Experimental evaluation of evolution and coevolution as agents of ecosystem change in Trinidadian streams

Eric P. Palkovacs[1,*], Michael C. Marshall[2], Brad A. Lamphere[3], Benjamin R. Lynch[1], Dylan J. Weese[1], Douglas F. Fraser[4], David N. Reznick[5], Catherine M. Pringle[2] and Michael T. Kinnison[1]

[1]*School of Biology and Ecology, University of Maine, Orono, ME 04469, USA*
[2]*Odum School of Ecology, University of Georgia, Athens, GA 30602, USA*
[3]*Department of Zoology, North Carolina State University, Raleigh, NC 27695, USA*
[4]*Department of Biology, Siena College, Loudonville, NY 12211, USA*
[5]*Department of Biology, University of California, Riverside, CA 92521, USA*

Evolution has been shown to be a critical determinant of ecological processes in some systems, but its importance relative to traditional ecological effects is not well known. In addition, almost nothing is known about the role of coevolution in shaping ecosystem function. Here, we experimentally evaluated the relative effects of species invasion (a traditional ecological effect), evolution and coevolution on ecosystem processes in Trinidadian streams. We manipulated the presence and population-of-origin of two common fish species, the guppy (*Poecilia reticulata*) and the killifish (*Rivulus hartii*). We measured epilithic algal biomass and accrual, aquatic invertebrate biomass, and detrital decomposition. Our results show that, for some ecosystem responses, the effects of evolution and coevolution were larger than the effects of species invasion. Guppy evolution in response to alternative predation regimes significantly influenced algal biomass and accrual rates. Guppies from a high-predation site caused an increase in algae relative to guppies from a low-predation site; algae effects were probably shaped by observed divergence in rates of nutrient excretion and algae consumption. *Rivulus*–guppy coevolution significantly influenced the biomass of aquatic invertebrates. Locally coevolved populations reduced invertebrate biomass relative to non-coevolved populations. These results challenge the general assumption that intraspecific diversity is a less critical determinant of ecosystem function than is interspecific diversity. Given existing evidence for contemporary evolution in these fish species, our findings suggest considerable potential for eco-evolutionary feedbacks to operate as populations adapt to natural or anthropogenic perturbations.

Keywords: eco-evolutionary dynamics; community genetics; ecosystem function; intraspecific variation; local adaptation; species invasion

1. INTRODUCTION

Recent evidence suggests that evolution may play a critical role in shaping ecological processes over contemporary time scales. This evidence is derived largely from studies of how phenotypic and genetic differences among individuals and populations influence the dynamics of populations and the characteristics of communities and ecosystems. Although ecological changes directly associated with ongoing evolution have rarely been observed in nature (but see Grant & Grant 2006; Pelletier *et al.* 2007; Kinnison *et al.* 2008), widespread contemporary evolution of ecologically important traits suggests that evolution could be an important driver of population, community and ecosystem dynamics (Thompson 1998, 1999; Hairston *et al.* 2005; Kinnison & Hairston 2007).

The majority of studies investigating the community and ecosystem effects of intraspecific diversity involve foundation plant species (i.e. plants that structure a community or ecosystem). Intraspecific variation in plant traits, especially leaf chemistry, has been shown to drive variation in soil processes and arthropod communities (Whitham *et al.* 2006; Bailey *et al.* 2009; Johnson *et al.* 2009). Variation among arthropod communities, in turn, can have implications for trophic interactions involving avian predators (Bailey *et al.* 2006). Recently, the ecological effects of evolutionary divergence have been extended to include the top-down effects of predators. Fish populations that are locally adapted to exploit certain prey resources selectively remove favoured prey, thereby reshaping community composition (Yonekura *et al.* 2007; Post *et al.* 2008; Palkovacs & Post 2009). Just as bottom-up effects can cascade up food webs from plants to arthropods to birds (Bailey *et al.* 2006), top-down effects can cascade down food webs from fishes to zooplankton to phytoplankton (Post *et al.* 2008). This bidirectionality suggests that

there are numerous mechanisms by which evolution may influence ecosystem processes by altering the nature of food-web interactions.

Although intraspecific diversity has been shown to be important for a variety of population, community and ecosystem processes, the relative importance of evolutionary effects compared with traditional ecological effects remains poorly known (Hairston *et al.* 2005; Ezard *et al.* 2009). In addition, prior studies have focused on the ecosystem consequences of diversity within single species, even though food webs present opportunities for evolution in multiple interacting species. As such, almost no empirical attention has been paid to potential ecological effects arising via local coevolutionary processes, even though coevolution is a ubiquitous feature of ecological communities (Thompson 2005) and can be a key driver of the evolution of ecologically important traits (e.g. Brodie *et al.* 2002; Benkman *et al.* 2003). Understanding the relative magnitude of ecological, evolutionary and coevolutionary effects in communities and ecosystems is a critical step towards assessing whether evolution deserves broad consideration from ecologists (Johnson & Stinchcombe 2007).

In the experiment described here, we evaluated the relative importance of species invasion (a traditional ecological effect), intraspecific evolution and local coevolution for ecosystem processes in model Trinidadian stream ecosystems. Species invasion changes species-level diversity in ecological communities, thereby providing a recognized interspecific baseline against which to measure the importance of intraspecific evolution and coevolution. We focused on two fish species that are native to Trinidad—the guppy (Poeciliidae: *Poecilia reticulata*) and the killifish (Rivulidae: *Rivulus hartii*). To examine the effects of species invasion, we compared experimental ecosystems containing *Rivulus* with experimental ecosystems containing *Rivulus* and guppies. To examine the effects of intraspecific evolution, we compared experimental ecosystems containing *Rivulus* from the same population and guppies originating from either of two evolutionarily divergent populations. To examine the effects of local coevolution, we compared ecosystems where the fish community comprised *Rivulus* and guppies collected either from the same site (locally coevolved) or from different sites (non-coevolved). For each ecosystem comparison, we calculated effect sizes to evaluate the magnitude of evolutionary and coevolutionary effects relative to the (ecological) effects of guppy invasion.

2. STUDY SYSTEM

We based our experiment on naturally occurring stream ecosystems in the Northern Range Mountains of Trinidad. Streams in this region are characterized by series of barrier waterfalls that create discrete community and habitat segments. Downstream segments contain large fish predators (e.g. *Crenicichla alta*, *Hoplias malabaricus*), open forest canopies and high-resource conditions (high standing stocks of epilithic algae), whereas upstream segments contain no large piscivorous fishes, closed forest canopies

and lower resource conditions (Grether *et al.* 2001; Reznick *et al.* 2001). Guppies and *Rivulus* are common in both downstream and upstream habitats. However, *Rivulus* also occupy headwater streams that lack guppies (Gilliam *et al.* 1993). Therefore, three identifiable fish communities exist within most drainages: species-rich high-predation communities (HP); *Rivulus*–guppy low-predation communities (LP); and *Rivulus*-only communities (RO). Historically, guppies from HP and LP sites have probably invaded RO sites, setting the stage for evolutionary and coevolutionary effects in stream ecosystems.

There are several reasons to suspect that guppy invasion, guppy evolution and *Rivulus*–guppy coevolution might alter ecosystem properties. One reason involves the potential influence of guppy invasion and evolution on the body size structure of the fish community. Size structure may have important implications for excretion rates, nutrient dynamics and primary production. For a given biomass of fishes, an assemblage of smaller individuals is expected to produce higher excretion-driven nutrient fluxes than an assemblage of larger individuals, which can serve to stimulate primary production (Vanni & Layne 1997; Vanni 2002; Hall *et al.* 2007). Surveys of HP, LP and RO sites across four Northern Range drainages (Arima, Aripo, Guanapo and Marianne) indicate that the average size of an adult guppy is approximately one-fifth the average size of an individual *Rivulus* (0.15 g versus 0.75 g; E. P. Palkovacs 2008, unpublished data). Thus, the invasion of guppies into RO streams changes the body size distribution of the fish community. Similarly, guppy populations show evolutionary divergence in life-history traits that also shape body size distributions. Compared with the guppies from LP environments, guppies from HP environments experience higher mortality rates due to predation, driving the evolution of smaller size (and earlier age) at maturity and greater reproductive investment in the form of more, smaller offspring (Reznick 1982; Reznick & Endler 1982; Reznick & Bryga 1996; Reznick *et al.* 1996). Experimental introductions of guppies from HP sites transplanted to RO sites demonstrate that such life-history differences evolve on contemporary (ecological) time scales (Reznick & Bryga 1987; Reznick *et al.* 1990, 1997).

