Assessing the effects of guppy life history evolution on nutrient recycling: from experiments to the field

RANA W. EL-SABAAWI*, MICHAEL C. MARSHALL[†], RONALD D. BASSAR[‡],
ANDRÉS LÓPEZ-SEPULCRE[§,¶], ERIC P. PALKOVACS** AND CHRISTOPHER DALTON[††]

Department of Biology, University of Victoria, Victoria, BC, Canada
[†]*Odum School of Ecology, University of Georgia, Athens, GA, U.S.A.*
[‡]*Department of Environmental Conservation, University of Massachusetts, Amherst, MA, U.S.A.*
[§]*Institute of Ecology and Evolutionary Science of Paris, CNRS UMR 7618, Paris, France*
[¶]*Center of Excellence in Biological Interactions, University of Jyväslylä, Jyväslylä, Finland*
***Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, CA, U.S.A.*
[††]*Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY, U.S.A.*

SUMMARY

1. Trait evolution can occur in response to anthropogenic alterations to ecosystems and can occur on timescales similar to those of ecological processes suggesting that it could alter ecosystem function. In this study, we characterise the effects of life history evolution on nutrient recycling using the Trinidadian guppy (*Poecilia reticulata*) as a model system.

2. Guppy life history traits evolve in response to predation pressure. When predation pressure is removed, guppy population density and average body size of the population also increase. Therefore, the evolution of guppy life histories involves changes in individual traits and demographic characteristics, both of which can alter nutrient recycling. The relative contributions of these variables to guppy-driven nutrient recycling are unknown.

3. We synthesise data from published experiments to disentangle how differences in individual traits, population characteristics and environmental conditions contribute to differences in guppy excretion rates. Individual guppies adapted to the absence of predators [low-predation (LP) guppies] have lower nitrogen and phosphorus excretion rates than individual guppies adapted to predators [high-predation (HP) guppies]. However, LP guppy populations excrete twice as much nitrogen as HP populations because of their larger average body size and higher population densities.

4. We compare these findings to guppy excretion data collected from HP and LP sites in four rivers in Trinidad. Phenotypic and population differences in excretion rates are consistent with those observed in the experiments.

5. Our study demonstrates that life history evolution can alter nutrient recycling in freshwater ecosystems. Characterizing the combined effects of traits and demographics is essential for understanding the effects of life history evolution on ecosystem processes.

Keywords: ammonium, light, phosphorus, eco-evolutionary interactions, stoichiometry

Introduction

Animals can play important roles in the biogeochemical cycles of aquatic ecosystems. Fish and invertebrates excrete dissolved inorganic nutrients as metabolic waste, which is immediately available to primary producers, and which can support a significant fraction of primary production (Vanni, 2002). There have been numerous studies on the effects of fish and other consumers on nutrient recycling in lakes and rivers (Sereda, Hudson & Mcloughlin, 2008; Benstead *et al.*, 2010; Johnson *et al.*, 2010; Sereda & Hudson, 2011). Most of these studies seek to link differences in nutrient recycling to interspecific differences in organism traits such as elemental

body composition or body size. Fewer studies examine intraspecific variability in nutrient recycling, which has been linked to ontogenetic variability in dietary quality, body size or elemental composition (Pilati & Vanni, 2007).

Differences in nutrient recycling among populations raise the possibility that microevolutionary trait differences are important determinants of nutrient recycling rates. Trait evolution could alter excretion by changing organismal physiology, behaviour, morphology or tissue elemental composition. Studies show that trait evolution frequently occurs on the short timescales formerly thought to be the exclusive domain of ecological dynamics (Reznick *et al.*, 1997; Thompson, 1998; Hendry & Kinnison, 1999). Such rapid contemporary evolution has the capacity to interact dynamically with and alter the expected outcomes of ecological processes (Palkovacs *et al.*, 2009; Bassar *et al.*, 2010). Evolutionary change can occur in response to common anthropogenic stressors such as species invasions, species losses or habitat alterations (Palkovacs *et al.*, 2012). Characterising the effects of evolutionary change on ecosystem function is therefore important for understanding the consequences of anthropogenic stress and for crafting effective mitigation and conservation strategies (Hendry *et al.*, 2010).

Evolutionary changes in trait distribution might influence the response of ecosystem processes to biodiversity losses. The loss of top predators due to habitat alteration or exploitation threatens aquatic ecosystems globally (Estes *et al.*, 2011). Predators can have cascading effects on ecosystem function by changing the density and biomass of consumer populations (Pace *et al.*, 1999). Cascading effects of predators can also result from plastic responses in prey behaviour metabolism, body size and life history (Peckarsky *et al.*, 2001; Hawlena & Schmitz, 2010). However, predators can also act as agents of natural selection causing prey life history and morphology to evolve (Reznick & Endler, 1982). The rapid evolution of prey traits can alter prey population dynamics and community interactions (Yoshida *et al.*, 2003; Becks *et al.*, 2010). Adaptive changes in prey traits affect a range of ecosystem processes including nutrient recycling, which shapes emergent ecosystem responses to evolutionary changes in prey phenotype (Palkovacs *et al.*, 2009; Bassar *et al.*, 2010, 2012).

Currently, we do not know the extent to which trait evolution affects nutrient recycling, which might also depend on population characteristics such as body size and density (Elser & Urabe, 1999; Elser *et al.*, 2000; Hall *et al.*, 2007; Benstead *et al.*, 2010). Although trait evolution almost invariably co-occurs with changes in demographic

variables such as population density and body size distribution (Post *et al.*, 2008; Coulson, Tuljapurkar & Childs, 2010), most studies examining the effects of trait evolution on ecosystem function have focused on the role of individual traits. In addition, most information on eco-evolutionary interactions comes from small-scale experiments (Yoshida *et al.*, 2003; Harmon *et al.*, 2009; Bassar *et al.*, 2010). These experiments allow us to measure the importance of specific factors and to isolate effects that might be confounded in nature, but the benefit of these experiments comes at a cost of realism and applicability to real-world ecosystems. The results of these experiments are rarely compared to observations from natural ecosystems (but see Palkovacs & Post, 2009).

