main effect: net contrast, direct contrast, and indirect contrast. The net (direct + indirect) contrast between two treatments was constructed to represent the difference between nonelectrified plots of the treatments of interest. The indirect contrast was calculated from the difference between the electrified plots of the treatments of interest. We calculated the direct contrast by subtracting the indirect contrast from the net contrast.

Mechanistic Model

We built a mathematical model of ecosystem dynamics to estimate the indirect effects of phenotypic divergence that are mediated by one or more ecosystem variables between fish and algae. The model describes the dynamics through time of the concentration of dissolved nutrients (nitrogen), the biomass of benthic organic material (BOM), primary producers, invertebrates, and fish (fig. 1). As intended by experimental design, in the model the effect of leaf packs was neglected. We parameterized and initialized the model with data from the experiment and other independent data sets (table A1). Parameters for which no reliable measurements were available were estimated by fitting the ecosystem model to all the experimental data at once (see appendix). Model fitting followed the standard scheme of sensitivity analysis to select identifiable parameters and estimation of identifiable parameters by least squares. This was done here by using the R package FME (Soetaert and Petzoldt 2010). The FME algorithm computes approximate estimates of parameter uncertainty by linearization of the model around the best-fit parameters and provides samples of the data-dependent probability distribution of the parameters by using a Markov chain Monte Carlo (MCMC) method.

We used the fitted model to repeat the experiment in silico and compute the outcome of fish exclusion by elec-

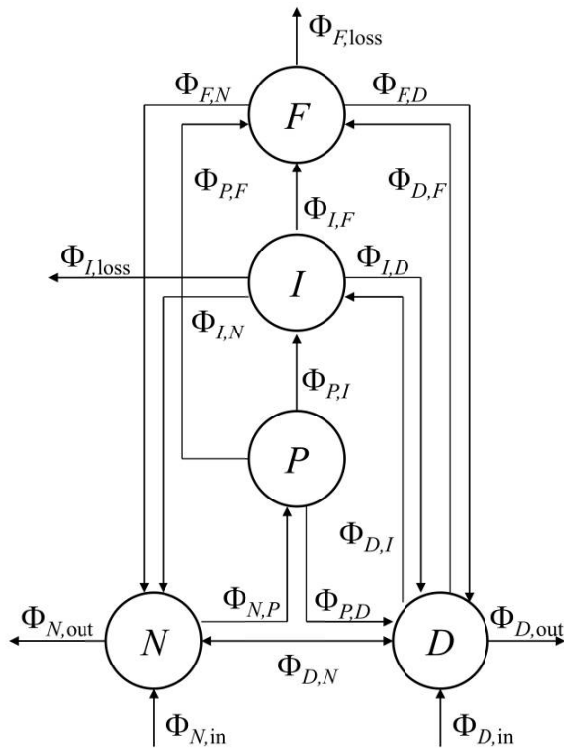


Figure 1: Structure of the ecosystem model. Primary producers are restricted to periphyton (P) and are possibly limited by nitrogen (N). Other ecosystem compartments are detritus as benthic organic matter (D), invertebrates (I), and fish (F). Nutrients move up the food chain by fixation by primary producers ($\Phi_{N,P}$), predation on primary producers and detritus by invertebrates ($\Phi_{P,D}$, $\Phi_{D,I}$) and fish ($\Phi_{P,P}$, $\Phi_{D,F}$), and predation on invertebrates by fish ($\Phi_{I,P}$). Fish and invertebrates return nutrient to N by respiration and excretion ($\Phi_{F,N}$, $\Phi_{I,N}$). The nutrient flow $\Phi_{P,D}$ from primary producers to detritus is driven by mortality; $\Phi_{I,D}$ from invertebrates to detritus is driven by egestion and mortality; $\Phi_{F,D}$ from fish to detritus is driven by egestion only; fish biomass lost by mortality is small and leaves the system ($\Phi_{F,loss}$). Invertebrates that emerge are lost from the system ($\Phi_{I,loss}$). Decomposition causes a two-way flow between N and D ($\Phi_{D,N}$) involving mineralization from D to N and immobilization from N to D . The water flow brings nutrient and detritus in ($\Phi_{N,in}$, $\Phi_{D,in}$) and out ($\Phi_{N,out}$, $\Phi_{D,out}$) of the system.

tric enclosures for each fish treatment. We assumed that nitrogen, BOM, and invertebrates were well mixed throughout the entire system, including both plot types, and that the exclusion of fish from the (small) electrified enclosures and its impact on periphyton within the enclosures had no significant feedback effect on the global ecosystem dynamics. Thus, periphyton dynamics in both types of enclosures were driven by the global mesocosm dynamics of nitrogen, BOM, invertebrates, and fish, and the

model predicted the effect of fish exclusion on algal growth and stock (see appendix). Indirect and direct contrasts were measured via the same contrast matrix used for the experimental analysis; confidence intervals were computed by using the MCMC-generated sample of the parameter distribution.

Since we explicitly modeled the mechanisms contributing to the fluxes between ecosystem compartments, we were able to directly evaluate the effect of fish phenotype mediated by fish predation (direct effect) and the two components of the total indirect effect (see fig. 1): the effect mediated by invertebrate predation (trophic cascade) and the effect mediated by nutrient uptake (i.e., production). The mathematical derivation is presented in the appendix, and the general result is encapsulated in equation (A11). The model further allowed us to partition the latter into its three components: the effects mediated by fish excretion, invertebrate excretion, and remineralization via decomposition. This was done by recalculating the indirect effect mediated by nutrient uptake while controlling for the change in fish excretion, in invertebrate excretion, and in decomposition due to fish phenotype (see supplementary information and eqq. [S18]–[S21], available online). The differences between the uncontrolled effect and each controlled effect provided an estimate of the contribution of the corresponding ecosystem pathway (fish excretion or invertebrate excretion or decomposition) to the indirect effect mediated by nutrient uptake. We performed Monte Carlo simulations using samples of the probability distribution of model parameters to obtain estimates of uncertainty (standard error) for all estimated direct and indirect effects.

Results

Experimental Results: Net, Direct, and Total Indirect Effects

Algae. The net effect of adding guppies was to decrease algal biomass, a result driven by a large negative direct effect of guppies consuming algae that was 333% larger than the indirect effect (fig. 2A; tables 1, S1 [tables S1–S8 are available in the supplementary information]). The total indirect effect of adding guppies to the mesocosms was also negative (fig. 2A; tables 1, S1), which is contrary to the expectations of a trophic cascade or nutrient enrichment by guppies, whether we assume that the consumption of invertebrates reduces invertebrate consumption of algae or whether we assume that the addition of guppies causes an increase in the rate of nitrogen cycling. Doubling the density of guppies reduced the amount of algae in the mesocosms, which also was driven mainly by a large negative direct effect that was 250% larger than the total in-