A second reason to suspect that guppy invasion, guppy evolution and *Rivulus*–guppy coevolution may influence ecosystem properties involves interspecific and intraspecific differences in diets and trophic interactions. Guppies are omnivores, consuming both epilithic algae and aquatic invertebrates (Dussault & Kramer 1981; Grether *et al.* 2001). By contrast, *Rivulus* are strict predators, foraging on aquatic and terrestrial invertebrates and small fishes (Gilliam *et al.* 1993; Fraser *et al.* 1999). The introduction of omnivores into food webs may decrease the biomass of primary producers by reducing the strength of trophic cascades (Bruno & O'Connor 2005; Duffy *et al.* 2007; Ho & Pennings 2008). At the intraspecific level, guppy populations show divergence in feeding morphology that could underlie dietary differences. Guppies from HP localities have shallower heads with mouths oriented dorsally, whereas guppies from LP sites

have deeper heads and more terminal mouths (Langerhans & DeWitt 2004; Hendry *et al.* 2006; C. K. Ghalambor, J. A. Walker & D. N. Reznick 2004, unpublished data). Similar morphological differences are evident in pelagic versus benthic forms of many lacustrine fish species (Robinson & Wilson 1994) and may have important consequences for guppy foraging behaviour (Robinson & Wilson 1995). In addition, ecological interactions between guppies and *Rivulus*, including predation by adult *Rivulus* on small guppies (primarily males and juveniles) and competition for shared prey resources, may set the stage for local coevolution to influence ecosystem properties.

3. MATERIAL AND METHODS
(a) *Experimental design and analysis*
We assembled experimental mesocosms to test the relative effects of species invasion, evolution and coevolution on stream ecosystems. Our experimental treatments were: (i) RO *Rivulus*-only, (ii) RO *Rivulus*+HP guppies, (iii) RO *Rivulus*+LP guppies, and (iv) sympatric LP *Rivulus*+LP guppies. We employed two methods of statistical analysis. First, treatments were compared using *a priori* orthogonal contrasts. Contrast 1 (invasion) tested the effects of a traditional ecological variable, species invasion, on ecosystem properties. The invasion contrast, designed to test the mean effect of guppy invasion into RO sites (ignoring intraspecific differences), compared the mean of the RO treatment (treatment 1) with the mean of the three *Rivulus*–guppy treatments (treatments 2–4). Contrast 2 (evolution) tested the effects of guppy evolution on ecosystem properties, keeping the *Rivulus* population-of-origin constant. The evolution contrast compared the mean of the HP guppy treatment (treatment 2) with the mean of the LP guppy treatment (treatment 3). Contrast 3 (coevolution) tested the effects of *Rivulus*–guppy coevolution on ecosystem properties. The coevolution contrast compared the mean of the non-coevolved *Rivulus*–guppy treatments (treatments 2 and 3) with the mean of the coevolved *Rivulus*–guppy treatment (treatment 4). Second, we used ANOVA with *post hoc* Tukey's honestly significant difference (HSD) tests to examine pairwise differences between treatments. Using pairwise tests, we examined the alternative scenarios by which guppies can invade RO streams—from an HP site (treatment 1 versus treatment 2) or an LP site (treatment 1 versus treatment 3). We also tested for differences between the RO treatment and the *Rivulus*–guppy coevolved treatment (treatment 1 versus treatment 4) because these ecosystem states represent the eco-evolutionary equilibrium states exhibited in this system. Note that the pairwise comparison for guppy evolution (treatment 2 versus treatment 3) was already performed as one of our orthogonal contrasts. Orthogonal contrasts and pairwise tests were evaluated at $\alpha = 0.05$. Statistical analyses were performed using SPSS v. 11.0.4 for Mac OS X (SPSS Inc., Chicago, IL, USA).

Response variables of primary interest included algal standing stocks at the end of the experiment, algal accrual rates over the duration of the experiment, total invertebrate biomass (and the biomass of predators and grazers) at the end of the experiment and decomposition rates over the duration of the experiment. In order to examine the mechanisms by which evolutionarily divergent guppy and *Rivulus* populations shaped experimental ecosystems, we estimated size-specific guppy and *Rivulus* excretion rates and examined the algal content of guppy diets. Experimental methods are described in detail below. Variables were log

transformed for analysis to satisfy the assumption of normality. To aid the comparison of the magnitude of ecological, evolutionary and coevolutionary effects for each response variable, we calculated means for all treatments and effect sizes for orthogonal contrasts. Effect sizes were calculated as Cohen's *d* (Cohen 1988).

(b) *Stream mesocosms*
We constructed 16 experimental stream mesocosms adjacent to Ramdeen Stream, a second-order tributary of the Arima River on the south slope of Trinidad's Northern Range Mountains. Each mesocosm was 2.6 m long×0.5 m wide and received flow from the adjacent stream, piped through a 1500 l settling tank to remove silt and debris. A mixture of gravel and sand was placed at the bottom of each mesocosm. Water depth was maintained at approximately 5 cm at the inflow and approximately 20 cm at the outflow to simulate natural habitat variation. Flow rates in each mesocosm were adjusted daily, and flow was maintained at approximately 67 l h^{-1} throughout the duration of the experiment. Vertical shade cloth (80 cm high) was erected around the sides of each channel. Light levels, monitored throughout the experiment using HOBO light loggers (Onset Computer Corp., Bourne, MA, USA), indicated that the light environment in the mesocosms was typical of open canopy conditions. Two hollow bricks (30.5 cm×19.5 cm×10 cm) were placed in each mesocosm to serve as habitat structure for fishes. The tops of all mesocosms remained open to allow terrestrial infall, but large leaf debris was removed daily. The experiment was allowed to run for a total of 28 days, starting on 27 June 2007.

We used two source populations for guppies (HP and LP) and two source populations for *Rivulus* (LP and RO). The LP site was the same for both species. All fishes were collected from the Guanapo River (HP: 10°41′46″ N, 61°15′43″ W; LP: 10°42′37″ N, 61°16′2″ W; RO: 10°43′17″ N, 61°16′51″ W). Collecting sites were identified based on surveys of fish communities conducted by Gilliam *et al.* (1993). Ten *Rivulus* (treatment 1) or five male guppies, five female guppies and five *Rivulus* (treatment 2–4) were introduced into each mesocosm to maintain equal biomass of fishes across all mesocosms. Fishes were introduced on 27 June 2007. Natural size distributions were approximated, with the exception that *Rivulus* greater than 55 mm in total length were excluded due to the risk of predation on guppies (which would have altered our treatments). Before being introduced into the experiment, all fishes were individually marked with injected elastomer (Northwest Marine Technology Inc., Shaw Island, WA, USA), photographed and weighed. All fishes were collected at the termination of the experiment (28 July 2007), photographed and weighed. Juvenile guppies born during the experiment were collected from all mesocosms at the end of the experiment. No juvenile *Rivulus* were found in the mesocosms. Differences between treatments in total fishes (numbers and biomass) and guppy reproductive output (numbers and biomass) were evaluated using ANOVA with Tukey's HSD tests.

We introduced invertebrates into each mesocosm one week before the introduction of fishes. An area of Ramdeen Stream equal to the area of all mesocosms was sampled for invertebrates using a 63 μm sieve, and predators (primarily Odonata) were removed. The remaining live invertebrates were homogenized and added in equal amounts to each mesocosm one week before the introduction of fishes. At the termination of the experiment, invertebrate communities were sampled. Two passes along the length of each mesocosm were performed using a kicknet (15 cm×15 cm, 1 mm mesh).

This procedure was followed by a 5 min visual inspection to collect any large, mobile macroinvertebrates that escaped the net. Samples were preserved in 70 per cent ethanol.

We identified invertebrates to the level of family according to Merritt & Cummins (1996) and Maharaj & Alkins-Koo (2007). Length was determined for each specimen. Mass was found using length–mass regressions from Benke *et al.* (1999). Mass for Calamoceratidae was found using the regression equation for Limnephilidae, according to McNeely *et al.* (2007). Total invertebrate biomass for each mesocosm was estimated by extrapolating from the area sampled to the total mesocosm area.

We added 11 litterbags (10 cm × 10 cm, 1 mm mesh, containing 3–4 g freshly picked, dried *Pachystachys coccinea* leaves) to each mesocosm at the start of the experiment and removed one, weekly, to estimate decomposition rates. Five bags were retained as handling controls (mean handling loss = 0.344 g). Decomposition rate was calculated as the slope of the ln-transformed per cent remaining leaf mass regressed on the incubation period in days since the start of the experiment (processing coefficient; Hauer & Lamberti 2006).