In this study, we ask how important are the effects of trait evolution on nutrient recycling, particularly when we consider that trait evolution co-occurs with changes in demographic characteristics of populations. We first use experimental data compiled from our recent work on the Trinidadian guppy (*Poecilia reticulata*) to disentangle the phenotypic (i.e. trait) and demographic effects (density, size structure) of predators on nutrient recycling. We then compare these findings against newly collected measurements of guppy excretion from four rivers in Trinidad. Guppies have become a model system for studying the effects of life history evolution on ecosystem function, but almost all guppy studies have so far been conducted in experimental mesocosms (Palkovacs *et al.*, 2009; Bassar *et al.*, 2010, 2012; El-Sabaawi *et al.*, 2015). This is the first study to explore how life history evolution might affect nutrient recycling patterns in nature.

Methods

The study system

On the island of Trinidad, guppy phenotypic traits evolve in response to predator removal (Reznick & Endler, 1982; Reznick, 1997). In sites where guppies are found with large fish predators (e.g. *Crenicichla alta*, *Hoplias malabaricus*), they mature early and produce numerous, small offspring (the high-predation 'HP' phenotype) (Reznick & Bryga, 1996; Reznick, Rodd & Cardenas, 1996b; Reznick *et al.*, 1996a). In sites where they co-exist only with a single weak predator (*Rivulus hartii*), guppies mature late and produce few but large offspring (the low-predation 'LP' phenotype) (Reznick & Bryga, 1996; Reznick *et al.*, 1996a,b). Predation has been invoked to explain a suite of guppy traits including metabolic rates, male colour, growth, body shape and

trophic morphology (Magurran, 2005; Burns, Di Nardo & Rodd, 2009; Huizinga, Ghalambor & Reznick, 2009; Palkovacs, Wasserman & Kinnison, 2011; Zandona *et al.*, 2011). In many cases, differences in traits observed between HP and LP guppies in the wild persist in the second generation of laboratory-grown fish, indicating that trait changes are heritable and that trait evolution has occurred in response to predation (Reznick, 1982; Reznick & Bryga, 1996; Huizinga *et al.*, 2009).

Rivers in the Northern Range Mountains of Trinidad are punctuated by waterfalls that limit the upstream movement of fish (Magurran, 2005). High-predation sites are often found downstream of waterfalls, while LP sites are found upstream. The evolution of guppies can be induced experimentally by transplanting guppies from downstream HP sites to upstream guppy-free sites, and LP life history traits begin to evolve within 2–3 years of introduction (Reznick, Bryga & Endler, 1990; Reznick *et al.*, 1997). Several demographic characteristics of guppy populations also change as LP phenotypes evolve. Because of their lower mortality rates, LP populations have higher densities than HP populations (Reznick *et al.*, 1996a,b). Because they also mature later and have higher survival rates, average body size in LP populations is bigger than in HP populations (Reznick & Endler, 1982; Reznick, Butler & Rodd, 2001). There are also differences in light availability between HP and LP sites. High-predation sites are typically second- or third-order streams with open canopies and relatively high levels of primary production, whereas LP sites are smaller first- to second-order streams with closed canopies and low levels of primary production (Reznick *et al.*, 2001). Therefore, the evolution of guppy traits co-occurs with changes to population demographics and environmental variables. Some of these differences are shaped by predation (density and size structure), while others (e.g. light) are extrinsic. Density, body size and light are all key variables for structuring ecosystem function that have also been shown to affect consumer-mediated nutrient recycling rates in freshwater ecosystems (Hall *et al.*, 2007; Sereda *et al.*, 2008; Benstead *et al.*, 2010; Moslemi *et al.*, 2012). However, the role of these variables in mediating the effects of life history evolution on nutrient recycling has not been studied.

Recently, we conducted a series of artificial stream mesocosm experiments designed to assess whether guppy life history phenotype had significant and meaningful effects on ecosystem function (Palkovacs *et al.*, 2009; Bassar *et al.*, 2010; El-Sabaawi *et al.*, 2015). In all of these experiments, wild-caught HP and LP guppies were introduced into artificial stream channels, and the response of

several ecosystem variables was measured over a month (see Appendix S1). These experiments showed consistent trends. Mesocosms with HP guppies had higher algal standing stocks, higher primary production and more ecosystem demand for N than those with LP guppies. There were also significant differences in nutrient recycling between phenotypes because HP guppies excreted more N per fish (and per unit biomass) than LP guppies. In each study, the guppy phenotype treatment was crossed with a population, community or an environmental variable, and to determine whether phenotypic effects on ecological response variables were meaningful, each of the studies compared the effect size of the phenotype to the effect size of the population, community or environmental treatment. Bassar *et al.* (2010, 2012) compared the ecosystem effects of guppy phenotype to the ecosystem effects of doubling guppy density, using a high- and low-density treatment that mimicked natural densities in LP and HP populations, respectively. El-Sabaawi *et al.* (2015) compared the effects of guppy phenotype under two light treatments (high and low, *c.* 26 and *c.* 111 mmol quanta m^{-2} day^{-1}, respectively), corresponding to light levels in HP and LP environments. These experiments showed that guppy phenotype had a significant effect on N excretion, which was either nearly equal to (El-Sabaawi *et al.*, 2015) or larger than (Bassar *et al.*, 2010) the environmental or population variable.

All of these experiments were conducted using the same experimental stream channels and were set up and assessed using identical methods (Appendix S1). Across these experiments, guppy excretion rates (N-NH$_4$ and P-SRP) were also measured using the same method (Appendix S1). Therefore, although the experiments were conducted in different years and by different investigators, they were otherwise similar. Up to this point, there has been no systematic effort to synthesise the findings from these experiments, or to compare them to excretion measurements from natural stream systems. We begin by presenting a synthesis of these experimental data, which we then use to make predictions about how guppy-mediated nutrient recycling (i.e. excretion) varies when we account for phenotypic (individual) as well as population or environmental differences between HP and LP populations. We then compare findings from these experiments to measurements of nutrient recycling from HP and LP populations in four rivers in Trinidad.

Synthesis of experimental data

Throughout our study, we will refer to two different estimates of nutrient recycling: an individual excretion

rate, which describes excretion rates of individual fish (measured in μg N or μg P fish^{-1} h^{-1}), and a population excretion rate, which is the sum of excretion rates of all individuals in a specified population (measured in total μg N or μg P m^{-2} h^{-1}). The phenotypic, demographic and environmental changes that vary across predation sites and that co-occur with guppy evolution can shape either of these estimates. For example, previous experiments have shown that in common garden conditions HP guppies excrete more N per fish than LP guppies, but environmental differences in light can enhance these differences in nature because high light (typical of HP environments) enhances N excretion, while low light (typical of LP environments) depresses N excretion (Bassar *et al.*, 2012; El-Sabaawi *et al.*, 2015). However, we predict that as a population, HP guppies contribute less recycled N than LP guppies because LP populations occur at higher densities than HP populations and because LP populations also have more large individuals than HP populations (Reznick *et al.*, 2001).