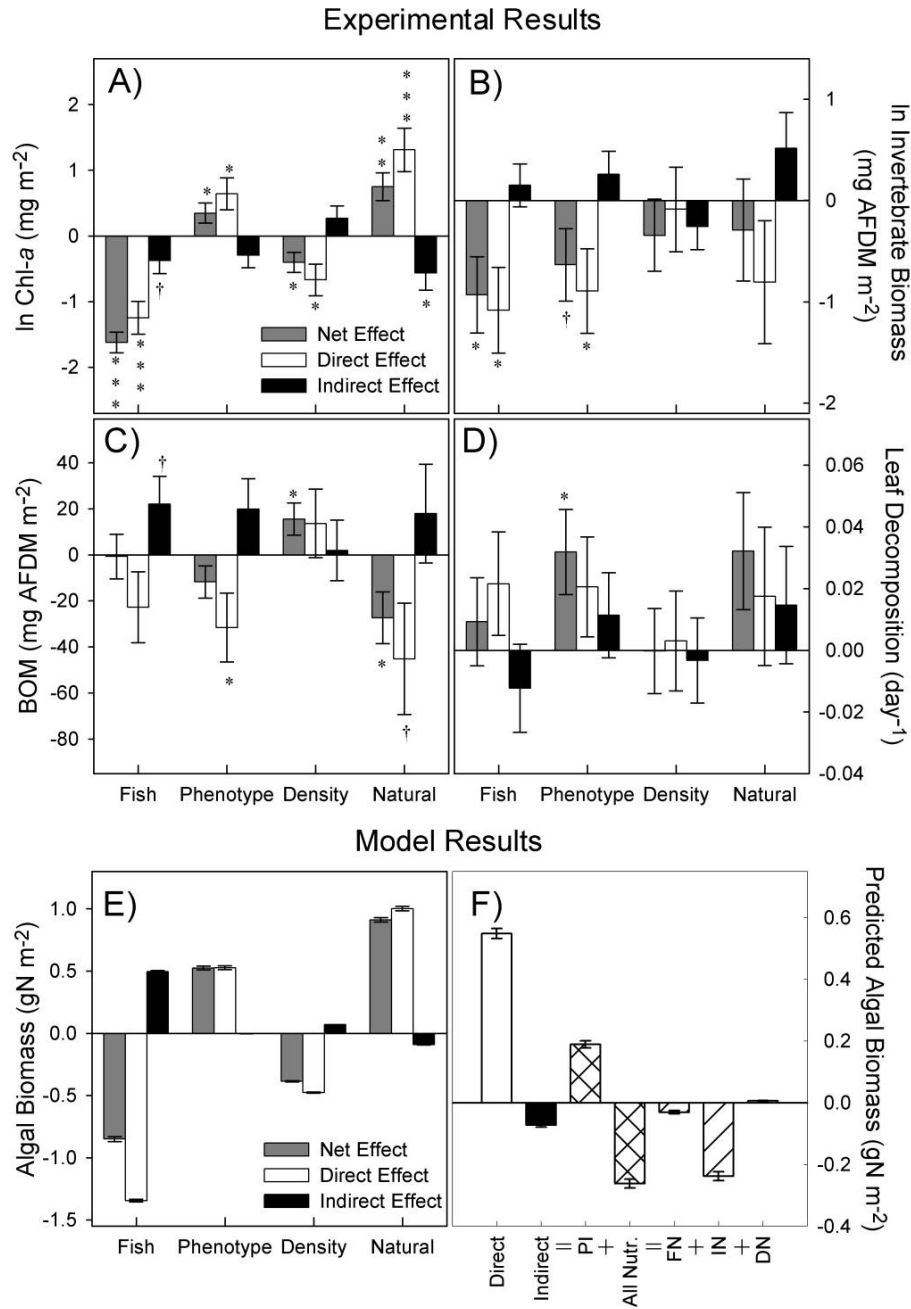


Figure 2: A–D, Contrast estimates (± 1 SE) for planned comparisons of fish, phenotype, density, and mean difference estimates for phenotypes at natural density for primary producer chlorophyll *a* (A), invertebrate biomass (B), benthic organic matter (BOM; C), and leaf decomposition rates (D). Contrasts are constructed as fish (fish–no fish), phenotype (high predation [HP]–low predation [LP]), density (high density–low density), and natural (HP at low density–LP at high density). Positive contrast estimates reflect higher means for mesocosms with guppies, HP, high density, and HP at low density. Natural represents the contributions of the combined density- and trait-mediated effects as the difference between HP phenotype at low densities (HPLD) and LP phenotype at high densities (LPHD; HPLD – LPHD). These differences

direct effect. Increased guppy density increased algal standing stocks slightly through indirect effects (fig. 2A; tables 1, S1) and, although nonsignificant, was able to slightly offset the negative direct effect.

HP guppies consumed substantially fewer diatoms than LP guppies (Bassar et al. 2010) and caused an increase on algal stocks compared with mesocosms with LP guppies. This increase was caused mostly by a significant, positive direct effect of the phenotype that was 218% larger than the total indirect effect (fig. 2A; tables 1, S1). Either increased consumption of invertebrates or increased nutrient excretion rates of HP guppies should yield positive indirect effects of the phenotype on chlorophyll *a* if either of these pathways were important. However, we found that the total indirect effect of the HP phenotype was to decrease the amount of chlorophyll *a* in the mesocosms (fig. 2A; tables 1, S1). This is the case even though HP guppies directly decreased the biomass of invertebrates (fig. 2B; tables 1, S2) and excreted nitrogen at a higher rate than LP guppies (Bassar et al. 2010).

Invertebrates. Adding guppies to the mesocosms decreased invertebrate biomass via a large direct effect that was 720% larger than the corresponding total indirect effect (fig. 2B; tables 1, S2). HP guppies decreased invertebrate biomass more relative to LP guppies, again mostly through a direct effect that was 345% larger than the corresponding nonsignificant total indirect effect (fig. 2B; tables 1, S2). There were no significant effects of variation in guppy density on invertebrate biomass (fig. 2B; tables 1, S2). The superficially similar effects of adding guppies and exchanging LP with HP guppies on invertebrate biomass were actually caused by different mechanisms. The decrease in invertebrate biomass caused by adding guppies to mesocosms arose from a decrease in the average individual mass of invertebrates (tables 1, S3). In contrast, exchanging HP guppies for LP guppies produced no change in the average size of individual chironomids but, instead, decreased the total number of chironomids (tables 1, S4).

Benthic Organic Matter and Leaf Decomposition. Adding guppies to the mesocosms decreased BOM through direct

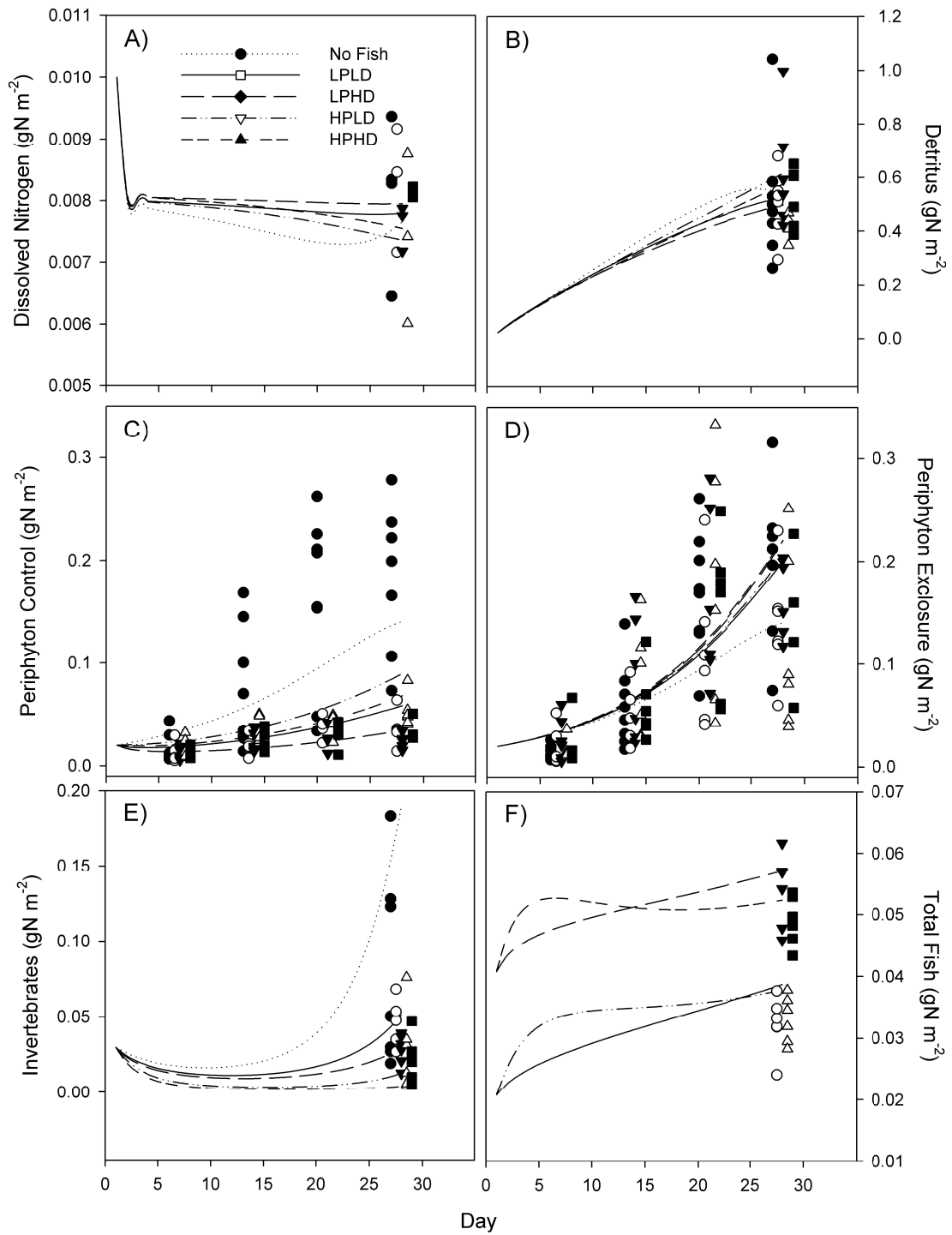
pathways and increased BOM by the same amount through indirect pathways, leading to no observed net effect BOM (fig. 2C; tables 1, S5). Doubling guppy density increased BOM, mostly as a function of direct effects that were 703% larger than the total indirect effect (fig. 2C; tables 1, S5). HP guppies caused a decrease in the total amount of BOM (fig. 2C; tables 1, S5). This decrease was mostly caused by a significant direct effect that was 159% larger than the opposing total indirect effect (fig. 2C). Exchanging HP guppies for LP guppies decreased the rate at which leaves decomposed (fig. 2D; tables 1, S6) in the mesocosms mostly as a function of a direct effect that was 181% larger than the total indirect effect (fig. 2D; tables 1, S6).