We added 12 unglazed tiles (5 cm × 5 cm) to each channel at the start of the experiment. Two tiles were chosen at random and removed each week to measure algal accumulation in terms of chlorophyll *a* concentrations (chl a). The tiles were placed in plastic bags in a −20° C freezer for at least 24 hours prior to extraction to facilitate algal cell lysing. We added 10 ml of 95 per cent ethanol to each bag and extracted in the dark at room temperature for 24 hours. We measured the fluorescence of the diluted extract using an Aquafluor handheld fluorometer (Turner Designs, Sunnyvale, CA, USA) calibrated with a solid standard. The chl *a* concentration was calculated using the total tile area and extract volume corrected for dilution. The algal accrual rate was calculated as the slope of the regression of chl *a* concentration on days since tile introduction.

We measured nutrient concentrations at the outflow of each mesocosm on 27 July 2007. Nitrogen was measured as NH_4 using an automated phenate method and as NO_3 using an automated cadmium reduction method (USEPA 1983). Phosphorus was measured as PO_4 using an automated ascorbic acid method (USEPA 1983). Nutrient analyses were performed at the Odum School of Ecology Analytical Chemistry Laboratory at the University of Georgia. Nutrient concentrations in source water entering the mesocosms were also measured on this date: $PO_4 = 1.7 \, \mu g P \, l^{-1}$, $NH_4 = 4.4 \, \mu g N \, l^{-1}$ and $NO_3 = 202 \, \mu g N \, l^{-1}$.

(c) Excretion rates
We estimated size- and sex-specific excretion rates for guppies and *Rivulus* using short-term excretion trials. *Rivulus* excretion trials were performed after dark on 26 July 2007, and guppy excretion trials were performed during daylight hours on 27 July 2007. For *Rivulus*, three individuals were selected from each mesocosm and placed independently into three different plastic ziplock bags. For guppies, individuals were selected from each mesocosm and grouped into four sex/size classes (1 adult female, 2 adult males, 3–5 subadults and 5–8 juveniles). Guppies smaller than 0.1 g wet mass were categorized as juveniles, and guppies larger than 0.1 g wet mass, but unidentifiable as either male or female, were categorized as subadults. Sex/size class groups were placed independently into four different plastic ziplock plastic bags. Each bag contained 200 ml filtered water from the mesocosm source water. Plastic bags containing fishes were placed into buckets containing stream water to prevent temperature

stress. After 15 min, 60 ml of water was removed from each bag, filtered through a Pall AE glass fibre filter (Pall Corp., East Hills, NY, USA) and frozen until analysis.

NH_4 and PO_4 concentrations from each excretion bag were measured as described above. To estimate excretion rates, we calculated the difference between nutrient concentrations in excretion bags after incubation minus the average concentration for two control bags (incubated without fishes). We divided this difference by the number of fishes per bag and multiplied by bag volume to obtain an excretion rate per fish. We calculated mass-specific excretion as the per fish rate divided by the average wet mass of fishes per bag. We then estimated the total excretion flux driven by *Rivulus* and guppies in each mesocosm by applying mass-specific excretion rate estimates to the empirical sex/size distributions obtained via fish censuses conducted on 28 July 2007. We calculated the contribution of nutrients to each mesocosm via guppy and *Rivulus* excretion relative to nutrient concentrations in source water (as measured on 27 July 2007 and assuming a constant flow rate of $67 \, l \, h^{-1}$). Differences in total N- and P-excretion (for *Rivulus* and guppies) were examined using orthogonal contrasts and pairwise tests, as described above.

(d) Guppy diets
Algal consumption was measured from adult guppies collected from the mesocosms at the termination of the experiment and frozen for preservation. We extracted chl *a* from guppy diets by making a transverse incision across each abdomen with a scalpel to expose stomach contents and incubating them in 95 per cent ethanol for 24 hours before measuring chl *a* concentrations as described above. Dietary algal content was expressed in terms of chl *a* mass per fish wet mass. Differences in dietary algae were examined using two-way ANOVA with Tukey's HSD tests, with treatment and sex included as fixed effects. Sample sizes (females, males) were: treatment 2, $n = 13, 15$; treatment 3, $n = 12, 15$; treatment 4, $n = 9, 13$.

4. RESULTS
(a) Fish biomass and guppy offspring production
Total fish biomass at the end of the experiment did not differ among treatments (ANOVA: $F_{3,12} = 1.820$, $p = 0.20$; table 1). However, the addition of guppies and the source of guppies did alter the overall size structure of the fish community (i.e. the total number of fishes comprising that biomass differed significantly; ANOVA: $F_{3,12} = 62.349$, $p < 0.01$; table 1). The number of fishes was the lowest in the RO treatment (Tukey's HSD: treatment 1 versus treatment 2, $p < 0.01$; treatment 1 versus treatment 3, $p < 0.01$; treatment 1 versus treatment 4, $p < 0.01$), the greatest in the treatment containing HP guppies (Tukey's HSD: treatment 2 versus treatment 3, $p < 0.01$; treatment 2 versus treatment 4, $p < 0.01$) and did not differ between the treatments containing LP guppies (Tukey's HSD: treatment 3 versus treatment 4, $p = 0.99$). Guppy source effects resulted from significant differences in reproductive output, in terms of both offspring numbers (ANOVA: $F_{2,9} = 13.877$, $p < 0.01$; table 1) and offspring biomass (ANOVA: $F_{2,9} = 10.653$, $p < 0.01$; table 1). Compared with LP guppies, HP guppies had more numerous offspring (Tukey's HSD: treatment 2 versus treatment 3, $p < 0.01$; treatment 2 versus treatment 4, $p < 0.01$) and a greater biomass of offspring (Tukey's HSD: treatment 2 versus treatment 3, $p < 0.01$; treatment 2 versus treatment 4, $p = 0.02$).

Table 1. Numbers ($n\,m^{-2}$) and biomass ($g\,m^{-2}$) for all fishes and for guppy offspring born in the mesocosms (mean$\pm$s.e.), collected at the end of the experiment.

treatment	total fishes		guppy offspring	
	($n\,m^{-2}$)	($g\,m^{-2}$)	($n\,m^{-2}$)	($g\,m^{-2}$)
1. RO *Rivulus*	7.83 (0.18)	9.49 (0.39)	—	—
2. RO *Rivulus*+HP guppies	53.55 (6.85)	10.51 (0.64)	42.05 (6.49)	2.02 (0.32)
3. RO *Rivulus*+LP guppies	23.60 (2.63)	8.80 (0.66)	11.76 (2.43)	0.38 (0.14)
4. LP *Rivulus*+LP guppies	24.45 (2.05)	9.15 (0.48)	12.66 (2.18)	0.47 (0.09)

These findings are consistent with previously documented life-history divergence between guppy populations locally adapted to HP versus LP habitats.

(b) *Algae, invertebrates and decomposition rates*
Orthogonal contrasts. Guppy invasion, a traditional ecological factor, did not have a significant effect on algal biomass, algal accrual rates, invertebrate biomass (total, grazers or predators) or decomposition rates. Guppy evolution had a significant impact on algal biomass (ANOVA: $F_{1,12}=6.463$, $p=0.03$; figure 1b) and algal accrual rates (ANOVA: $F_{1,12}=7.989$, $p=0.02$), with HP guppies causing increases in algae compared with LP guppies. *Rivulus*–guppy coevolution had a significant effect on total invertebrate biomass (ANOVA: $F_{1,12}=6.364$, $p=0.03$; figure 1c), with the coevolved fish community reducing aquatic invertebrate biomass relative to the non-coevolved community. The difference in invertebrate biomass between the coevolved and non-coevolved treatments resulted primarily from a change in the biomass of invertebrate predators (ANOVA: $F_{1,12}=8.162$, $p=0.02$). Predatory invertebrates were not included in the invertebrate introduction stock. Therefore, those present at the end of the experiment colonized the mesocosms during the course of the experiment (adult odonates were frequently observed depositing eggs into the mesocosms). Decomposition rates were not significantly different for any contrast. However, the absence of any differences in decomposition rates may have been due to the small mesh size of the litterbags, which prevented many invertebrate shredders and fishes from accessing the leaf litter. Full results for orthogonal contrasts are provided in appendix A.