We collated individual guppy excretion rates from previous experiments into a single data set and then used Monte Carlo simulations to estimate population excretion rates from these data. We focused on the El-Sabaawi (phenotype × light, P×L) and Bassar (phenotype × density, P×D) experiments because they reported individual guppy excretion rates, rather than total excretion rates of all guppies in a single artificial stream channel, as measured and reported in Palkovacs *et al.* (2009). Details of these experiments (manipulations, replicates, populations used, etc.) are in Appendix S2. Each experiment ran for 28 days, and guppy excretion rates were measured on the second-to-last day using methods described in Appendix 1. The P×D experiment was run at low light comparable to the low light treatment in P×L, while P×L was run at low guppy density comparable to the low-density treatment in P×D (Appendix S2). Therefore, both experiments had a low guppy density/low light treatment, which we used to show that interexperimental differences in observed excretion rates were small (Figure S1). The same initial average body size distributions (*c.* 14 mm) and sex ratios (1 : 1) were used in both experiments. Both studies revealed a statistically significant phenotypic difference in N excretion rates between size-matched HP and LP guppies, with individual HP guppies excreting more N per hour than individual LP guppies. In contrast, both studies found that phenotypic differences in P excretion were consistently low. In both studies, significant differences in recycling rates were observed only for female and juvenile guppies, so we limited our analysis of experimental data to females and juveniles. Because male excretion rates were not significantly different between phenotypes, and because they were only weakly correlated with body size, we assumed that the contributions of male guppies to nutrient recycling would not vary between LP and HP populations in the experiments. Because males are generally small compared to females, and because guppy populations are female biased, the total contribution of males to nutrient recycling is likely to be smaller than the contributions of female guppies in nature (Rodd & Reznick, 1997; Reznick *et al.*, 2001).

We analysed these combined experimental data starting with a linear mixed-effect model with phenotype (HP versus LP), density (high versus low) and light (high versus low) as categorical fixed effects. Interactions between phenotype and density, and phenotype and light were also included. Body size (measured in wet mass) was included as a continuous variable, and interactions between body size and the main fixed effects were included in initial model runs. Both body size and excretion rates were natural log-transformed prior to analysis because excretion rate scale allometrically with body size. The numbers/identities of the experimental channel nested within each experiment were included as categorical random effects. To identify the combination of variables that best predict excretion rates, the full model (including all described fixed effects, covariates and interactions) was contrasted to alternative models with a reduced subset of parameters but with the same random effect structure. Because our goal was to specifically address the effects of phenotype, light and density on individual recycling rates, and to project these effects into population estimates, we considered only model selection results that retained phenotype, light and density as main effects and used the model selection procedure to decide on including interactions. Model comparisons and selection of the final model were performed using the Akaike Information Criterion (AIC$_c$) and maximum-likelihood testing (Burnham & Anderson, 2002; Bolker, 2008). We decided on including interactions only if they were present in the top model and if their removal had effects on model fit as illustrated by maximum-likelihood analysis. We used this conservative approach to decide on including interactions because properly assessing interactions typically requires large data sets, and including weak interactions in the predictive model would introduce large errors in the parameter estimates, and in population estimates based on those parameters.

To calculate population excretion rates while also accounting for estimation error, we used Monte Carlo

simulations drawn from the coefficients generated from the linear mixed-effects model described above to estimate population excretion rates for guppy populations that had either HP or LP population characteristics (size distributions and densities). For each population type, we ran 1000 simulations; each simulation drew a random sample of coefficients of the linear mixed-effects model from a multivariate normal distribution using the estimated coefficient means and variance–covariance matrix. We predicted individual excretion values for a continuous range of sizes for two scenarios reflecting how guppies are found in nature: HP phenotypes at high light and low density, and LP phenotype at low light and high density. We then integrated these values over the known frequency distribution of size classes and then multiplied the integrated values by density estimates of HP and LP populations. One of the advantages of the P×D and P×L experiments was that they measured guppy growth and life history traits (reproductive allotment, fecundity, etc.) from individually marked guppies and then used these values to estimate a population growth rate using an internal projection model (IPM). This approach generated a stable size distribution that is based on individually measured growth and life history traits, which we used in our calculation of population excretion. For total guppy density, we used six guppies per 1.2 m^2 (for HP/low-density population) and 12 guppies per 1.2 m^2 (for LP/high density population), which corresponded to low and high density treatments across all experiments (1.2 m^2 is the benthic area of the experimental channels). These values were chosen based on a previous survey of guppy populations (Reznick *et al.*, 2001). For each population, we report the mean and standard errors of the simulation results.

Measurements from natural stream ecosystems

We measured guppy excretion rates from high- and low-predation sections of four major rivers: the Aripo, Guanapo, Arima and Marianne. The environmental characteristics of these sites are reported in Table S1. We chose these particular rivers because they had been the focus of previous studies and were therefore well characterised. The predation communities within each river were identified by the presence or absence of major predators. Guppy life history trait differences from these rivers were confirmed in previous studies (Reznick *et al.*, 1996a,b; Zandona *et al.*, 2011). In all of these rivers, HP sites had higher light availability (more open canopies) than LP sites (Table S1).

We caught *c.* 12–16 guppies from each population using dip nets and measured nitrogen (N-NH$_4$) and phosphorus (P-SRP) excretion rates using the same methods used in the experiments (Appendix S1). This subsample was dominated by females and juvenile guppies, but also included some males that we were not able to exclude during the trial. We analysed natural log-transformed excretion rates as a linear mixed-effects model that included predation community (HP versus LP) as a fixed effect, natural log-transformed body mass as a covariate and river identity as a random effect. We compared subsets of this model using AIC$_c$ and maximum-likelihood tests. We generated model coefficients and total population excretion estimates as described for the experimental data. We estimated densities at each site by seining a section of stream of known dimensions until depletion and then estimating catch per unit effort. Using this method, average density estimates were 9.2 ($\pm$2.9 SE) guppies m^{-2} in LP sites and 2.2 ($\pm$0.75 SE) guppies m^{-2} in HP sites (Table S1). For size distribution of LP and HP sites, we used the size structures of HP and LP guppies reported in Reznick *et al.* (2001), which were based on mark–recapture experiments in multiple HP-LP populations (see also Rodd & Reznick, 1997).