Model Analysis: Estimating Indirect Effects of Phenotype on Algal Stock

The simulations of the experimental approach yielded net, direct, and indirect effects of fish, phenotype, density, and natural treatments on algal biomass (fig. 2E) that are consistent with the experimental results (fig. 2E). For fish phenotype, the net positive effect is almost entirely due to the direct effect mediated by fish predation, in agreement with the experimental result (total indirect effect of phenotype not significantly different from 0; fig. 2A). The model allows direct calculation of these effects (appendix; eq. [A11]) and partitioning the total indirect effect into first- and higher-order indirect effects (fig. 2F). Although the total indirect effect is very small compared with the net and direct effects (<0.5% of the net effect; fig. 2E), the model reveals that there are two potentially large indirect effects of guppies on algae that tend to compensate one another (PI and All Nutr. in fig. 2F). First, because guppies also eat invertebrates and some invertebrates graze on algae, this indirect effect causes an increase in algae abundance (trophic cascade). However, the positive effect on algae mediated by guppy predation on invertebrates is more than compensated by the negative indirect effect mediated by nutrient uptake. These effects are similar in size, and because they oppose each other in direction, they almost cancel.

The greater attack rate of HP fish on invertebrates is

represent the combined effects that would be seen in natural populations of these phenotypes and hence represent the combination of ecological and evolutionary effects. *E*, Partition of net effects of guppies, phenotype, and density on algal biomass from model mimicking the enclosure experimental design. *F*, Exact partition (eq. [A11]) of guppy phenotype net effect on algal biomass into the direct effect mediated by fish predation on algae, the total indirect effect, and the indirect effects mediated by invertebrate predation on algae (PI) and by algal nutrient uptake (All Nutr.). The effect mediated by algal nutrient uptake has itself three components: change in fish excretion (FN), change in invertebrate excretion (IN), and change in decomposition (DN). In *E* and *F*, mean values were computed by Monte Carlo simulations sampling the probability distribution of model parameters. Small differences in the calculation of the direct and indirect effects between *E* and *F* result from *E* being calculated from contrasts and *F* from exact partitioning of effects. For mathematical definition and derivation of indirect effects, see appendix and supplementary information (available online). Three asterisks, $P < .001$; two asterisks, $P < .01$; one asterisk, $P < .05$; dagger, $P < .10$.



critical for generating this result. The model shows that the total influence of HP fish on nutrient dynamics was negative (All Nutr. in fig. 2*F*). But this and other mesocosm experiments have also shown that HP guppies excrete nutrients at higher rates than LP guppies in mesocosm experiments (Palkovacs et al. 2009; Bassar et al. 2010), which implies that HP guppies may produce positive effects on nutrient uptake. Using the model to partition the total effect of HP fish on nutrient uptake among its ecological pathways (fish excretion, invertebrate excretion, decomposition; fig. 2*F*), we found that the effect mediated by fish excretion was, in fact, small (in magnitude <10% of the direct effect) and negative (FN in fig. 2*F*). This reflects the fact that the effect of faster excretion in HP was offset by lower density compared with LP (see fig. 3). Furthermore, this negative effect was dramatized by the addition of an eightfold negative effect mediated by invertebrate excretion (IN in fig. 2*F*). Thus, the total effect of HP guppies on algal stocks includes significant loss of nutrient input by invertebrates (IN in fig. 2*F*) that nearly balances the trophic cascade from fish to invertebrates to algae (PI in fig. 2*F*), while the indirect effects mediated by fish excretion (FN in fig. 2*F*) and decomposition (DN in fig. 2*F*) make only minor contributions to the total indirect effect.

Discussion

In a previous article, we showed that guppies modify community structure and ecosystem processes as a function of their phenotype and density (Bassar et al. 2010). However, because guppy phenotypes differ in both the food resources consumed and the rates of nutrient excretion, we were unable to distinguish these separate contributions to the ecosystem effects. Here we combine experimental and modeling approaches to partition these effects into direct and indirect pathways shaped by trophic links and the nutrient cycle.

The experimental results show that omnivorous guppies primarily influence the stream communities and ecosystem processes by directly consuming invertebrates and periphyton but that indirect processes modulate the magnitude of the overall net effect. Further, we show that all net, direct, and total indirect effects of phenotype difference are as strong as or stronger than the corresponding net, direct, and total indirect effects of doubling the density of fish. The ecosystem model allowed us to evaluate the con-

tribution of four indirect pathways to the effect of guppy phenotype on primary production: trophic cascade, guppy nutrient excretion, invertebrate nutrient excretion, and decomposition (fig. 4). The model outputs suggest that at least the first three of these effects are relatively large, but because they act in opposition to one another, they combine to produce a small total indirect effect of the phenotype. HP guppies caused a trophic cascade that was almost compensated by the indirect effect on nutrient cycling. The compensation was possible because the effect on nutrient cycling mediated by invertebrate excretion was negative and sufficiently large. Consuming invertebrates not only releases predation pressure on algae, it also decreases fertilization by invertebrate excretion (or decomposition).

Our ability to interpret the patterns produced by the exclosures as the net indirect effect on algae relies on there being no effects of the exclosure on invertebrates. For example, had invertebrates increased their grazing on algae in response to the absence of guppies in the electrified plots, then our measured indirect effects of guppies on algae would be confounded by a direct effect of invertebrates on algae that is an experimental artifact. This artifact would have shown up as an indirect effect on the invertebrates. However, our results show that the indirect effect on compartments other than algae were not significant (table 1; fig. 2).

Direct versus Indirect Ecological Effects of Adaptation

Understanding the interactions among species in communities is important because “Without them, predictions and insights into the nature and dynamics of complex food webs or communities are crude at best” (Laska and Wootton 1998, p. 473). To this end, community ecologists have long struggled with determining the relative role of direct and indirect interactions among organisms in structuring and determining the stability of communities. One key problem has been generalizing the results from simple one-predator and two-prey systems to more complex communities. When more than two functional groups are present in the system, the number of possible indirect links increases in a combinatorial fashion. This increases the likelihood that indirect effects will become more important in determining the dynamics of the system, but it is generally unclear whether the dynamics would be determined

Figure 3: Observed and predicted trajectories for the state variables (g N m^{-2}) in all five treatments. Predicted values are for least squares best fit of the ecosystem model given by equations (A1)–(A7). Fish state variables J , K , and L are estimated independently but added up in the graph to express total fish biomass. Although fish biomass is expressed as total dry mass g m^{-2} in the model, it is here converted, for illustrative purposes, to g N m^{-2} (multiplying by $c_F = 0.09$). Data is jittered on the X -axis for clarity. HD, high density; LD, low density; HP, high predation; LP, low predation.

by many indirect effects, each of small size, or by a few of large size. Empirically measuring the direction and magnitude of these links has been elusive in most systems. Answering this question requires a combination of experimental manipulations and mathematical modeling of the interactions among community members, as we have done here (Laska and Wootton 1998).