Pairwise comparisons. Aside from the above significant effect of guppy evolution on algal biomass and accrual, pairwise comparisons were not statistically significant. Neither guppy invasion from an HP site (treatment 1 versus treatment 2) nor guppy invasion from an LP site (treatment 1 versus treatment 3) had a significant effect on algal biomass, algal accrual rates, invertebrate biomass (total, grazers or predators) or decomposition rates. Likewise, we did not find a significant difference between the RO treatment and the coevolved *Rivulus*–guppy treatment (treatment 1 versus treatment 4) for algal biomass, algal accrual rates, invertebrate biomass (total, predators and grazers) or decomposition rates. However, the pattern of treatment means for invertebrate biomass (total, predators and grazers) was consistent with the findings from the orthogonal contrasts (i.e. the lowest biomass in the coevolved treatment, treatment 4). Treatment
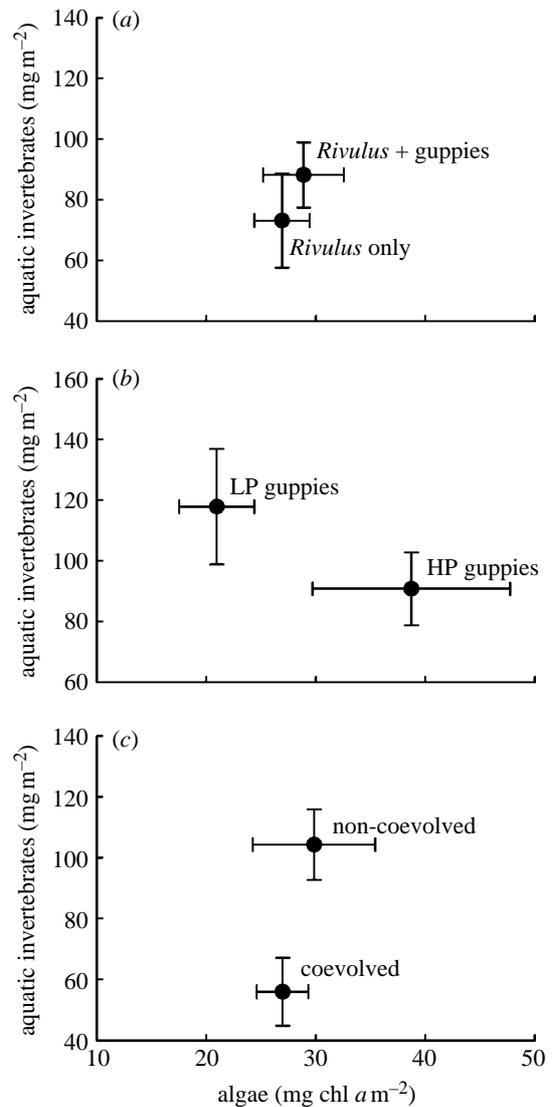

Figure 1. Effects of (a) guppy invasion, (b) guppy evolution and (c) *Rivulus*–guppy coevolution on aquatic invertebrates and epilithic algae (mean$\pm$s.e.) in stream mesocosms.

means ($\pm$s.e.) and results of pairwise comparisons are provided in appendix B.

(c) *Excretion rates*
Orthogonal contrasts. Fish excretion contributed substantially to nutrient concentrations in the mesocosms, and guppy excretion contributed more nutrients than did *Rivulus* excretion. Orthogonal contrasts revealed a significant effect of guppy invasion on the total nutrient flux due to fish excretion for NH_4 (ANOVA: $F_{1,12}=17.205$, $p<0.01$) and a marginally significant
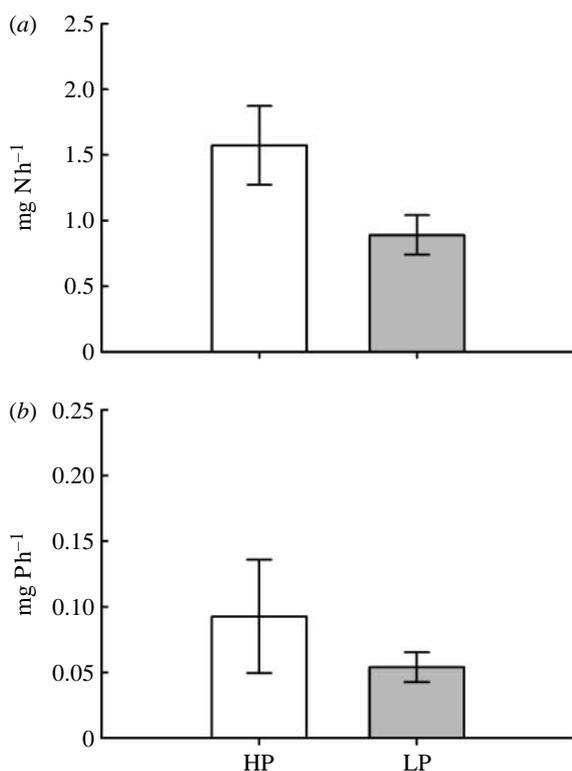
Figure 2. Mesocosm-level excretion rates (mean $\pm$ s.e.) for guppies from high-predation (HP) and low-predation (LP) populations coexisting with *Rivulus* from the *Rivulus*-only (RO) site ((*a*) N-excretion and (*b*) P-excretion).
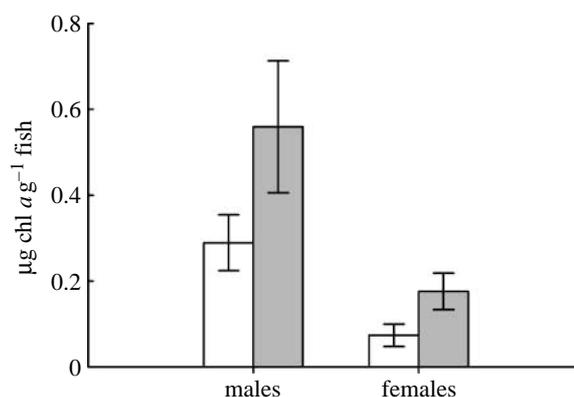

Figure 3. Algal content of guppy diets (mean $\pm$ s.e.) for males and females from high-predation (HP, white bars) and low-predation (LP, grey bars) populations coexisting with *Rivulus* from the *Rivulus*-only (RO) site.

effect for PO$_4$ (ANOVA: $F_{1,12} = 3.600$, $p = 0.08$). Relative to nutrient concentrations in source water, guppy excretion increased total N concentrations (measured as NH$_4$+NO$_3$) by an average of 7.5 per cent and P concentrations (measured as PO$_4$) by an average of 58.5 per cent. *Rivulus* excretion increased N concentrations by an average of just 0.9 per cent and P concentrations by an average of 6.1 per cent. The effect of guppy evolution on total excretion was significant for NH$_4$ (ANOVA: $F_{1,12} = 5.656$, $p = 0.04$; figure 2*a*), with HP guppies contributing more NH$_4$ via excretion than LP guppies. The effect of guppy evolution was not significant for PO$_4$ (ANOVA: $F_{1,12} = 1.001$, $p = 0.34$; figure 2*b*). Relative to source water, HP guppy excretion increased N concentrations by an average of 11.4 per cent and P concentrations by an average of 82.9 per cent, whereas LP guppy excretion increased N concentrations by an average of 5.6 per cent and P concentrations by an average of 46.4 per cent. *Rivulus*–guppy coevolution had a significant effect on NH$_4$ excretion (ANOVA: $F_{1,12} = 5.407$, $p = 0.04$), with the non-coevolved fish community contributing more NH$_4$ to the mesocosms than the coevolved community. Coevolution did not have a significant effect on PO$_4$ excretion (ANOVA: $F_{1,12} = 0.496$, $p = 0.50$). For the evolution and coevolution contrasts, differences in total fish excretion were driven overwhelmingly by differences in guppy excretion rates, not by differences in *Rivulus* excretion rates, which did not differ among treatments for either NH$_4$ or PO$_4$.

Pairwise comparisons. Guppy invasion from an HP site had a significant effect on total NH$_4$ excretion (Tukey's HSD: treatment 1 versus treatment 2,

$p < 0.01$) but not on total PO$_4$ excretion (Tukey's HSD: treatment 1 versus treatment 2, $p = 0.14$). Guppy invasion from an LP site had a marginally significant effect on total NH$_4$ excretion (Tukey's HSD: treatment 1 versus treatment 3, $p = 0.06$) but no effect on total PO$_4$ excretion (Tukey's HSD: treatment 1 versus treatment 3, $p = 0.57$). Somewhat surprisingly, based on overall differences in *Rivulus* versus guppy excretion rates, we did not find a significant difference between the RO treatment and the coevolved *Rivulus*–guppy treatment for either NH$_4$ excretion (Tukey's HSD: treatment 1 versus treatment 4, $p = 0.23$) or PO$_4$ excretion (Tukey's HSD: treatment 1 versus treatment 4, $p = 0.64$). However, mean values were greater for the coevolved treatment (appendix B).