All statistical analyses were conducted in the R environment using packages lmer4, car and MuMIn (Fox & Weisberg, 2011; Bates, Maechler, Bolker & Walker, 2014; R Development Core Team, 2012; Bartoń, 2014)

Dissecting the phenotypic, demographic and environmental contributions to population excretion

We estimated the relative influences of phenotype, demography and light on population excretion rates by calculating how LP population excretion would change if each consecutive variable were unaltered in the course of guppy life history evolution (i.e. held at the HP value). Using the same simulation procedures described above, we calculated LP population excretion in four scenarios: (i) where individual excretion rates did not evolve (i.e. remained at HP levels) but where guppy populations took on LP characteristics through increases in density and average body size; (ii) where the LP guppies evolved the measured different excretion rates and population density increased, but size structure remained HP-like (i.e. dominated by small guppies); (iii) where LP excretion rates evolved, average size structure increased, but population density remained at low, HP-like levels; and (iv) where both excretion rates and population characteristics became LP like, but light levels remained as high as they were in HP sites. We

compared the calculated LP population excretion rate from each scenario with LP population excretion calculated from simulations that assume that individual excretion rates, population demographics and environmental conditions all become LP like after the removal of predators. We conducted this analysis on N excretion data from the experiments and natural streams. However, because life history phenotype and light were confounded in natural stream systems, we were not able to tease apart their relative influences on population excretion.

Results

The analysis of experimental data showed that two models were nearly equally good at fitting N excretion (Table S2). Both models contained phenotype, density and light as fixed effects, but the second model also included the interaction between phenotype and body size (Table S2). However, because removing this interaction did not alter model fit ($\chi^2 = 1.9$, d.f. = 1, $P = 0.168$), we chose the first and simplest model to simulate population excretion rates. This model showed that HP guppies had larger individual N excretion rates than LP guppies, that guppies at high light excreted more N than guppies at low light and that density had a only a small effect on N excretion of individual guppies (Table 1, Fig. 1a). The net effect was that an HP guppy recycled more N than an LP guppy of a comparable body size (Fig. 1a). However, as a population, LP guppies were estimated to recycle *c.* 2× more N than HP guppies (Fig. 1b).

The best models for individual N excretion measured from natural streams included body size, predation community and their interaction (Table S3). We retained the interaction because a maximum-likelihood ratio test showed that removing the interaction had a significant effect on model fit ($\chi^2 = 4.1$, d.f. = 1, $P = 0.04$). The interaction suggested that the slope of the relationship between body size and N excretion was steeper for individual HP guppies than individual LP guppies (Table 1, Fig. 1c). However, total N excretion in LP sites was estimated to be *c.* 3× more than in HP guppy sites (Fig. 1d).

The best model describing P excretion from the experiments included body size, light, phenotype, density as well as two interactions: body size × phenotype and body size × density (Table S4). We retained the body size × density interaction because removing it had a significant effect on model fit ($\chi^2 = 4.1$, d.f. = 1, $P = 0.03$). At small body sizes, an LP guppy excreted less P than

Table 1 Models and parameter estimates for nitrogen (N-NH4) excretion and phosphorus (P-SRP) excretion based on experimental data and measurements from natural streams

Model	Variable	Estimate	SE	t value
a. N	(experiments)			
	Intercept	3.97	0.19	20.54
	Body size	0.73	0.08	9.31
	Phenotype (LP)	−0.14	0.09	−1.63
	Light (Low)	−0.29	0.11	−2.73
Density (Low)		0.11	0.20	
		0.02		
b. N (natural streams)	Intercept	3.15	0.27	11.61
	Body size	0.58	0.08	7.09
	Predation community (LP)	−0.92	0.37	−2.48
	Predation community (LP) × Body size	−0.24	0.12	−2.04
c. P	(experiments)			
	Intercept	1.45	0.67	2.15
	Body Size	0.14	0.24	0.57
	Phenotype (LP)	−0.93	0.42	−2.21
	Light (Low)	−0.78	0.30	−2.56
	Density (Low)	1.25	0.62	2.01
	Body size × Phenotype (LP)	−0.43	0.18	−2.40
Body	size × Density (Low)	0.51	0.25	1.99
d. P (natural streams)	Intercept	0.22	0.35	0.63
	Body size	0.18	0.09	2.06
	Predation community (LP)	−1.03	0.21	−5.04

All parameter estimates are for body size = 0.

an HP guppy of the same size, and the opposite was true for large guppies (Table 1, Fig. 2a). However, there were no differences in mean population P excretion rates between HP and LP guppy populations (Fig. 2b).

The best model for P excretion from natural streams included body size, predation community and their interaction (Table S5). We did not retain the interaction because removing it did not affect model fit ($\chi^2 = 0.7$, d.f. = 1, $P = 0.40$). According to this model, HP guppies excreted more P per fish than LP guppies, and body size was positively correlated with P excretion in both phenotypes (Table 1, Fig. 2c). However, as observed in the experimental data, differences in P recycling between HP and LP populations were relatively small (Fig. 2d).

Excretion rates in natural streams were lower (between 50 and 20%) than excretion rates measured in the experiments. The median of per capita N excretion rates was *c.* 4.2 μg N fish^{-1} h^{-1} in natural streams compared to *c.* 6.6 μg N fish^{-1} h^{-1} in the experiments. Similarly, the median P excretion rate was 0.4 μg P fish^{-1} h^{-1} in natural streams and *c.* 2.2 μg P fish^{-1} h^{-1} in the experiments.