While community interactions that are a function of numerical dynamics between species are well studied (Wootton 1994), we know far less about how interactions change in response to local adaptation by members of the community (Fussmann et al. 2007; Haloin and Strauss 2008). We have shown with regard to guppies that such phenotype-mediated effects can often be larger than numerical effects. However, numerical and phenotype-mediated effects are often confounded in nature (Strauss et al. 2008), as they are in natural guppy populations. LP guppies live at higher densities than HP guppies in natural streams. There are readily observable differences in the ecosystem parameters between upstream (where LP guppies reside) and downstream (where HP guppies reside) locations; the associations of those differences with substantial differences in guppy density could suggest that the differences in guppy density would be likely responsible for the ecosystem distinctions. However, we have shown here that there are substantially different effects of each phenotype on the ecosystem, effects that are often as strong or stronger than the effects of doubling density. Thus, exchanging LP for HP guppies creates qualitatively different effects on the ecosystem than the effects of simply doubling the density of a hypothetical “average” guppy phenotype. As a result, accurately assessing the role of guppies in these ecosystems requires separating the effects of phenotype from those of density. If our results represent a more general phenomenon in adaptation, they may add to the call for a heavier component of evolutionary biology in community and ecosystem ecology (Ferriere et al. 2004;

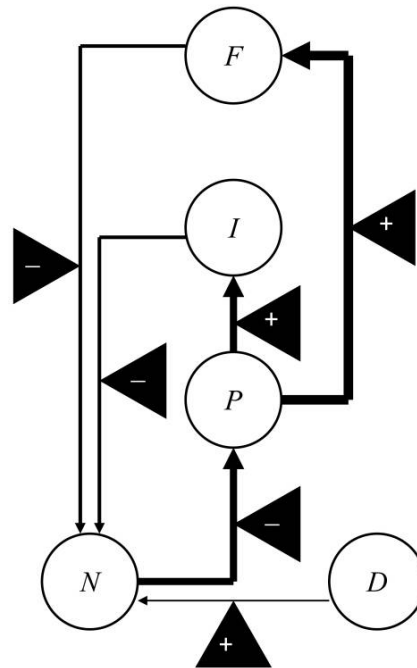


Figure 4: Model estimation of the direct and four indirect effects of fish phenotype on algal biomass. The direct effect is fish (*F*) predation on algae (*P*). The indirect effects are invertebrate (*I*) predation, uptake of nutrient (*N*) cycled by fish, invertebrates, and decomposition (*D*). Arrow thickness is proportional to the effect size, as measured by the expected value of the corresponding contrast (see fig. 2E, 2F). A positive effect indicates higher algal biomass with the high-predation phenotype (HP) compared with the low-predation phenotype (LP).

Johnson and Stinchcombe 2007; Haloin and Strauss 2008; Loreau 2010; Schoener 2011).

Our mechanistic model suggests that the direct ecolog-

Table 1: Contrast *F* values from linear mixed models analysis of experimental results

Variable	Fish contrast			Phenotype			Density		
	Net	Direct	Indirect	Net	Direct	Indirect	Net	Direct	Indirect
Algal stocks	105.60***	25.11***	3.62 [†]	5.17*	7.10*	2.40	6.89*	7.69*	1.97
Nonpredatory inverts	6.14*	6.51*	.50	3.18 [†]	4.59*	1.29	.93	.04	1.27
Chironomid:									
Count	2.87	1.38	.02	9.00**	3.30 [†]	.12	2.59	1.23	.00
Size	5.68*	6.64*	1.48	.00	.92	1.74	.54	1.71	1.17
Benthic organic matter	.00	2.20	3.42 [†]	2.85	4.49*	2.26	4.99*	.84	.02
Leaf decomposition	.42	1.66	.74	5.35*	1.61	.68	.00	.04	.06

* $P < .05$.

** $P < .01$.

*** $P < .001$.

[†] $P < .10$.

ical effect and the indirect ecological effects (second-order or higher-order effects) caused by phenotypic change in one member of the community may actually be of similar magnitude. We were only able to detect these potential indirect effects by combining experimental manipulations of organismal characters and mechanistic modeling of ecosystem dynamics. With the experiment alone, we would have concluded that the largest effect of the guppy phenotype is the direct effect of the difference in guppy diet on algal biomass and that the (total) indirect effect is relatively small and in opposite direction to a trophic cascade. Adding the mechanistic model revealed that the relatively small total indirect effect may in fact be the sum of large indirect effects that are in the direction predicted by observed differences in guppy diet but almost cancel. Thus, empirical studies comparing the relative magnitudes of net effects and direct effects in ecological webs may erroneously conclude that indirect pathways are unimportant.

The various effects of the phenotype on the ecosystem are determined by differences in dietary preference and nutrient excretion rates. Nutrient recycling consequences of trophic cascades are rarely considered in studies of indirect effects but may be important when nutrient cycling is fast or when the consumer is omnivorous (Leroux and Loreau 2010). This may be particularly important when

the primary invertebrate prey are detritivores that do not eat algae (Leroux and Loreau 2010), as in our mesocosms, where the most abundant invertebrates are chironomids. Because our model does not distinguish between fine and coarse detrital matter, with chironomids playing a key role in the transition from the latter to the former, the contribution of decomposition to the indirect pathway through invertebrates and nutrient may even have been underestimated. Indeed, invertebrate effects on detritus (e.g., variation in shredding rate among invertebrates, an effect not included in the model) could also contribute significantly to the indirect effects of fish adaptation on primary production.

Differences in the dietary preference cause the changes in the consumptive effects of guppies and their invertebrate prey. However, it is unclear whether the differences in guppy excretion rates are themselves driven by the dietary preference as well. HP guppies consume more invertebrates and excrete nitrogen at higher rates than LP guppies. If consuming invertebrates leads to higher rates of nutrient excretion, then the indirect effect of guppy nutrient excretion could itself be explained by adaptations related to dietary preference. Alternatively, higher excretion rates in HP guppies could be independent from diet and may reflect broader physiological adaptations (e.g.,

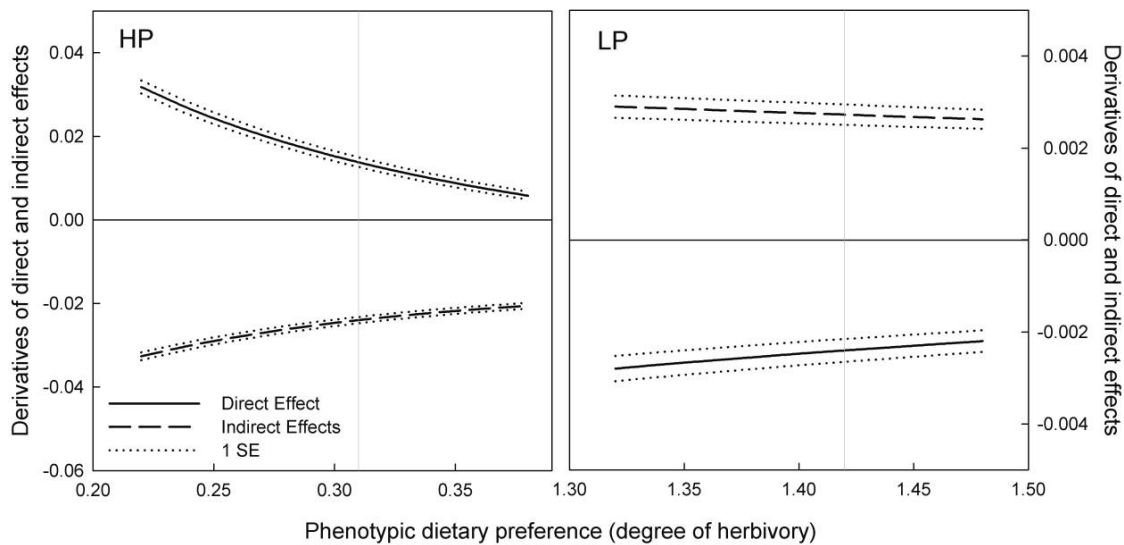


Figure 5: Derivatives of the direct and indirect effects of phenotype on algal biomass with respect to guppy dietary preference (degree of herbivory). HP, high-predation fish; LP, low-predation fish. Derivatives are defined as responses to a small decrease in herbivory in HP and a small increase in herbivory in LP; negative derivatives thus indicate selection for less herbivory in HP and more herbivory in LP. The derivatives of the net effects (not shown) obtained by summing derivatives of direct and indirect effects. The derivatives' uncertainty is estimated by using Markov chain Monte Carlo samples of the probability distribution of the estimated model parameters; dotted lines indicate ± 1 SE. Derivatives and their uncertainty are calculated around the observed (estimated) degree of herbivory (0.31 for HP, 1.41 for LP, indicated by thin dotted vertical lines). For computational details, see supplementary information (available online).

metabolic rate) to living in high-resource and high-mortality-risk environments (Ricklefs and Wikelski 2002).

Evolutionary Feedback

As the phenotypic state of a population evolves, its ecological state (e.g., density) and its environment change. As the environment changes, selective pressures on the phenotype may change, resulting in an evolutionary feedback of ecological dynamics on trait evolution. The reciprocal interplay of ecological and evolutionary dynamics has been analyzed in mathematical models (Mylius and Diekmann 1995; Metz et al. 2008; Jones et al. 2009) and demonstrated in the laboratory (Yoshida et al. 2003). There are a few experimental studies in the wild (or in in situ mesocosms) that suggest that the coupling of ecological and evolutionary dynamics may play an important role in jointly shaping adaptation and ecosystem function (Post et al. 2008; Harmon et al. 2009; Palkovacs et al. 2009; Bassar et al. 2010). But there is not a single system in nature where a complete characterization of direct and indirect dynamic feedbacks between ecology and evolution has been achieved.