Differences in fish excretion rates were somewhat related to differences in nutrient concentrations (measured at the outflow of each mesocosm) for NH$_4$ and PO$_4$, but not for total N (measured as NH$_4$+NO$_3$). The HP guppy treatment (treatment 2) had the highest excretion rates and the highest nutrient concentrations for NH$_4$ and PO$_4$, but the lowest concentrations for total N. Nutrient concentrations in the water are the product of both excretion and uptake via algal and heterotrophic microbial activity. Therefore, increased algal growth in the HP guppy treatment may have reduced NO$_3$ concentrations in the water, despite higher rates of fish excretion.

(d) Guppy diets
Overall, algae consumption by guppies differed significantly among treatments (ANOVA: $F_{2,71} = 5.039$, $p = 0.01$). The amount of algae consumed was significantly greater for LP guppies than for HP guppies (Tukey's HSD: treatment 2 versus treatment 3, $p = 0.04$; treatment 2 versus treatment 4, $p = 0.01$; figure 3) and significantly greater for males than for females (ANOVA: $F_{2,71} = 17.257$, $p < 0.01$; figure 3). The interaction between treatment and sex was not significant (ANOVA: $F_{2,71} = 1.086$, $p = 0.34$).

(e) Effect sizes
Compared with the effects of guppy invasion, guppy evolution and *Rivulus*–guppy coevolution had relatively large effects on ecosystem properties (figure 4). For algal accrual rates, guppy evolution had the largest
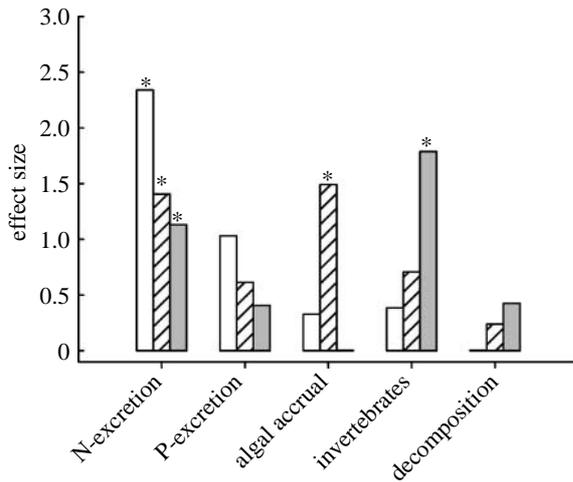
Figure 4. Effect sizes (Cohen's *d*) for guppy invasion (white bars), guppy evolution (hatched bars) and *Rivulus*–guppy coevolution (grey bars) derived from orthogonal contrasts. (Contrasts that were significant at the $\alpha = 0.05$ level are indicated by an asterisk.)

effect size (4.6 times as large as guppy invasion), followed by guppy invasion. For invertebrate biomass, *Rivulus*–guppy coevolution had the largest effect size (4.7 times as large as guppy invasion) followed by guppy evolution (1.8 times as large as guppy invasion). For decomposition rates, effect sizes were relatively small for all contrasts, although the effects of guppy evolution and *Rivulus*–guppy coevolution tended to be slightly larger than the effect of guppy invasion. For N- and P-excretion rates, guppy invasion had the largest effect size, followed by guppy evolution and *Rivulus*–guppy coevolution.

5. DISCUSSION
(a) *Effects of invasion, evolution and coevolution*
Ecologists have traditionally focused on functional diversity at or above the species level to explain community and ecosystem patterns and processes (Hooper *et al.* 2005). For this reason, species introductions and invasions have garnered much attention. Invading species introduce new phenotypes into communities, thereby initiating novel ecological interactions and modifying existing ones (White *et al.* 2006). However, phenotype distributions and ecological interactions can also change as a result of evolutionary and coevolutionary processes including those occurring over contemporary (ecological) time scales (Thompson 1999; Hairston *et al.* 2005). We examined the effects of guppy evolution and *Rivulus*–guppy coevolution on model Trinidadian stream ecosystems and compared these effects with the effects of guppy invasion. Our results show that the magnitude of evolutionary and coevolutionary effects can exceed those of traditional ecological effects.

Guppies and *Rivulus* show substantial interspecific differences in body size, excretion rates and trophic interactions. Owing to body size differences, guppies drove higher nutrient excretion rates than did *Rivulus*. In addition, guppies consume epilithic algae, whereas *Rivulus* do not. Due to these interspecific differences, guppy invasion might be expected to have important

impacts on ecosystem properties. However, our results show that guppy invasion (the substitution of half the biomass of *Rivulus* for an equal biomass of guppies) did not drastically alter algal dynamics, invertebrate biomass or decomposition rates (figures 1*a* and 4). The lack of a significant effect of guppy invasion on algae may be due to the opposing forces of nutrient excretion and algal consumption. Algal standing stocks represent primary production minus consumption. Guppy invasion caused a significant increase in N-excretion and a marginally significant increase in P-excretion (figure 4), which are expected to increase primary production. However, algae consumption by guppies may have offset any increases in primary production, resulting in no net change in algal biomass or accrual rates. We observed a small increase in aquatic invertebrate biomass caused by guppy invasion, but the effect was not significant (figures 1*a* and 4). This lack of a significant effect may have been due to either substantial invertebrate consumption by guppies and/or compensatory feeding by *Rivulus* when released (somewhat) from intraspecific competition.

Guppy populations display substantial intraspecific variation in life-history traits and morphology as a result of local adaptation to different predation regimes. HP guppies drove significantly higher rates of N-excretion compared with LP guppies (figure 2), perhaps as a consequence of life-history evolution on fish community body size structure. LP guppies consumed greater quantities of algae compared with HP guppies (figure 3), perhaps due to differences in morphology and foraging behaviour. Owing to these differences, guppy evolution might be expected to have important effects on ecosystem properties. Our results show that guppy evolution did indeed have a significant influence on algal biomass and accrual rates, with HP guppies driving increases in algae relative to LP guppies (figures 1*b* and 4).

Why did guppy evolution cause significant changes in algal biomass and accrual rates while guppy invasion did not? For guppy invasion, the effects of nutrient excretion and algal consumption appeared to work in opposite directions on algal biomass. However, for guppy evolution, these factors appeared to work in concert. Compared with LP guppies, HP guppies excreted N at higher rates, increasing primary production, and fed on algae at lower rates, decreasing algal consumption. Both effects served to increase algal standing stocks, and the net result was a significant increase in algal biomass and accrual rates for mesocosms containing HP guppies (figures 1*b* and 4).

Interestingly, guppy populations from both HP and LP localities showed consistent sex differences in algal consumption, with males of both types consuming more algae than females (figure 3). Previous studies have suggested that male and female guppies show different patterns of morphological divergence across predation regimes and other habitat features (Hendry *et al.* 2006) and show different degrees of phenotypic plasticity when faced with alternative food presentations (Robinson & Wilson 1995). One possible explanation for sex-specific aspects of phenotypic divergence and plasticity in trophic morphology is the link between algae consumption and male guppy colour patterns, which are important as mating cues and

respond to the quantity of algae-derived carotenoids in the diet (Grether 2000; Grether *et al.* 2005).

In streams where *Rivulus* and guppies coexist in the absence of other fish species, predation and competition may drive coevolution between these species. We found that the coevolved *Rivulus*–guppy treatment had a lower biomass of aquatic invertebrates than the non-coevolved treatments (figure 1*c* and 4). The reduction of a shared prey resource in the coevolved treatment suggests that competition between guppies and *Rivulus* may have selected for niche convergence (Scheffer & van Nes 2006) and enhanced competitive ability (Hairston 1980). If coevolved guppies and *Rivulus* can more efficiently exploit aquatic invertebrates, this hypothesis could explain why coevolution significantly decreased invertebrate biomass. Behavioural observations conducted in the mesocosms provide some support for this interpretation. Guppies and *Rivulus* showed less habitat segregation and interspecific avoidance behaviour in the coevolved treatment than in the non-coevolved treatments (B. A. Lamphere 2007, unpublished data). However, further work is needed to test this hypothesized mechanism.

The current paradigm for predicting ecological function is based primarily on overall phenotypic similarity or phylogenetic relatedness—the more dissimilar or distantly related two forms, the more probable they will be functionally distinct (Webb *et al.* 2002; Hooper *et al.* 2005). This assumption underlies the use of species as functional units, as overall phenotypic differences found among species are generally larger than differences within species. Based on this assumption, one would predict that the relative effect sizes in our experiment would be: guppy invasion > guppy evolution > *Rivulus*–guppy coevolution. However, our results did not generally conform to this pattern (figure 4). The effect of guppy evolution was greater than the effect of guppy invasion for algal biomass, algal accrual rates, invertebrate biomass and decomposition rates. The effect of *Rivulus*–guppy coevolution was greater than the effect of guppy invasion for invertebrate biomass and decomposition rates. These results suggest that phenotypic dissimilarity and phylogenetic relatedness are not always reliable predictors of ecological function. Given that contemporary evolution is prevalent in many species (Kinnison & Hendry 2001), including those examined in this study, our findings also suggest that ongoing evolution may merit greater consideration in both basic and applied ecology.