Experiments

(a)

Natural streams

(c)

(b)

(d)
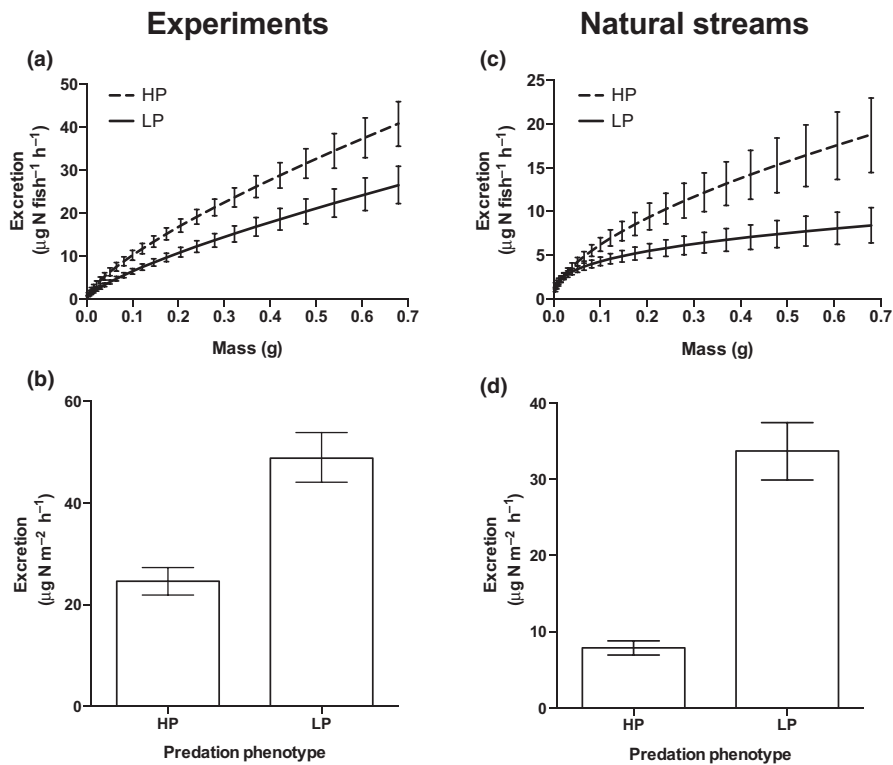
Fig. 1 Nitrogen (NH$_4$-N) excretion rates estimated from experiments (a, b) and from natural streams (c, d). Panels a and c represent individual excretion rates (and SEs). High-predation (HP) guppies are shown using dashed lines, and low-predation guppies (LP) using solid lines. Panels (b) and (d) represent estimated total N-NH4 excretion of all individuals in HP and LP populations (means and SEs).

Experiments

(a)

Natural streams

(c)

(b)

(d)
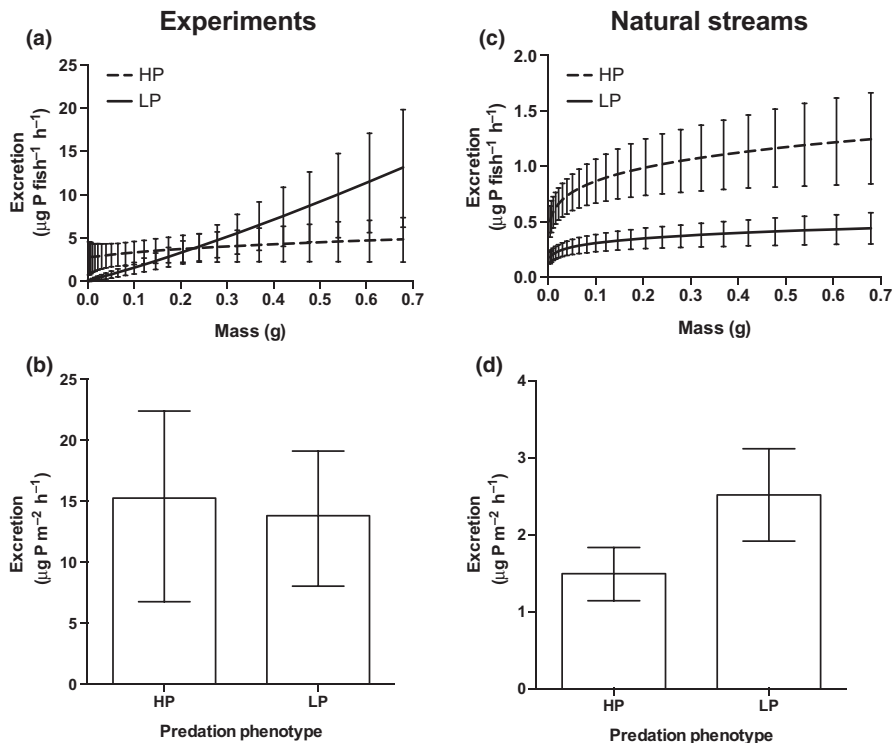
Fig. 2 Phosphorus (SRP-P) excretion rates estimated from experiments (a, b) and from natural streams (c, d). Panels a and c represent individual excretion rates (and SEs). High-predation (HP) guppies are shown using dashed lines, and low-predation (LP) guppies using solid lines. Panels (b) and (d) represent estimated total P-SRP excretion of all individuals in HP and LP populations (means and SEs).

Although individual HP guppies excreted more N per fish than LP guppies, they contributed less as a population because of their relatively low densities and low average body size (Table 2, Fig. 1b,d). The analysis of N excretion from the experiments suggested that demographic changes had a larger effect on population excretion than changes in individual excretion rates (Table 2). Changes in density and average size structure from HP

Table 2 The results of an analysis that assesses the relative influences of the phenotypic and ecological correlates of guppy life history evolution on the total N-NH4 recycled by the guppy population. 'Change' is defined as the relative change of total population N-NH4 excretion by low-predation (LP) guppies when 'parameter manipulated' is assumed to not vary with guppy life history evolution. This means that the parameter is held at the high-predation (HP) value in model simulation. Change is calculated relative to total population N-NH4 excretion estimated when all parameters are held at LP values. A negative value in the change column indicates a reduction in LP total population excretion in response to variable manipulation, and a positive value indicates an increase