With the guppy system, we can take a step closer to predicting the eco-evolutionary dynamics driven by the full eco-evolutionary feedback loop. Our mechanistic model can be used to predict how much the direct and indirect ecological effects of phenotype difference can feed back evolutionarily and contribute to the evolution of that divergence. While a comprehensive analysis is beyond the scope of this article, some initial insights are possible. Let us assume that, all else being equal, reduced algal stock selects against herbivory and increased algal stock selects for herbivory; let us assume further that the degree of herbivory can evolve independently of other adaptive traits that are known to differ between LP and HP phenotypes. Then, to predict the direction of selection on LP and HP herbivory, we examine how the difference in algal stock between phenotypes changes if HP becomes less herbivorous or LP becomes more herbivorous. This is achieved by computing the derivatives of the net ecological effect to a small decrease of HP herbivory or a small increase of LP herbivory (fig. 5).

The results show that our predictions about further evolution in the system are altered when we take the indirect ecological effects seriously. To see this, recall that the derivative of the net ecological effect is the sum of derivatives of the direct and indirect effects. By computing the direct and indirect effects derivatives over a range of LP and HP degrees of herbivory that includes current values, we find that the sign of the derivatives of the indirect effect to either higher LP herbivory or lower HP herbivory is always opposite of the sign of the derivatives of the direct effect

(fig. 5). In HP, the direct effect's derivative is positive, while the indirect effect's derivative is negative and larger in magnitude. The net effect's derivative is therefore negative, which means that less herbivory in HP reduces the difference in algal stock. Thus, algal stock in the HP population decreases, which may select for even less herbivory in HP. Had we neglected the indirect ecological effect and equated the net effect with the direct effect, we would have made the exact opposite prediction. Likewise, in LP, the selection pressure generated by the indirect effect dominates and opposes selection by the direct effect. The net ecological effect's derivative is positive, which indicates that less herbivory is favored. In contrast, the direct effect's derivative is negative and would have by itself predicted selection for more herbivory.

The general lesson from this computational exercise is that although the total indirect ecological effect may be small in magnitude, its derivative with respect to phenotype can be large—and it is the derivative, not the raw value, that determines the contribution to the evolutionary feedback. In the mathematical model of our system, the derivative of the total indirect effect is large because several indirect effects are large and vary strongly with respect to phenotype. This underscores the importance for the correct assessment of evolutionary feedbacks of extending the analysis of the total indirect effect to its mechanistic components along each ecological pathway. Future studies also need to investigate how indirect ecological effects feed back evolutionarily on genetic variation in fish traits other than foraging behavior.

Acknowledgments

We are indebted to D. F. Fraser and S. A. Thomas for constructing the mesocosms and advice on the experiment. We thank the Ramdeen family for the use of their land and water. S. Grabinski, Y. Reynoso, and many field and laboratory technicians helped to process samples. S. Connelly provided advice on the use of electric exclosures. D. DeAngelis and J. Stegen advised the model construction and helped gather parameter estimates. C. Dalton, R. El-Sabaawi, and T. Heatherly provided unpublished data. M. Alkins-Koo, R. Hernandez, Simla Tropical Research Station, and the University of the West Indies provided housing and logistical support. We are grateful to three anonymous reviewers for their constructive criticisms. One reviewer's insightful suggestions helped us to greatly improve the scope of the mathematical method for partitioning direct and indirect effects. This research was funded by a National Science Foundation Frontiers in Integrative Biological Research grant (EF0623632), the French Agence Nationale de la Recherche (EVORANGE project), and the Institut Universitaire de France.

APPENDIX

Mathematical Ecosystem Model

Model Description

The ecosystem structure shown in figure 1 translates into a system of ordinary differential equations in which flow rates determine the rates of variation of each ecosystem state variable. Given mesocosm depth (mean, 160 mm), N is measured as a concentration per unit area (g m^{-2}). The nutrient (N) content of periphyton (c_p), invertebrates (c_i), and fish (c_f) is assumed constant, and P , I , and D are measured as grams of nutrient (N) per unit area. The fish population is structured into three stages, and the model describes the dynamics of female biomass (g dry mass per unit area) in each stage: immatures, J ; small adults, K ; and large adults, L . Transitions between stages are driven by individual growth and reproduction. The fish population is connected to the rest of the ecosystem by fish predation (on invertebrates, periphyton, and detritus), fish excretion, and fish egestion. Although the state variables J , K , and L measure the biomass of the female population, ecosystem processes are influenced by the total (female and male) fish biomass, under the assumption of a constant, even sex ratio in all three stages. Model construction is summarized hereafter and expounded in more detail in the supplementary information.

The rates of change of N (dissolved nitrogen), P (periphyton), I (invertebrates), and D (detritus) are given by

$$\frac{dN}{dt} = \Phi_{N,\text{in}} - \Phi_{N,\text{out}} + \Phi_{D,N} - \Phi_{N,P} + \Phi_{I,N} + \Phi_{F,N}, \quad (\text{A1})$$

$$\frac{dP}{dt} = \Phi_{N,P} - \Phi_{P,D} - \Phi_{P,I} - \Phi_{P,F}, \quad (\text{A2})$$

$$\frac{dI}{dt} = \Phi_{P,I} + \Phi_{D,I} - \Phi_{I,N} - \Phi_{I,D} - \Phi_{I,\text{loss}} - \Phi_{I,F}, \quad (\text{A3})$$

$$\frac{dD}{dt} = \Phi_{D,\text{in}} - \Phi_{D,\text{out}} - \Phi_{D,N} + \Phi_{P,D} - \Phi_{D,I} + \Phi_{I,D} - \Phi_{D,F} + \Phi_{F,D}. \quad (\text{A4})$$

The flow rates denoted generically by Φ are modeled under the following assumptions.

1. The system is well mixed and spatially homogeneous. Import and export of material (nutrient, detritus) occur at constant rates. Hence $\Phi_{N,\text{in}} = N_0$, $\Phi_{N,\text{out}} = j_N N$, $\Phi_{D,\text{in}} = D_0$, and $\Phi_{D,\text{out}} = j_D D$. Decomposition assumes a constant microbial fraction q in detritus and constant rates μ and ν of mineralization and immobilization; hence $\Phi_{D,N} = qD(\mu - \nu N)$.

2. Nutrient limitation of algal growth $\Phi_{N,P}$ is described by a Monod function; maximal growth rate g and half-saturation constant h are constant. Light limitation is characterized by a constant light extinction coefficient k . Hence $\Phi_{N,P} = g[(1 - e^{-kP})/(h + N)]N$.

3. The functional (trophic) response of invertebrates on detritus and periphyton is Holling Type I, with constant, prey-specific feeding rates f_{ID} and f_{IP} : $\Phi_{P,I} = f_{IP}P(I/c_I)$, $\Phi_{D,I} = f_{ID}D(I/c_I)$. Assimilation rates are constant and prey specific: σ_{ID} and σ_{IP} for invertebrates feeding on detritus and periphyton, respectively.

4. The fish feeding rate f_{YZ} ($Y = \text{stage} = J, K, L$; $Z = \text{prey type} = D, P, I$) is stage and prey specific and best described by the Crowley-Martin functional response (de Villemereuil and López-Sepulcre 2011; see below). Assimilation rates σ_{FD} , σ_{FP} , and σ_{FI} are constant and prey specific; they are independent of stage but differ between phenotypes uniformly across prey types, with a reduction factor δ for assimilation in LP compared with HP. Hence

$$\Phi_{Z,F} = c_Z[f_{JZ}(1 + x_J)J + f_{KZ}(1 + x)K + f_{LZ}(1 + x)L],$$

$$\Phi_{F,D} = \left[\sum_{Z=D,P,I} (1 - \sigma_{FZ})c_Z f_{JZ} \right] (1 + x_J)J + \left[\sum_{Z=D,P,I} (1 - \sigma_{FZ})c_Z f_{KZ} \right] (1 + x)K + \left[\sum_{Z=D,P,I} (1 - \sigma_{FZ})c_Z f_{LZ} \right] (1 + x)L,$$

where x_J and x are the male-to-female ratios of individual mass in stage J and in stages K and L .

5. Mortality of algae and invertebrates occurs at constant rates and fluxes nutrient to the detritus compartment. Invertebrates that emerge (individual probability e_I) are lost from the system at constant rate m_I . Assumptions 4 and 5 lead to $\Phi_{P,D} = m_P P$, $\Phi_{I,D} = (1 - \sigma_{IP})\Phi_{P,I} + (1 - \sigma_{ID})\Phi_{D,I} + m_I e_I I$, $\Phi_{I,\text{loss}} = m_I(1 - e_I)I$. In the experiment, dead fish

are immediately replaced by fish of similar mass, making fish mortality negligible in effect; the mortality rate m_F is thus small and independent of stage or phenotype, and the contribution to detritus is neglected.