The results of our experiment show that coevolution may be an important factor shaping ecosystem processes. The existence of coevolutionary effects in ecosystems is not unexpected. Ecological processes are often determined not by the phenotypic traits of one species (although the traits of some species may have inordinately large effects; Post & Palkovacs 2009), but by how traits mediate interactions among many species (Urban & Skelly 2006). What is unexpected about our results is the magnitude of coevolutionary effects. For invertebrate biomass and decomposition rates, the effect of coevolution was larger than the effects of either invasion or evolution (figure 4).

Interestingly, our results suggest that it is the source of invading guppies, rather than the invasion of guppies

per se, which determines algal biomass and accrual rates, key ecosystem properties. Relative to the RO condition, HP and LP guppy invasions had opposite effects on algal biomass and accrual rates. Perhaps equally intriguing, *Rivulus*–guppy coevolution returned algal biomass and accrual rates to levels similar to their pre-guppy states. This finding suggests a note of caution for non-experimental studies of invasion. If we were to have compared algal biomass and accrual rates in natural streams at the eco-evolutionary equilibrium states that occur in nature (RO and coevolved *Rivulus*–guppy), we may have concluded that the addition of guppies to the ecosystem has no significant effects. This scenario provides a potential example of how evolutionary processes may mask ecological dynamics (Yoshida *et al.* 2007), an area of research that certainly warrants further investigation.

(b) *Eco-evolutionary feedbacks*
In this study, we examined the ecological effects of phenotypic divergence—the approach commonly used in community and ecosystem genetics (Whitham *et al.* 2006, 2008; Johnson & Stinchcombe 2007). We are able to draw inferences about the effects of evolution on ecosystem dynamics because previous research has shown that the phenotypes we examined are heritable and subject to contemporary evolution. However, it remains a frontier to experimentally examine the ecosystem effects of dynamically evolving (and coevolving) populations in the wild. Our results suggest that the ecosystem consequences of species invasions may derive, in part, from post-invasion evolution to new environmental conditions and that coevolution with other community members may contribute to community and ecosystem resilience. However, one potentially critical element that can only be entirely captured using dynamic experiments is the eco-evolutionary feedback (Fussmann *et al.* 2007; Post & Palkovacs 2009).

Eco-evolutionary feedbacks occur when organisms change the biotic or abiotic conditions of their environment and those changes then influence the direction of evolution (Laland *et al.* 1999; Palkovacs & Post 2008; Post & Palkovacs 2009). Such feedbacks can change the ecological and evolutionary trajectories of systems, causing them to deviate from expectations based on fixed phenotype experiments (Habets *et al.* 2006). We did not directly test eco-evolutionary feedbacks in this experiment, but we can use the results to speculate about potential feedback mechanisms. As revealed by previous studies, guppies from HP habitats typically experience greater resource availability due to increased algal standing stocks than do guppies from LP habitats (Grether *et al.* 2001; Reznick *et al.* 2001). The availability of algal resources appears to contribute to the evolution of female growth rates (Arendt & Reznick 2005) and can influence the evolution of male guppy colour patterns (Grether 2000; Grether *et al.* 2005). Increases in algal biomass associated with HP environments have previously been interpreted as extrinsically determined features of the ecosystem—features to which guppies respond evolutionarily. By contrast, our findings suggest that guppy populations can influence algal availability as a by-product of the evolution of life-history traits, body size differences,

morphological traits and dietary preferences. The scope for guppies to shape their ecosystem in natural streams is currently unknown. However, the experiment reported here raises the real possibility that previously documented evolutionary responses to increased algal availability may represent (at least in part) eco-evolutionary feedbacks. To examine this possibility, we have recently undertaken a series of dynamic experiments tracking the eco-evolutionary consequences of guppy invasion, guppy evolution and *Rivulus*–guppy coevolution as they unfold in the wild.

Humans are a long-recognized global driver of species invasions. Therefore, conservation biologists focus much attention on the ecology of species invasions and on developing policies to slow the rate of species introductions. Only relatively recently has it been recognized that humans are also a global driver of intraspecific phenotypic change (Palumbi 2001; Hendry *et al.* 2008). Eco-evolutionary dynamics—the effects of ecology on contemporary evolution and the reciprocal effects of evolution (and coevolution) on ecological processes—provide a new framework to understand natural systems. However, broader integration of eco-evolutionary dynamics into applied ecology and conservation biology will probably depend upon additional studies similar to ours that provide empirical insights into the relative importance of evolution and coevolution in areas of conservation concern, such as species invasions, over-harvesting, habitat alteration and global climate change.

APPENDIX A

Table 2. ANOVA tables for *a priori* orthogonal contrasts (SS, sum of squares; MS, mean squares; $*p < 0.05$).

source	d.f.	SS	MS	*F*-ratio	*p*-value
algal biomass					
treatments	3	0.129	0.043	2.131	0.15
C1 (invasion)	1	1.097×10^{-5}	1.097×10^{-5}	0.001	0.98
C2 (evolution)	1	0.129	0.129	6.463	*0.03**
C3 (coevolution)	1	5.797×10^{-5}	5.797×10^{-5}	0.003	0.96
residual	12	0.243	0.020		
total	15	0.372			
algal accrual rate					
treatments	3	0.133	0.044	2.768	0.09
C1 (invasion)	1	0.005	0.005	0.329	0.58
C2 (evolution)	1	0.128	0.128	7.989	*0.02**
C3 (coevolution)	1	5.058×10^{-7}	5.058×10^{-7}	3.161×10^{-5}	0.99
residual	12	0.192	0.016		
total	15	0.325			
total invertebrate biomass					
treatments	3	0.240	0.080	2.602	0.10
C1 (invasion)	1	0.021	0.021	0.666	0.43
C2 (evolution)	1	0.022	0.022	0.710	0.41
C3 (coevolution)	1	0.197	0.197	6.364	*0.03**
residual	12	0.369	0.031		
total	15	0.609			
predator invertebrate biomass					
treatments	3	0.405	0.135	3.001	0.07
C1 (invasion)	1	0.027	0.027	0.599	0.46
C2 (evolution)	1	0.011	0.011	0.237	0.64
C3 (coevolution)	1	0.367	0.367	8.162	*0.02**
residual	12	0.540	0.045		
total	15	0.945			
grazer invertebrate biomass					
treatments	3	0.021	0.007	0.025	0.99
C1 (invasion)	1	0.011	0.011	0.038	0.85
C2 (evolution)	1	4.322×10^{-4}	4.322×10^{-4}	0.002	0.97
C3 (coevolution)	1	0.010	0.010	0.036	0.85

(*Continued.*)

Table 2. (*Continued.*)

source	d.f.	SS	MS	F-ratio	p-value
residual	12	3.334	0.278		
total	15	3.355			
decomposition rate					
treatments	3	0.007	0.002	0.197	0.90
C1 (invasion)	1	7.500×10^{-7}	7.500×10^{-7}	6.250×10^{-5}	0.99
C2 (evolution)	1	0.002	0.002	0.148	0.71
C3 (coevolution)	1	0.005	0.005	0.429	0.53
residual	12	0.140	0.012		
total	15	0.147			
N-excretion rate					
treatments	3	4.551	1.517	24.943	*<0.01**
C1 (invasion)	1	2.770	2.770	17.205	*<0.01**
C2 (evolution)	1	0.911	0.911	5.656	*0.04**
C3 (coevolution)	1	0.871	0.871	5.407	*0.04**
residual	12	1.929	0.161		
total	15	6.481			
P-excretion rate					
treatments	3	0.015	0.005	24.943	*<0.01**
C1 (invasion)	1	0.011	0.011	3.600	0.08
C2 (evolution)	1	0.003	0.003	1.001	0.34
C3 (coevolution)	1	0.001	0.001	0.496	0.50
residual	12	0.033	0.003		
total	15	0.048			

APPENDIX B

Table 3. Response variables (mean $\pm$ s.e.) for all treatments and *post hoc* pairwise comparisons between treatments from Tukey's HSD tests (*$p < 0.05$; see text for details).