Data	Parameter manipulated	Simulation scenario	Change (%)
Experiments	Phenotype	Phenotype = HP; Density, Light, and Size structure = LP	36
	Size structure	Size structure = HP; Phenotype, Density, and Light = LP	−35
	Density	Density = HP; Phenotype, Light, and Size structure = LP	−49
	Light	Light = HP; Phenotype, Density, and Size structure = LP	17
Natural streams	Individual Excretion Rate	Individual excretion rates = HP; Size structure and Density = LP	43
	Size structure	Size structure = HP; Individuals and Density = LP	−21
	Density	Density = HP; Individuals and size structure = LP	−76

to LP populations increased the N recycled by *c.* 35–50%. Excretion differences among HP-LP individuals were still important, causing *c.* 36% change in population excretion rates. Light availability affected N recycling, because low light conditions caused LP populations to contribute *c.* 20% less than they would have under high light conditions (Table 2). In the analysis of N excretion from natural streams, density and size structure had similar effects on population excretion as they did in the experiments, but individual excretion rate was more important than they were in the experiments. Because light and life history traits were confounded in natural systems (Table S1), it was not possible to tease apart their influences on individual recycling rates. Shifting individual excretion rates from LP to HP levels increased the amount of N recycled by natural stream LP populations by *c.* 43%. This amount was comparable to the combined effects of phenotype and light on population N excretion observed in the analysis of experimental data (*c.* 53%). We caution that this analysis would be sensitive to the selection of model parameters and should therefore be sensitive to changes in model selection criteria.

Discussion

Our study provides novel insight into the role of life history evolution in nutrient recycling in stream ecosystems by: (i) scaling previous experimental findings to provide estimates of total population excretion, (ii) comparing these findings to guppy excretion rates measured in natural stream ecosystems and (iii) dissecting the relative influences of phenotypic, demographic and environmental variables on the contributions of fish populations to nutrient recycling. To the best of our knowledge, this is the first integrative analysis of how individual life history traits, demographics and environmental conditions influence the contribution of consumers to nutrient recycling in freshwater ecosystems, and the first attempt to scale the effects of life history evolution on ecosystem function from small-scale experiments to natural ecosystems.

Our study shows that the overall contribution of guppy populations to N recycling is influenced by the phenotypic and demographic responses to predator removal. Individual HP and LP guppies excrete N at different rates, with LP guppies recycling less nutrients per individual than HP guppies. However, as a population, LP guppy populations recycle more N than HP guppies because their populations contain more large individuals and because they have higher densities than HP guppy populations. Therefore, as guppy populations respond phenotypically and demographically to predator release, their overall contribution to nutrient recycling is likely to increase, even as individual-level excretion rates evolve to decrease. Our analysis indicates that demographic differences in density and body size distribution can affect population nutrient recycling more than the individual-level phenotypic effects, although the latter are relatively important (Table 2).

Previous studies have shown that body size is an important predictor of individual recycling rates in aquatic consumers (Sereda *et al.*, 2008) and that density affects the overall contribution of consumers to nutrient recycling (Benstead *et al.*, 2010). Given that the contribution of guppies to nutrient recycling is heavily influenced by body size and density, two variables whose importance is widely known to aquatic ecologists, does knowing the evolutionary history of the target species improve our understanding of nutrient recycling? Clearly, individual excretion rates are important for determining the total contribution of the population N recycling (Table 2). However, the answer to the question also depends on whether density and body size distribution differences

between HP and LP populations are simply a numerical (non-evolutionary) effect of predator removal, or whether they are also affected by the evolution of life history phenotypes.

Although research in this area is continuously growing, it is increasingly evident that guppy population density and body size distribution are not simply numeric consequences of predator removal, but are also driven by evolutionary changes in individual traits (Reznick *et al.*, 1996; Reznick *et al.*, 2012; Bassar *et al.*, 2013). Removing a population from predators is likely to increase its density, regardless of its life history phenotype. However, LP adapted life histories are less sensitive to the negative effects of density dependence than predator-adapted life history, which can influence equilibrium densities in LP and HP guppy populations (Bassar *et al.*, 2013). Studies also show that there are no differences in the size specificity of mortality between HP and LP guppy populations in nature, suggesting that the size distribution observed in these populations is the product of life history traits (HP guppies producing more numerous but smaller offspring more frequently than LP guppies), in addition to changes in predator-mediated mortality rates (Reznick *et al.*, 1996a,b, 2001). Clearly, a better understanding of the effects of life history evolution on population demographics and size structure is critical, but it is also clear that density and size structure are significantly influenced by the evolution of individual traits and that trait evolution is therefore an important driver of nutrient recycling.

It is also evident from our experiment that environmental variability is important for determining nutrient recycling rates (Tables 1 and 2). Increased variability in light levels can affect the contributions of LP populations to nutrient recycling (Table 2). Because light levels can potentially vary among and within LP sites (Grether *et al.*, 2001), and because light can also affect the nutrient uptake by stream primary producers (Moslemi *et al.*, 2012), it will likely also determine how important guppy-mediated nutrient recycling is in stream ecosystems. However, despite the effect of light, differences in light among HP and LP environments do not on their own explain differences in total population N recycling (Figs 2b and 2d). Using the general linear model (a) in Table 1, we would estimate that simply moving an HP population from a high light (HP-like environment) to a low light (LP-like environment) without changing phenotypic or demographic characteristics would actually decrease the overall N recycling of the population by *c.* 30%. Light therefore is important in concert with phenotypic and demographic characteristics of the population.

Experimental findings and natural stream observations generally produced comparable individual and population patterns in N excretion (Fig. 1), but not in P excretion (Fig. 2). El-Sabaawi *et al.* (2012) predicted that LP guppies would recycle less P per guppy because their diets are generally poor in P compared to HP guppies. High-predation guppies are thought to select for high quality diet items (e.g. insects), whereas LP guppies are generalist feeders with poor quality diets containing large amounts of algae and detritus (Zandona *et al.*, 2011). Although phenotypic differences in diet persist in experiments (Bassar *et al.*, 2010), they do not appear to produce large differences in P excretion among HP-LP individuals or populations (Fig. 2b). One potential explanation is that the quality (i.e. %N and %P) of algae and invertebrates is higher in mesocosms than in natural streams (R. El-Sabaawi, unpubl. data). Differences in food quality between mesocosms and natural streams might also explain why N and P excretion rates are generally higher per capita in the experiments than the natural stream survey.