6. The excretion rate of invertebrates ρ_I is constant, and $\Phi_{I,N} = \rho_I I$. The excretion rate of fish is constant and stage specific; hence $\Phi_{F,N} = c_F[\rho_J(1+x)J + \rho_{KL}(1+x)(K+L)]$, where ρ_J and ρ_{KL} are the respiration rates of juvenile and adult fish, respectively.

The fish population dynamics are driven by

$$\frac{dJ}{dt} = (1+x)^{-1}(vr_K K + r_L L) + b_J - \alpha J - m_F J, \quad (\text{A5})$$

$$\frac{dK}{dt} = \alpha J + b_K K - vr_K K - \beta K - m_F K, \quad (\text{A6})$$

$$\frac{dL}{dt} = \beta K + b_L L - r_L L - m_F L. \quad (\text{A7})$$

Fish dynamics are linked to the rest of the ecosystem through the balance between assimilation and respiration, $b_Y = (1/c_F)(\sigma_{FD}c_D f_{YD} + \sigma_{FP}c_P f_{YP} + \sigma_{FI}c_I f_{YI}) - \rho_Y(Y = J, K, L)$. Crowley-Martin functional responses are given by

$$f_{YZ} = \frac{a_{YZ}(Z/c_Z)}{[1 + \sum_{X=D,P,I} a_{YX}\tau_{YX}(X/c_X)][1 + s(1+x)J/w_J + s(1+x)(K+L)/w_{KL}]} \quad (\text{A8})$$

($Y = J, K, L$; $Z = D, P, I$). Attack rates a_{YZ} and handling times τ_{YZ} are stage and prey specific; they also differ between phenotypes (de Villemereuil and López-Sepulcre 2011; see below). Periphyton and detritus represent a greater fraction of the diet in LP compared with HP (Zandonà et al. 2011); attack rates on periphyton and detritus were thus modeled by multiplicatively applying degrees of herbivory, H_{LP} and H_{HP} , to the estimated attack rates on invertebrates. Degrees of herbivory are assumed to be stage independent. The handling time of periphyton and detritus was shorter than the handling time of invertebrates and thus modeled by applying a reduction factor ζ to the latter (with ζ independent of stage or phenotype). Finally, the Crowley-Martin functional response (eq. [A8]) describes competition between fish occurring within stage and between juveniles and adults, with an intensity denoted by s . w_J and w_{KL} denote mean individual mass in juveniles ($w_J = (w_0 + w)/2$) and in adults ($w_{KL} = [(w + w_{\max})/2 + w_{\max}]/2$).

Defining $r_Y = \max\{0, b_Y\}$, we have $r_Y = b_Y$ when the assimilation-respiration balance is positive and $r_Y = 0$ when the balance is negative. Positive assimilation-respiration balance results in growth and/or reproduction. In juveniles, growth leads to reproductive maturity at rate α , given by (de Roos et al. 2007, 2008)

$$\alpha = \frac{r_J - m_J}{1 - (w_0/w)^{1-m_J/r_J}}, \quad (\text{A9})$$

where m_J denotes intrinsic mortality in juveniles, w_0 is mass at birth, and w is mass at maturity. Small adult females channel a fraction ν of their biomass production, r_K , into reproduction. Females in stage K grow into stage L at rate β , given by (de Roos et al. 2007, 2008)

$$\beta = \frac{(1-\nu)r_K - m_K}{1 - (w/w_{\max})^{1-m_K/(1-\nu)r_K}}, \quad (\text{A10})$$

where m_K denotes intrinsic mortality in small adults and w_{\max} is the asymptotic mass.

Parameters and Initial Values

Parameter values are listed with their units in table A1 and commented on in table S8. Parameters for which no reliable estimates were available from this or other experiments, natural streams, or literature data were estimated by model fitting. To this end, we used the experimental data that were available for each replicate of each treatment: upstream concentration of nitrogen (day 28); chlorophyll a measured on tiles sampled on days 7, 14, 21, and 28 (clean tiles are introduced on day 0); invertebrates ash-free dry mass (AFDM) measured on day 28; BOM AFDM measured on day 28; and wet mass and size structure of guppy population on day 28. Estimates of initial values were available for detritus (D) and fish (J, K, L in low-density and high-density treatments). Assuming a relatively small amount of

algal growth between day 0 and day 7, we approximated initial periphyton (P) by a value (0.02 g N m^{-2}) close to the minimum of P that was measured on day 7 across treatments and replicates. Initial values for nutrient concentration (N) and invertebrates (I) were chosen to be consistent with measurements at the end of the experiment (for more details, see supplementary information). The overall fit of the model is the result of an optimization process over all state variables and therefore a compromise among state variables.

Analysis of Direct and Indirect Effects

We analyzed the phenotype effects on algal biomass first by mimicking the electric enclosure experimental design. To simulate the dynamics of periphyton within electric enclosures, we use equation (A2) as a submodel in which consumption by fish (Φ_{pf}) is set to 0, and N and I are externally forced by the $N(t)$ and $I(t)$ trajectories that the full model generates. Then, we calculated the contrasts on day 28, as done with the experimental results.

Then, we used the full model to directly calculate the net, direct, and total indirect effects of fish phenotype and to partition the latter into first- and second-order indirect effects (i.e., that involve two or three links in the flow network between fish and periphyton (fig. 1). To this end, we use equation (A2) to establish (for details, see supplementary information)

$$\log P_{HP}(T) - \log P_{LP}(T) = \int_{t=0}^T \frac{-\Phi_{P,F}^{HP}}{P_{HP}} - \frac{-\Phi_{P,F}^{LP}}{P_{LP}} dt + \int_{t=0}^T \frac{-\Phi_{P,I}^{HP}}{P_{HP}} - \frac{-\Phi_{P,I}^{LP}}{P_{LP}} dt + \int_{t=0}^T \frac{\Phi_{N,P}^{HP}}{P_{HP}} - \frac{\Phi_{N,P}^{LP}}{P_{LP}} dt, \quad (\text{A11})$$

where T is time at the end of the experiment ($T = 28$), the left-hand side is the net effect on algal stock measured on log scale, the first integral on the right-hand side measures the direct effect of fish predation, and the second and third integrals sum up to the total indirect effect. The latter is thereby partitioned into the (first-order) indirect effect mediated by invertebrate predation (second integral) and the indirect effect mediated by nutrient uptake (third integral).

The indirect effect mediated by nutrient uptake can be partitioned further into (first- and higher-order) indirect effects mediated by the flows that contribute to N , that is, fish excretion $\Phi_{F,N}$, invertebrate excretion $\Phi_{I,N}$, and decomposition $\Phi_{D,N}$. To this end, we recalculate the third integral while removing the effect of fish phenotype on each flow in turn. Then, for each flow, the indirect effect mediated by that flow is estimated by the difference between the original integral and the recalculated integral. For details about numerical implementation, see supplementary information.