	RO *Rivulus*; treatment 1	RO *Rivulus*, HP guppies; treatment 2[a]	RO *Rivulus*, LP guppies; treatment 3[b]	LP *Rivulus*, LP guppies; treatment 4[c]
algal biomass (mg chl a m^{-2})	26.92 (2.53)	38.73 (9.03)	20.96 (1.17)	26.98 (1.17)
algal accrual[d]	0.88 (0.07)	1.36 (0.26)	0.75 (0.11)	0.96 (0.04)
invertebrate biomass (mg m^{-2})	73.11 (15.50)	90.76 (11.98)	117.82 (19.03)	55.96 (11.20)
predator biomass (mg m^{-2})	49.85 (9.08)	73.38 (9.22)	98.29 (24.11)	36.26 (9.81)
grazer biomass (mg m^{-2})	11.47 (8.52)	6.17 (3.32)	9.58 (7.52)	4.31 (1.51)
decomposition rate[e]	0.14 (0.02)	0.15 (0.02)	0.14 (0.02)	0.15 (0.02)
N-excretion rate (mg N^{-1})	0.18 (0.01)	*1.66* (0.30)*	0.99 (0.15)	0.76 (0.21)
P-excretion rate (mg P h^{-1})	0.01 (0.00)	0.10 (0.04)	0.06 (0.01)	0.06 (0.01)

[a]Guppy invasion from HP (Tukey's HSD: treatment 1 versus treatment 2).
[b]Guppy invasion from LP (Tukey's HSD: treatment 1 versus treatment 3).
[c]Eco-evolutionary equilibrium states (Tukey's HSD: treatment 1 versus treatment 4).
[d]Slope (chl a concentration on days since introduction).
[e]Slope (ln per cent remaining leaf mass on days since introduction).

REFERENCES

Arendt, J. D. & Reznick, D. N. 2005 Evolution of juvenile growth rates in female guppies (*Poecilia reticulata*): predator regime or resource level? *Proc. R. Soc. B* **272**, 333–337. (doi:10.1098/rspb.2004.2899)

Bailey, J. K., Wooley, S. C., Lindroth, R. L. & Whitham, T. G. 2006 Importance of species interactions to community heritability: a genetic basis to trophic-level interactions. *Ecol. Lett.* **9**, 78–85. (doi:10.1111/j.1461-0248.2005.00844.x)

Bailey, J. K. *et al.* 2009 From genes to ecosystems: a synthesis of the effects of plant genetic factors across levels of organization. *Phil. Trans. R. Soc. B* **364**, 1607–1616. (doi:10.1098/rstb.2008.0336)

Benke, A. C., Huryn, A. D., Smock, L. A. & Wallace, J. B. 1999 Length–mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. *J. North Am. Benthol. Soc.* **18**, 308–343. (doi:10.2307/1468447)

Benkman, C. W., Parchman, T. L., Favis, A. & Siepielski, A. M. 2003 Reciprocal selection causes a coevolutionary arms race between crossbills and lodgepole pine. *Am. Nat.* **162**, 182–194. (doi:10.1086/376580)

Brodie, E. D. J., Ridenhour, B. J. & Brodie, E. D. I. 2002 The evolutionary response of predators to dangerous prey: hotspots and coldspots in the geographic mosaic of coevolution between garter snakes and newts. *Evolution* **56**, 2067–2082. (doi:10.1111/j.0014-3820.2002.tb00132.x)

Bruno, J. F. & O'Connor, M. I. 2005 Cascading effects of predator diversity and omnivory in a marine food web. *Ecol. Lett.* **8**, 1048–1056. (doi:10.1111/j.1461-0248.2005.00808.x)

Cohen, J. 1988 *Stastical power analysis for the behavioral sciences*, 2nd edn. Hillsdale, NJ: Lawrence Erlbaum Associates.

Duffy, J. E., Cardinale, B. J., France, K. E., McIntyre, P. B., Thébault, E. & Loreau, M. 2007 The functional role of biodiversity in ecosystems: incorporating trophic complexity. *Ecol. Lett.* **10**, 522–538. (doi:10.1111/j.1461-0248.2007.01037.x)

Dussault, G. V. & Kramer, D. L. 1981 Food and feeding behavior of the guppy, *Poecilia reticulata* (Pisces: Poeciliidae). *Can. J. Zool.* **59**, 684–701. (doi:10.2307/176638)

Ezard, T. H. G., Côté, S. D. & Pelletier, F. 2009 Eco-evolutionary dynamics: disentangling phenotypic, environmental and population fluctuations. *Phil. Trans. R. Soc. B* **364**, 1491–1498. (doi:10.1098/rstb.2009.0006)

Fraser, D. F., Gilliam, J. F., MacGowan, M. P., Arcaro, C. M. & Guillozet, P. H. 1999 Habitat quality in a hostile river corridor. *Ecology* **80**, 597–607. (doi:10.2307/176638)

Fussmann, G. F., Loreau, M. & Abrams, P. A. 2007 Eco-evolutionary dynamics of communities and ecosystems. *Funct. Ecol.* **21**, 465–477. (doi:10.1111/j.1365-2435.2007.01275.x)

Gilliam, J. F., Fraser, D. F. & Alkinskoo, M. 1993 Structure of a tropical stream fish community: a role for biotic interactions. *Ecology* **74**, 1856–1870. (doi:10.2307/1939943)

Grant, P. R. & Grant, B. R. 2006 Evolution of character displacement in Darwin's finches. *Science* **313**, 224–226. (doi:10.1126/science.1128374)

Grether, G. F. 2000 Carotenoid limitation and mate preference evolution: a test of the indicator hypothesis in guppies (*Poecilia reticulata*). *Evolution* **54**, 1712–1724. (doi:10.1111/j.0014-3820.2000.tb00715.x)

Grether, G. F., Millie, D. F., Bryant, M. J., Reznick, D. N. & Mayea, W. 2001 Rain forest canopy cover, resource availability, and life history evolution in guppies. *Ecology* **82**, 1546–1559. (doi:10.2307/2679799)

Grether, G. F., Cummings, M. E. & Hudon, J. 2005 Countergradient variation in the sexual coloration of guppies (*Poecilia reticulata*): drosopterin synthesis balances carotenoid availability. *Evolution* **59**, 175–188. (doi:10.1111/j.0014-3820.2005.tb00904.x)

Habets, M. G. J. L., Rozen, D. E., Hoekstra, R. F. & de Visser, J. A. G. M. 2006 The effect of population structure on the adaptive radiation of microbial populations evolving in spatially structured environments. *Ecol. Lett.* **9**, 1041–1048. (doi:10.1111/j.1461-0248.2006.00955.x)

Hairston, N. G. 1980 Evolution under interspecific competition: field experiments on terrestrial salamanders. *Evolution* **34**, 409–420. (doi:10.2307/2408210)

Hairston Jr, N. G., Ellner, S. P., Geber, M. A., Yoshida, T. & Fox, J. A. 2005 Rapid evolution and the convergence of ecological and evolutionary time. *Ecol. Lett.* **8**, 1114–1127. (doi:10.1111/j.1461-0248.2005.00812.x)

Hall, R. O., Koch, B. J., Marshall, M. C., Taylor, B. W. & Tronstad, L. M. 2007 How body size mediates the role of animals in nutrient cycling in aquatic ecosystems. In *Body size: the structure and function of aquatic ecosystems* (eds A. Hildrew, D. Raffaelli & R. Edmonds-Brown), pp. 286–305. Cambridge, UK: Cambridge University Press.

Hauer, F. R. & Lamberti, G. A. 2006 *Methods in stream ecology*. Amsterdam, The Netherlands: Academic Press.

Hendry, A. P., Kelly, M. L., Kinnison, M. T. & Reznick, D. N. 2006 Parallel evolution of the sexes? Effects of predation and habitat features on the size and shape of wild guppies. *J. Evol. Biol.* **19**, 741–754. (doi:10.1111/j.1420-9101.2005.01061.x)

Hendry, A. P., Farrugia, T. J. & Kinnison, M. T. 2008 Human influences on rates of phenotypic change in wild animal populations. *Mol. Ecol.* **17**, 20–29. (doi:10.1111/j.1365-294X.2007.03428.x)

Ho, C. & Pennings, S. C. 2008 Consequences of omnivory for trophic interactions on a salt marsh shrub. *Ecology* **89**, 1714–1722. (doi:10.1890/07-1069.1)

Hooper, D. U. et al. 2005 Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecol. Monogr.* **75**, 3–35. (doi:10.1890/04-0922)

Johnson, M. T. J. & Stinchcombe, J. R. 2007 An emerging synthesis between community ecology and evolutionary biology. *Trends Ecol. Evol.* **22**, 250–257. (doi:10.1016/j.tree.2007.01.014)

Johnson, M. T. J., Vellend, M. & Stinchcombe, J. R. 2009 Evolution in plant populations as a driver of ecological changes in arthropod communities. *Phil. Trans. R. Soc. B* **364**, 1593–1605. (doi:10.1098/rstb.2008.0334)