Our study focuses on dissecting the ways in which life history evolution influence nutrient recycling of individuals and populations. Ultimately, for fish-mediated nutrient recycling to be important to the ecosystem, fish have to supply a significant proportion of nutrient demand by primary producers and microbes. Guppies reach high densities, especially in LP sites, and are likely to supply large concentrations of ammonium and SRP, both of which can limit primary production in Trinidadian streams (Moslemi *et al.*, 2012). Preliminary findings indicate that guppies in upstream locations can supply up to 30% of total ammonium demand within 1 year of their introduction into guppy-free locations (M.C. Marshall, unpubl. data). It is important to note that guppies can also influence ecosystem demand through their foraging activities and diet selectivity, which vary between life history phenotypes (Palkovacs *et al.*, 2009; Bassar *et al.*, 2010). The effects of guppies on nutrient recycling are likely to change in complex ways during the course of evolution as their traits, population characteristics and foraging activities change.

An interesting question is whether guppy elemental composition (organismal stoichiometry), which was not measured in this study, affects nutrient recycling patterns. Guppy organismal stoichiometry varies widely among rivers in Trinidad, but does not correlate strongly with predation phenotype (El-Sabaawi *et al.*, 2012). However, we currently do not know how organismal stoichiometry, diet elemental composition or metabolic demand shape guppy nutrient recycling, although studies about this topic are in progress. Finally, studies

of nutrient recycling rarely comment on the role of egestion, likely because egestion needs to be broken down to be immediately available to primary consumers. However, nutrient levels in egested waste might vary with guppy diet or life history phenotype, and egested waste might be an important source of dissolved nutrients in the long term (Halvorson *et al.*, 2015).

Freshwater ecosystems are currently suffering from a myriad of anthropogenic threats. Because of the capacity of aquatic consumers to evolve rapidly in response to these perturbations, it is important to consider evolutionary as well as ecological responses to anthropogenic impacts. The vast majority of studies that have assessed the ecological consequences of evolution and intraspecific diversity have focused on population dynamics and community dynamics (Bailey *et al.*, 2009; Travis, Leips & Rodd, 2013). Studies on ecosystem processes are less common (Post *et al.*, 2008; Harmon *et al.*, 2009; Palkovacs *et al.*, 2009; Bassar *et al.*, 2010). In addition, almost all eco-evolutionary studies involve small-scale experiments without no reference to natural ecosystems (but see Palkovacs & Post, 2009). Our study shows for the first time that the effects of fish life history evolution on nutrient recycling observed in experiments are also evident in natural ecosystems. These effects are influenced by differences in excretion rates between life history phenotypes, differences in demographics and differences in environmental condition. Our study therefore calls for a better integration between evolutionary biology and ecosystem science in freshwater ecosystems, and a more integrated understanding of interactions between ecological and evolutionary processes that spans from small-scale experiments to natural ecosystems.

Acknowledgments

References

Bailey J.K., Hendry A.P., Kinnison M.T., Post D.M., Palkovacs E.P., Pelletier F. *et al.* (2009) From genes to ecosystems: an emerging synthesis of eco-evolutionary dynamics. *New Phytologist*, **184**, 746–749.

Bartoń K. (2014). MuMIn: Multi-Model Inference. R package version 1.10.5. http://CRAN.R-project.org/package=MuMIn

Bassar R.D., Ferriere R., Lopez-Sepulcre A., Marshall M.C., Travis J., Pringle C.M. *et al.* (2012) Direct and indirect ecosystem effects of evolutionary adaptation in the Trinidadian guppy (*Poecilia reticulata*). *American Naturalist*, **180**, 167–185.

Bassar R.D., Lopez-Sepulcre A., Reznick D.N. & Travis J. (2013) Experimental evidence for density-dependent regulation and selection on Trinidadian guppy life histories. *American Naturalist*, **181**, 25–38.

Bassar R.D., Marshall M.C., Lopez-Sepulcre A., Zandona E., Auer S.K., Travis J. *et al.* (2010) Local adaptation in Trinidadian guppies alters ecosystem processes. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 3616–3621.

Bates D., Maechler M., Ben Bolke & Walker S. (2014) *lme4: Linear mixed-effects models using Eigen*. R package version 1.1-6. http://CRAN.R-project.org/package=lme4

Becks L., Ellner S.P., Jones L.E. & Hairston N.G. Jr (2010) Reduction of adaptive genetic diversity radically alters eco-evolutionary community dynamics. *Ecology Letters*, **13**, 989–997.

Benstead J.P., Cross W.F., March J.G., Mcdowell W.H., Ramirez A. & Covich A.P. (2010) Biotic and abiotic controls on the ecosystem significance of consumer excretion in two contrasting tropical streams. *Freshwater Biology*, **55**, 2047–2061.

Bolker B.M. (2008) *Ecological Models and Data in R*. Princeton University Press, Princeton New Jersey USA.

Burnham K.P. & Anderson D.R. (2002) *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. Springer, New York USA.

Burns J., Di Nardo P. & Rodd F. (2009) The role of predation in variation in body shape in guppies *Poecilia reticulata*: a comparison of field and common garden phenotypes. *Journal of Fish Biology*, **75**, 1144–1157.

Coulson T., Tuljapurkar S. & Childs D.Z. (2010) Using evolutionary demography to link life history theory, quantitative genetics and population ecology. *Journal of Animal Ecology*, **79**, 1226–1240.

El-Sabaawi R.W., Bassar R.D., Rakowski C., Marshall M.C., Bryan B.L., Thomas S.A. *et al.* (2015) Intraspecific phenotypic differences in fish affect ecosystem processes as much as bottom-up factors. *Oikos*, 10.1111/oik.01769.

El-Sabaawi R.W., Zandona A., Kohler T.J., Marshall M.C., Moslemi J.M., Travis J. *et al.* (2012) Widespread intraspecific organismal stoichiometry among populations of the Trinidadian guppy. *Functional Ecology*, **26**, 666–676.

Elser J.J., O'Brien W.J., Dobberfuhl D.R. & Dowling T.E. (2000) The evolution of ecosystem processes: growth rate and elemental stoichiometry of a key herbivore in temperate and arctic habitats. *Journal of Evolutionary Biology*, **13**, 845–853.

Elser J.J. & Urabe J. (1999) The stoichiometry of consumer-driven nutrient recycling: theory, observations, and consequences. *Ecology*, **80**, 735–751.

Estes J.A., Terborgh J., Brashares J.S., Power M.E., Berger J., Bond W.J. et al. (2011) Trophic downgrading of planet earth. *Science*, **333**, 301–306.

Fox J. & Weisberg S. (2011) *An {R} Companion to Applied Regression*. R Package, Thousand Oaks, CA.