Table A1: Parameter values and initial conditions

Definition	Notation	Unit	Value
Nutrient:			
Input rate of nutrient (dissolved nitrogen) from upstream	N_0	g (nutrient) m ⁻² day ⁻¹	.217 ^a
Rate of nutrient loss ^b	j_N	g (nutrient) g ⁻¹ (nutrient) day ⁻¹	26.98
Detritus:			
Input rate of detritus (BOM) from upstream	D_0	g (nutrient) m ⁻² day ⁻¹	.0251 ^a
Rate of detritus loss ^b	j_D	g (nutrient) g ⁻¹ (nutrient) day ⁻¹	.024
Nitrogen content of detritus	c_D	g (nutrient) g ⁻¹ (biomass)	.0075
Microbe fraction	q	Dimensionless	.05
Mineralization rate	μ	g (inorganic nutrient) g ⁻¹ (organic nutrient) day ⁻¹	.05
Immobilization rate	ν	g ⁻¹ (inorganic nutrient) day ⁻¹	.5
Periphyton:			
Maximum growth rate ^b	g	g (nutrient) g ⁻¹ (nutrient) day ⁻¹	1.68
Monod response half-saturation constant	h	g (nutrient) m ⁻²	.01
Light extinction coefficient	k	g ⁻¹ (nutrient) m ⁻²	.4
Mortality rate	m_P	day ⁻¹	.2
Nutrient content	c_P	g (nutrient) g ⁻¹ (biomass)	.07543
Invertebrates:			
Respiration rate	ρ_I	g (nutrient) g ⁻¹ (nutrient) day ⁻¹	.15
Mortality rate	m_I	day ⁻¹	.01
Larval emergence probability	e_I	Dimensionless	.7
Feeding rate	$f_{ID} f_{IP} = .05f_{ID}$	m ² g ⁻¹ (biomass) day ⁻¹	
On detritus ^b			.6823
On periphyton			.0341
Assimilation efficiencies	σ_{IZ} with $Z = D P$	Dimensionless	
D			.1
P			.6
Nitrogen content	c_I	g (nutrient) g ⁻¹ (biomass)	.11
Fish:			
Intraspecific competition intensity	s	g ⁻¹ (biomass)	.0368 ^a
Male : female mass ratio:			
At birth	x_I	Dimensionless	.9
At maturity	x	Dimensionless	.4
Asymptotic mass	w_{max}	g	.07
Mortality rate in mesocosm	m_F	day ⁻¹	.001
Nitrogen content	c_F	g (nutrient) g ⁻¹ (biomass)	.09
Handling time factor ^b	ζ	Dimensionless	.1
Assimilation factor ^b	δ	Dimensionless	.492
Phenotype-specific fish parameters:			
Respiration rate	ρ_Y with $Y = J K$ or L	g (biomass) g ⁻¹ (biomass) day ⁻¹	
LP:			
J, K			.06
L			.03
HP:			
J, K			.12
L			.06
Natural mortality rate	m_Y with $Y = J K$ or L	day ⁻¹	
LP:			
J, K			.025
L			.0018
HP:			
J, K			.042
L			.0014
Mass at birth	w_0	g	
LP			.0013
HP			.0009
Mass at maturity	w	g	
LP			.0223
HP			.0132
Reproductive effort	ν	Dimensionless	

Table A1 (Continued)

Definition	Notation	Unit	Value
LP			.5
HP			.8
Attack rate (on invertebrates)	a_y with $Y = J K L$	$\text{m}^2 \text{day}^{-1}$	
LP:			
<i>J</i>			15.510 ^a
<i>K</i>			15.871 ^a
<i>L</i>			16.019 ^a
HP:			
<i>J</i>			31.424 ^a
<i>K</i>			32.347 ^a
<i>L</i>			32.738 ^a
Handling time (of invertebrates)	τ_y with $Y = J K L$	day g^{-1} (biomass prey)	
LP:			
<i>J</i>			2.023 ^a
<i>K</i>			1.446 ^a
<i>L</i>			1.211 ^a
HP:			
<i>J</i>			2.194 ^a
<i>K</i>			1.512 ^a
<i>L</i>			1.223 ^a
Degree of herbivory ^b		Dimensionless	
LP			1.408
HP			.31
Assimilation efficiencies	σ_{fz} with $Z = D P I$	Dimensionless	
LP:			
<i>D</i>			.148
<i>P</i>			.295
<i>I</i>			.443
HP:			
<i>D</i>			.3
<i>P</i>			.6
<i>I</i>			.9
Initial conditions (at $t = 0$):			
<i>N</i>		g m^{-2}	.01
<i>D</i>		$\text{g (nutrient) m}^{-2}$.022
<i>P</i>		$\text{g (nutrient) m}^{-2}$.02
<i>I</i>		$\text{g (nutrient) m}^{-2}$.03
<i>J</i>		$\text{g (biomass) m}^{-2}$	
LD			.045
HD			.098
<i>K</i>		$\text{g (biomass) m}^{-2}$	
LD			.148
HD			.278
<i>L</i>		$\text{g (biomass) m}^{-2}$	
LD			0
HD			0

Note: All biomass are measured in dry mass. BOM, benthic organic matter; LP, low predation; HP, high predation; LD, low density; HD, high density. For references, see table S8.

^aParameter values are averages over mesocosm replicates.

^bParameters were estimated by model fitting.

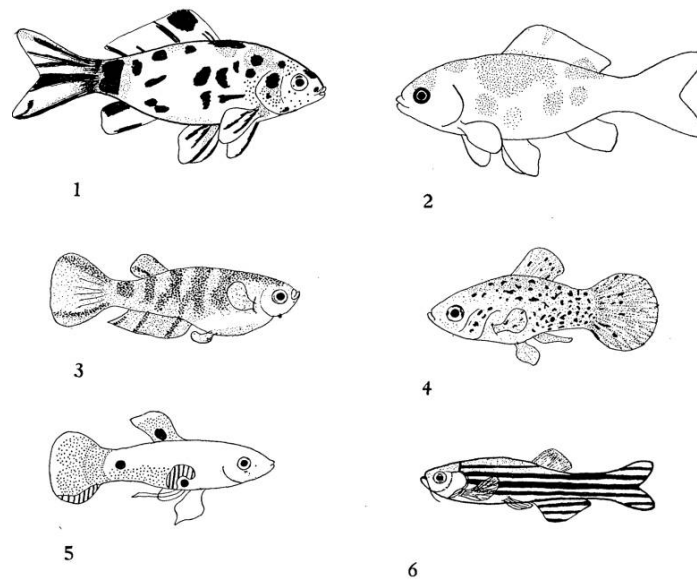
Literature Cited

- Bassar, R. D., M. C. Marshall, A. López-Sepulcre, E. Zandonà, S. K. Auer, J. Travis, C. M. Pringle, et al. 2010. Local adaptation in Trinidadian guppies alters ecosystem processes. *Proceedings of the National Academy of Sciences of the USA* 107:3616–3621.
- Baumgärtner, D., and K. Rothhaupt. 2003. Predictive length–dry mass regressions for freshwater invertebrates in a pre-alpine lake littoral. *International Review of Hydrobiology* 88:453–463.
- Benfield, E. F. 2006. Decomposition of leaf material. Pages 711–720 in F. R. Hauer and G. A. Lamberti, eds. *Methods in stream ecology*. Academic Press, San Diego.
- Benke, A. C., A. D. Huryn, L. A. Smock, and J. B. Wallace. 1999. Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. *Journal of the North American Benthological Society* 18:308–343.