Kinnison, M. T. & Hairston Jr, N. G. 2007 Eco-evolutionary conservation biology: contemporary evolution and the dynamics of persistence. *Funct. Ecol.* **21**, 444–454. (doi:10.1111/j.1365-2435.2007.01278.x)

Kinnison, M. T. & Hendry, A. P. 2001 The pace of modern life II: from rates of contemporary microevolution to pattern and process. *Genetica* **112**, 145–164. (doi:10.1023/A:1013375419520)

Kinnison, M. T., Unwin, M. J. & Quinn, T. P. 2008 Eco-evolutionary vs. habitat contributions to invasion in salmon: experimental evaluation in the wild. *Mol. Ecol.* **17**, 405–414. (doi:10.1111/j.1365-294X.2007.03495.x)

Laland, K. N., Odling-Smee, F. J. & Feldman, M. W. 1999 Evolutionary consequences of niche construction and their implications for ecology. *Proc. Natl Acad. Sci. USA* **96**, 10 242–10 247. (doi:10.1073/pnas.96.18.10242)

Langerhans, R. B. & DeWitt, T. J. 2004 Shared and unique features of evolutionary diversification. *Am. Nat.* **164**, 335–349. (doi:10.1086/422857)

Maharaj, L. D. & Alkins-Koo, M. 2007 Use of benthic macroinvertebrates to assess anthropogenic impacts in the rivers of Trinidad and Tobago. Report to the Environmental Management Authority. Appendix 11: A simplified taxonomic key to the benthic macroinvertebrates of Trinidad and Tobago. Port of Spain, Trinidad.

McNeely, C., Finlay, J. C. & Power, M. C. 2007 Grazer traits, competition, and carbon sources to a headwater-stream food web. *Ecology* **88**, 391–401. (doi:10.1890/0012-9658(2007)88[391:GTCACS]2.0.CO;2)

Merritt, R. T. & Cummins, K. W. 1996 *An introduction to the aquatic insects of North America*, 3rd edn. Dubuque, IA: Kendall/Hunt Publishing Co.

Palkovacs, E. P. & Post, D. M. 2008 Eco-evolutionary interactions between predators and prey: can predator-induced changes to prey communities feed back to shape predator foraging traits? *Evol. Ecol. Res.* **10**, 699–720.

Palkovacs, E. P. & Post, D. M. 2009 Experimental evidence that phenotypic divergence in predators drives community divergence in prey. *Ecology* **90**, 300–305. (doi:10.1890/08-1673.1)

Palumbi, S. R. 2001 Humans as the world's greatest evolutionary force. *Science* **293**, 1786–1790. (doi:10.1126/science.293.5536.1786)

Pelletier, F., Clutton-Brock, T., Pemberton, J., Tuljapurkar, S. & Coulson, T. 2007 The evolutionary demography of ecological change: linking trait variation and population growth. *Science* **315**, 1571–1574. (doi:10.1126/science.1139024)

Post, D. M. & Palkovacs, E. P. 2009 Eco-evolutionary feedbacks in community and ecosystem ecology:

interactions between the ecological theatre and the evolutionary play. *Phil. Trans. R. Soc. B* **364**, 1629–1640. (doi:10.1098/rstb.2009.0012)

Post, D. M., Palkovacs, E. P., Schielke, E. G. & Dodson, S. I. 2008 Intraspecific phenotypic variation in a predator affects community structure and cascading trophic interactions. *Ecology* **89**, 2019–2032. (doi:10.1890/07-1216.1)

Reznick, D. 1982 The impact of predation on life history evolution in Trinidadian guppies: genetic basis of observed life history patterns. *Evolution* **36**, 1236–1250. (doi:10.2307/2408156)

Reznick, D. & Endler, J. A. 1982 The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution* **36**, 160–177. (doi:10.2307/2407978)

Reznick, D., Butler, M. J. & Rodd, H. 2001 Life-history evolution in guppies. VII. The comparative ecology of high- and low-predation environments. *Am. Nat.* **157**, 126–140. (doi:10.1086/318627)

Reznick, D. A., Bryga, H. & Endler, J. A. 1990 Experimentally induced life-history evolution in a natural population. *Nature* **346**, 357–359. (doi:10.1038/346357a0)

Reznick, D. N. & Bryga, H. 1987 Life-history evolution in guppies (*Poecilia reticulata*): 1. Phenotypic and genetic changes in an introduction experiment. *Evolution* **41**, 1370–1385. (doi:10.2307/2409101)

Reznick, D. N. & Bryga, H. A. 1996 Life-history evolution in guppies (*Poecilia reticulata*: Poeciliidae). 5. Genetic basis of parallelism in life histories. *Am. Nat.* **147**, 339–359. (doi:10.1086/285855)

Reznick, D. N., Butler, M. J., Rodd, F. H. & Ross, P. 1996 Life-history evolution in guppies (*Poecilia reticulata*). 6. Differential mortality as a mechanism for natural selection. *Evolution* **50**, 1651–1660. (doi:10.2307/2410901)

Reznick, D. N., Shaw, F. H., Rodd, F. H. & Shaw, R. G. 1997 Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). *Science* **275**, 1934–1937. (doi:10.1126/science.275.5308.1934)

Robinson, B. W. & Wilson, D. S. 1994 Character release and displacement in fishes: a neglected literature. *Am. Nat.* **144**, 596–627. (doi:10.1086/285696)

Robinson, B. W. & Wilson, D. S. 1995 Experimentally induced morphological diversity in Trinidadian guppies (*Poecilia reticulata*). *Copeia* **1995**, 294–305. (doi:10.2307/1446893)

Scheffer, M. & van Nes, E. H. 2006 Self-organized similarity, the evolutionary emergence of groups of similar species. *Proc. Natl Acad. Sci. USA* **103**, 6230–6235. (doi:10.1073/pnas.0508024103)

Thompson, J. N. 1998 Rapid evolution as an ecological process. *Trends Ecol. Evol.* **13**, 329–332. (doi:10.1016/S0169-5347(98)01378-0)

Thompson, J. N. 1999 The evolution of species interactions. *Science* **284**, 2116–2118. (doi:10.1126/science.284.5423.2116)

Thompson, J. N. 2005 *The geographic mosaic of coevolution*. Chicago, IL: University of Chicago Press.

Urban, M. C. & Skelly, D. K. 2006 Evolving metacommunities: toward an evolutionary perspective on metacommunities. *Ecology* **87**, 1616–1626. (doi:10.1890/0012-9658(2006)87[1616:EMTAEP]2.0.CO;2)

USEPA 1983 Methods for Chemical Analysis of Water and Wastes, EPA-600/4-79-020. United States Environmental Protection Agency, Cincinnati, OH.

Vanni, M. J. 2002 Nutrient cycling by animals in freshwater ecosystems. *Annu. Rev. Ecol. Syst.* **33**, 341–370. (doi:10.1146/annurev.ecolsys.33.010802.150519)

Vanni, M. J. & Layne, C. D. 1997 Nutrient recycling and herbivory as mechanisms in the 'top-down' effect of fish on algae in lakes. *Ecology* **78**, 21–40. (doi:10.2307/2265976)

Webb, C. O., Ackerly, D. D., McPeek, M. A. & Donoghue, M. J. 2002 Phylogenies and community ecology. *Annu. Rev. Ecol. Syst.* **33**, 475–505. (doi:10.1146/annurev.ecolsys.33.010802.150448)

White, E. M., Wilson, J. C. & Clarke, A. R. 2006 Biotic indirect effects: a neglected concept in invasion biology. *Divers. Distrib.* **12**, 443–455. (doi:10.1111/j.1366-9516.2006.00265.x)

Whitham, T. G. *et al.* 2006 A framework for community and ecosystem genetics: from genes to ecosystems. *Nat. Rev. Genet.* **7**, 510–523. (doi:10.1038/nrg1877)

Whitham, T. G., DiFazio, S. P., Schweitzer, J. A., Shuster, S. M., Allan, G. J., Bailey, J. K. & Woolbright, S. A. 2008 Extending genomics to natural communities and ecosystems. *Science* **320**, 492–495. (doi:10.1126/science.1153918)

Yonekura, R., Kohmatsu, Y. & Yuma, M. 2007 Difference in the predation impact enhanced by morphological divergence between introduced fish populations. *Biol. J. Linn. Soc.* **91**, 601–610. (doi:10.1111/j.1095-8312.2007.00821.x)

Yoshida, T., Ellner, S. P., Jones, L. E., Bohannan, B. J. M., Lenski, R. E. & Hairston Jr, N. G. 2007 Cryptic population dynamics: rapid evolution masks trophic interactions. *PLoS Biol.* **5**, 1868–1879. (doi:10.1371/journal.pbio.0050235)