Grether G.F., Millie D.F., Bryant M.J., Reznick D.N. & May-ea W. (2001) Rain forest canopy cover, resource availability, and life history evolution in guppies. *Ecology*, **82**, 1546–1559.

Hall R.O., Koch B.J., Marshall M.C., Taylor B.R. & 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.G. Hildrew, D.G. Raffaelli & R. Edmonds-Brown), pp. 286–305. Cambridge University Press, Cambridge UK.

Halvorson H.M., Fuller C., Entrekin S.A. & Evans-White M.A. (2015) Dietary influences on production, stoichiometry and decomposition of particulate wastes from shredders. *Freshwater Biology*, **60**, 466–478.

Harmon L.J., Matthews B., Des Roches S., Chase J.M., Shurin J.B. & Schluter D. (2009) Evolutionary diversification in stickleback affects ecosystem functioning. *Nature*, **458**, 1167–1170.

Hawlena D. & Schmitz O.J. (2010) Herbivore physiological response to predation risk and implications for ecosystem nutrient dynamics. *Proceedings of the National Academy of Sciences*, **107**, 15503–15507.

Hendry A.P. & Kinnison M.T. (1999) Perspective: the pace of modern life: measuring rates of contemporary microevolution. *Evolution*, **53**, 1637–1653.

Hendry A.P., Lohmann L.G., Conti E., Cracraft J., Crandall K.A., Faith D.P. et al. (2010) Evolutionary biology in biodiversity science, conservation, and policy: a call to action. *Evolution*, **64**, 1517–1528.

Huizinga M., Ghalambor C. & Reznick D. (2009) The genetic and environmental basis of adaptive differences in shoaling behaviour among populations of Trinidadian guppies, *Poecilia reticulata*. *Journal of Evolutionary Biology*, **22**, 1860–1866.

Johnson C.R., Luecke C., Whalen S.C. & Evans M.A. (2010) Direct and indirect effects of fish on pelagic nitrogen and phosphorus availability in oligotrophic Arctic Alaskan lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, **67**, 1635–1648.

Magurran A.E. (2005) *Evolutionary Ecology: The Trinidadian Guppy*. Oxford University Press, Oxford, New York.

Moslemi J.M., Snider S.B., Macneill K., Gilliam J.F. & Flecker A.S. (2012) Impacts of an invasive snail (*Tarebia granifera*) on nutrient cycling in tropical streams: the role of riparian deforestation in Trinidad, West Indies. *PLoS One*, **7**, e38806.

Pace M.L., Cole J.J., Carpenter S.R. & Kitchell J.F. (1999) Trophic cascades revealed in diverse ecosystems. *Trends in Ecology and Evolution*, **14**, 483–488.

Palkovacs E.P., Kinnison M.T., Correa C., Dalton C.M. & Hendry A.P. (2012) Fates beyond traits: ecological consequences of human-induced trait change. *Evolutionary Applications*, **5**, 183–191.

Palkovacs E.P., Marshall M.C., Lamphere B.A., Lynch B.R., Weese D.J., Fraser D.F. 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.

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

Palkovacs E.P., Wasserman B.A. & Kinnison M.T. (2011) Eco-evolutionary trophic dynamics: loss of top predators drives trophic evolution and ecology of prey. *PLoS One*, **6**, e18879.

Peckarsky B.L., Taylor B.W., Mcintosh A.R., Mcpeek M.A. & Lytle D.A. (2001) Variation in mayfly size at metamorphosis as a developmental response to risk of predation. *Ecology*, **82**, 740–757.

Pilati A. & Vanni M.J. (2007) Ontogeny, diet shifts, and nutrient stoichiometry in fish. *Oikos*, **116**, 1663–1674.

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

R Development Core Team (2012) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.

Reznick D. (1982) Genetic determination of offspring size in the guppy (*Poecilia reticulata*). *American Naturalist*, **120**, 181–188.

Reznick D., Butler M.J. & Rodd H. (2001) Life-history evolution in guppies. VII. The comparative ecology of high- and low-predation environments. *American Naturalist*, **157**, 126–140.

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

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

Reznick D.N. (1997) Life history evolution in guppies (*Poecila reticulata*): Guppies as a model for studying the evolutionary biology of aging. *Experimental Gerontology*, **32**, 245–258.

Reznick D.N., Bassar R.D., Travis J. & Rodd F.H. (2012) Life history evolution in guppies VIII: the demographics of density regulation in guppies (*Poecilia reticulata*). *Ecology*, **66**, 2903–2915.

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

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

Reznick D.N., Rodd F.H. & Cardenas M. (1996b) Life-history evolution in guppies (*Poecilia reticulata*: Poeciliidae). 4. Parallelism in life-history phenotypes. *American Naturalist*, **147**, 319–338.

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.

Rodd F.H. & Reznick D.N. (1997) Variation in the demography of guppy populations: The importance of predation and life histories. *Ecology*, **78**, 405–418.

Sereda J.M. & Hudson J.J. (2011) Empirical models for predicting the excretion of nutrients (N and P) by aquatic metazoans: taxonomic differences in rates and element ratios. *Freshwater Biology*, **56**, 250–263.

Sereda J.M., Hudson J.J. & Mcloughlin P.D. (2008) General empirical models for predicting the release of nutrients by fish, with a comparison between detritivores and non-detritivores. *Freshwater Biology*, **53**, 2133–2144.

Thompson J.N. (1998) Rapid evolution as an ecological process. *Trends in Ecology and Evolution*, **13**, 329–332.

Travis J., Leips J. & Rodd F.H. (2013) Evolution in population parameters: density-dependent selection or density-dependent fitness? *The American Naturalist*, **181**, S9–S20.

Vanni M.J. (2002) Nutrient cycling by animals in freshwater ecosystems. *Annual Review of Ecology and Systematics*, **33**, 341–370.

Yoshida T., Jones L.E., Ellner S.P., Fussmann G.F. & Hairston N.G. (2003) Rapid evolution drives ecological dynamics in a predator–prey system. *Nature*, **424**, 303–306.

Zandona E., Auer S.K., Kilham S.S., Howard J.L., Lopez-Sepulcre A., O'Connor M.P. *et al.* (2011) Diet quality and prey selectivity correlate with life histories and predation regime in Trinidadian guppies. *Functional Ecology*, **25**, 964–973.

Supporting Information

(Manuscript accepted 22 October 2014)