- Chitty, D. 1960. Population processes in the vole and their relevance to general theory. *Canadian Journal of Zoology* 38:99–113.
- Connelly, S., C. M. Pringle, R. J. Bixby, R. Brenes, M. R. Whiles, K. R. Lips, S. Kilham, et al. 2008. Changes in stream primary producer communities resulting from large-scale catastrophic amphibian declines: can small-scale experiments predict effects of tadpole loss? *Ecosystems* 11:1262–1276.
- Dercole, F., and S. Rinaldi. 2008. Analysis of evolutionary processes: the adaptive dynamics approach and its applications. Princeton University Press, Princeton, NJ.
- de Roos, A. M., T. Schellekens, T. van Kooten, K. van de Wolfshaar, D. Claessen, and L. Persson. 2007. Food-dependent growth leads to overcompensation in stage-specific biomass when mortality increases: the influence of maturation versus reproduction regulation. *American Naturalist* 170:E59–E76.
- . 2008. Simplifying a physiologically structured population model to a stage-structured biomass model. *Theoretical Population Biology* 73:47–62.
- de Villemereuil, P. B., and A. López-Sepulcre. 2011. Consumer functional responses under intra- and inter-specific interference competition. *Ecological Modelling* 222:419–426.
- Dieckmann, U., and R. Ferriere. 2004. Adaptive dynamics and evolving biodiversity. Pages 188–224 in R. Ferriere, U. Dieckmann, and D. Couvet, eds. *Evolutionary conservation biology*. Cambridge University Press, Cambridge.
- Feller, R. J., and R. M. Warwick. 1988. Energetics. Pages 181–196 in R. P. Higgins and H. Thiel, eds. *Introduction to the study of meiofauna*. Smithsonian Institution Press, Washington, DC.
- Ferriere, R., U. Dieckmann, and D. Couvet. 2004. *Evolutionary conservation biology*. Cambridge University Press, Cambridge.
- Fussmann, G. F., M. Loreau, and P. A. Abrams. 2007. Eco-evolutionary dynamics of communities and ecosystems. *Functional Ecology* 21:465–477.
- Grant, P. R., and B. R. Grant. 2002. Unpredictable evolution in a 30-year study of Darwin's finches. *Science* 296:707–711.
- Haloin, J. R., and S. Y. Strauss. 2008. Interplay between ecological communities and evolution: review of feedbacks from microevolutionary to macroevolutionary scales. *Annals of the New York Academy of Sciences* 1133:87–125.
- Harmon, L. J., B. Matthews, S. Des Roches, J. M. Chase, J. B. Shurin, and D. Schluter. 2009. Evolutionary diversification in stickleback affects ecosystem functioning. *Nature* 458:1167–1170.
- Haskins, C. P., E. F. Haskins, J. J. McLaughlin, and R. E. Hewitt. 1961. Polymorphism and population structure in *Lebistes reticulata*, a population study. Pages 320–395 in W. F. Blair, ed. *Vertebrate speciation*. University of Texas Press, Austin.
- Heino, M., J. A. J. Metz, and V. Kaitala. 1998. The enigma of frequency-dependent selection. *Trends in Ecology & Evolution* 13:367–370.
- Hendry, A. P., and M. T. Kinnison. 1999. Perspective: the pace of modern life: measuring rates of contemporary microevolution. *Evolution* 53:1637–1653.
- Johnson, M. T. J., and J. R. Stinchcombe. 2007. An emerging synthesis between community ecology and evolutionary biology. *Trends in Ecology & Evolution* 22:250–257.
- Jones, E. I., R. Ferriere, and J. L. Bronstein. 2009. Eco-evolutionary dynamics of mutualists and exploiters. *American Naturalist* 174:780–794.
- Kutner, M. H., C. J. Nachtsheim, J. Neter, and W. Li. 2005. *Applied linear statistical models*. McGraw-Hill, New York.
- Laska, M. S., and J. T. Wootton. 1998. Theoretical concepts and empirical approaches to measuring interaction strength. *Ecology* 79:461–476.
- Leroux, S. J., and M. Loreau. 2010. Consumer-mediated recycling and cascading trophic interactions. *Ecology* 91:2162–2171.
- Loeuille, N., M. Loreau, and R. Ferriere. 2002. Consequences of plant-herbivore coevolution on the dynamics and functioning of ecosystems. *Journal of Theoretical Biology* 217:369–381.
- Loreau, M. 2010. Linking biodiversity and ecosystems: towards a unifying ecological theory. *Philosophical Transactions of the Royal Society B: Biological Sciences* 365:49–60.
- Metz, J. A. J., S. D. Mylius, and O. Diekmann. 2008. When does evolution optimize? *Evolutionary Ecology Research* 10:629–654.
- Metz, J. A. J., R. M. Nisbet, and S. A. H. Geritz. 1992. How should we define fitness for general ecological scenarios? *Trends in Ecology & Evolution* 7:198–202.
- Miller, T. E. 1994. Direct and indirect species interaction in an early old-field plant community. *American Naturalist* 143:1007–1025.
- Miller, T. E., and J. Travis. 1996. The evolutionary role of indirect effects in communities. *Ecology* 77:1329–1335.
- Mylius, S. D., and O. Diekmann. 1995. On evolutionarily stable life histories, optimization and the need to be specific about density dependence. *Oikos* 74:1–12.
- Palkovacs, E. P., M. C. Marshall, B. A. Lamphere, B. R. Lynch, D. J. Weese, D. F. Fraser, D. N. Reznick, et al. 2009. Experimental evaluation of evolution and coevolution as agents of ecosystem change in Trinidadian streams. *Philosophical Transactions of the Royal Society B: Biological Sciences* 364:1617–1628.
- Pimentel, D. 1961. Animal population regulation by the genetic feedback mechanism. *American Naturalist* 95:65–79.
- Polis, G. A., and D. R. Strong. 1996. Food web complexity and community dynamics. *American Naturalist* 147:813–846.
- Post, D. M., E. P. Palkovacs, E. G. Schielke, and S. I. Dodson. 2008. Intraspecific variation in a predator affects community structure and cascading trophic interactions. *Ecology* 89:2019–2032.
- Pringle, C. M., and G. A. Blake. 1994. Quantitative effects of atyid shrimp (Decapoda: Atyidae) on the depositional environment in a tropical stream: use of electricity for experimental exclusion. *Canadian Journal of Fisheries and Aquatic Sciences* 51:1443–1450.
- Reznick, D., M. J. Butler, and H. Rodd. 2001. Life-history evolution in guppies. VII. The comparative ecology of high- and low-predation environments. *American Naturalist* 157:126–140.
- Reznick, D., and J. A. Endler. 1982. The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution* 36:160–177.
- Reznick, D. N. 1982. The impact of predation on life history evolution in Trinidadian guppies: genetic basis of observed life history patterns. *Evolution* 36:1236–1250.
- Reznick, D. N., and H. Bryga. 1987. Life-history evolution in guppies (*Poecilia reticulata*). 1. Phenotypic and genetic changes in an introduction experiment. *Evolution* 41:1370–1385.
- Reznick, D. N., H. Bryga, and J. A. Endler. 1990. Experimentally induced life-history evolution in a natural population. *Nature* 346:357–359.
- Reznick, D. N., M. J. Butler, F. H. Rodd, and P. Ross. 1996. Life-history evolution in guppies (*Poecilia reticulata*). 6. Differential mortality as a mechanism for natural selection. *Evolution* 50:1651–1660.
- Reznick, D. N., and C. K. Ghalambor. 2001. The population ecology of contemporary adaptations: what empirical studies reveal about

- the conditions that promote adaptive evolution. *Genetica* 112:183–198.
- Reznick, D. N., F. H. Shaw, F. H. Rodd, and R. G. Shaw. 1997. Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). *Science* 275:1934–1937.
- Ricklefs, R. E., and M. Wikelski. 2002. The physiology/life-history nexus. *Trends in Ecology & Evolution* 17:462–468.
- Rodd, F. H., and D. N. Reznick. 1997. Variation in the demography of guppy populations: the importance of predation and life histories. *Ecology* 78:405–418.
- Roughgarden, J. 1971. Density-dependent natural selection. *Ecology* 52:453–468.
- Schmitz, O. 2009. Indirect effects in communities and ecosystems: the role of trophic and nontrophic interactions. Pages 289–295 in S. A. Levin, ed. *The Princeton guide to ecology*. Princeton University Press, Princeton, NJ.
- Schoener, T. W. 2011. The newest synthesis: understanding the interplay of evolutionary and ecological dynamics. *Science* 331:426–429.
- Sinervo, B., E. Svensson, and T. Comendant. 2000. Density cycles and an offspring quantity and quality game driven by natural selection. *Nature* 406:985–988.
- Soetaert, K., and T. Petzoldt. 2010. Inverse modelling, sensitivity and Monte Carlo analysis in R using package FME. *Journal of Statistical Software* 33:1–28.
- Steinman, A. D., G. A. Lamberti, and P. R. Leavitt. 2006. Biomass and pigments of benthic algae. Pages 357–380 in F. R. Hauer and G. A. Lamberti, eds. *Methods in stream ecology*. Academic Press, London.
- Stone, L., and A. Roberts. 1991. Conditions for a species to gain advantage from the presence of competitors. *Ecology* 72:1964–1972.
- Strauss, S. Y. 1991. Direct, indirect and cumulative effects of three native herbivores on a shared host plant. *Ecology* 72:543–558.
- Strauss, S. Y., J. A. Lau, T. W. Schoener, and P. Tiffin. 2008. Evolution in ecological field experiments: implications for effect size. *Ecology Letters* 11:199–207.
- terHorst, C. P. 2010. Evolution in response to direct and indirect ecological effects in pitcher plant inquiline communities. *American Naturalist* 176:675–685.
- Werner, E. E., and S. D. Peacor. 2003. A review of trait-mediated indirect interactions in ecological communities. *Ecology* 84:1083–1100.
- Wootton, J. T. 1994. The nature and consequences of indirect effects in ecological communities. *Annual Review of Ecology and Systematics* 25:443–466.
- Yoshida, T., L. E. Jones, S. P. Ellner, G. F. Fussmann, and N. G. Hairston. 2003. Rapid evolution drives ecological dynamics in a predator-prey system. *Nature* 424:303–306.
- Zandonà, E., S. K. Auer, S. S. Kilham, J. H. Howard, A. López-Sepulcre, M. P. O'Connor, R. D. Bassar, et al. 2011. Diet quality and prey selectivity correlate with life histories and predation regime in Trinidadian guppies. *Functional Ecology* 25:964–973.

Associate Editor: Franz J. Weissing
Editor: Mark A. McPeck



1, Common shubunkin, a variety of *Carassius auratus*; 2, gold and white goldfish, *Carassius auratus*; 3, variegated medaka, *Oryzias latipes*; 4, rubra platy, *Platypoecilus maculatus*; 5, male guppy, *Lebistes reticulatus*; 6, zebra fish, *Brachydanio rerio*. From “The Development of Hereditary Color Patterns in Fish” by H. B. Goodrich (*American Naturalist*, 1935, 69:267–277).