

Widespread intraspecific organismal stoichiometry among populations of the Trinidadian guppy

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Summary

1. Ecological stoichiometry expresses ecological interactions as the balance between multiple elements. It relates the ecological function of organisms to their elemental composition, or their organismal stoichiometry. Organismal stoichiometry is thought to reflect elemental investments in life history and morphology acting in concert with variability in abiotic or environmental conditions, but the relative contribution of these factors to natural variability in organismal stoichiometry is poorly understood.

2. We assessed the relative contribution of stream identity, predation, body size and sex to the organismal stoichiometry of guppies (*Poecilia reticulata*) in six streams in Trinidad. In this system, guppy life-history phenotype evolves in response to predation. Guppies adapted to high-predation (HP) pressure grow faster, mature earlier, produce fewer and smaller offspring and eat a higher-quality diet than guppies adapted to low-predation (LP) pressure. This pattern of life-history evolution is repeated in many rivers encompassing a wide range of abiotic conditions.

3. Organismal stoichiometry of guppies was widely variable, spanning up to ~70% of the range of variability reported across freshwater fish taxa. Streams from where guppies were sampled were the most important predictor of organismal stoichiometry. In many cases, guppy populations from sites within the same stream varied as much as from sites in different streams.

4. Surprisingly, predation regime was a minor predictor of % C, C : P and C : N in female guppies, despite its strong correlation with life-history phenotype and other organismal traits in this species. Body size and sex were not significant predictors of organismal stoichiometry.

5. Guppies from HP sites were more stoichiometrically balanced with their diets than guppies from LP sites. The latter appeared to be more vulnerable to phosphorus limitation than the former, suggesting that dietary specialization associated with guppy life-history phenotype may have stoichiometric consequences that can affect guppy physiology and nutrient recycling.

6. Our findings suggest that local environmental conditions are a stronger predictor of organismal stoichiometry than organismal traits. We recommend that future work should explicitly consider correlations between organismal traits and organismal stoichiometry in the context of environmental heterogeneity.

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Introduction

The balance between the nutritional demand of organisms and the supply of nutrients from their diets has significant consequences for ecological and physiological processes. Ecological stoichiometry provides a framework for studying the ecological and physiological consequences of mismatches between nutritional demand and nutritional supply (Sternler & Elser 2002). Stoichiometry expresses biological interactions in terms of the balance of energy or carbon (C) and essential elements such as nitrogen (N) and phosphorus (P). A central premise of ecological stoichiometry is that most animals maintain fixed ratios of these elements in their bodies, a process known as elemental homeostasis. Elements consumed in excess of the animal's requirement are excreted or respired, while those limiting in supply are retained (Sternler 1997). The elemental composition of animals, or their organismal stoichiometry, thus acts as a proxy for their nutritional demand and plays an important role in predicting the contribution of animals to ecosystem function [e.g. consumer-mediated nutrient recycling (Vanni *et al.* 2002)]. Organismal stoichiometry can also provide insight for understanding nutritional constraints on animal populations, community interactions and food web processes (Andersen, Elser & Hessen 2004; Moe *et al.* 2005; Hall 2009). Despite the proliferation of stoichiometric models in the literature, factors that contribute to variability in organismal stoichiometry in nature are poorly understood.

Studies of organismal stoichiometry have mostly focused on describing interspecific rather than intraspecific variability in elemental composition. Organismal stoichiometry correlates significantly with phylogeny in both insects and fish (Fagan *et al.* 2002; Hendrixson, Sternler & Kay 2007). Drivers of these patterns have not been explored in detail but are thought to relate to elemental investments in body tissues related to morphology, trophic position, body size or to large-scale changes in nutrient cycles that have coincided with significant episodes of animal evolution (Tanner, Brazner & Brady 2000; Fagan *et al.* 2002; Kay *et al.* 2005; Gonzalez *et al.* 2011).

In contrast, few studies have assessed the ranges and causes of intraspecific variability in organismal stoichiometry. Most stoichiometric ecological models assume that intraspecific variability in organismal stoichiometry is narrower than interspecific variability and average stoichiometric ratios across individuals from the same populations (Hall 2009). However, recent evidence from a variety of taxa shows that intraspecific variability in organismal stoichiometry is wider than was previously thought (Pilati & Vanni 2007; Bertram *et al.* 2008; Small & Pringle 2010), and that it can significantly affect the predictions of food web and population models

(Nakazawa 2011). It is therefore important to characterize the range of intraspecific variability in organismal stoichiometry and to understand factors that generate it.

The few existing studies on intraspecific variation in organismal stoichiometry suggest that it can be the product of variability in organismal traits such as life history or morphology (Sternler & Elser 2002). For example, growth rate correlates with % P in many species presumably because rapid growth rates require an investment in mRNA, a P-rich molecule (Vrede *et al.* 2004). Elemental composition is often correlated with body size; in some taxa, size correlates with P, while in others it correlates with N or C (Pilati & Vanni 2007; Bertram *et al.* 2008). Morphology can also drive differences in the allocation of %N and %P because it can drive trade-offs in the allocation to muscle (N-rich), bone (P-rich) or other tissues (Vrede *et al.* 2011). Moreover variability in lipid content (C-rich) can drive differences in %C between adult and juvenile stages (Pilati & Vanni 2007) or between males and females (Aksnes, Gjerde & Roald 1986). Recent evidence has also shown that variation in abiotic conditions (e.g. nutrient availability, algal productivity or light availability) significantly modulates elemental content in terrestrial insects (Schade *et al.* 2003), aquatic invertebrates (Small & Pringle 2010), and fish (Dickman *et al.* 2008). As such, intraspecific patterns in organismal stoichiometry are likely the products of life history and morphology interacting with environmental conditions, but the relative contributions of these factors to organismal stoichiometry in a single species are poorly understood.

Animals distributed along replicated environmental gradients have long been used to understand how organismal traits evolve in response to natural selection and provide a unique opportunity to characterize factors that influence intraspecific organismal stoichiometry. One of the most striking examples of these animals is the guppy, *Poecilia reticulata*. Guppies on the island of Trinidad are found in contrasting biotic environments: high-predation (HP) environments where guppies exist with a number of adept fish predators, and low-predation (LP) environments where they exist only with a single species of fish, *Rivulus hartii*, which competes with guppies but only rarely preys on them (Reznick, Rodd & Cardenas 1996). Guppy life-history phenotypes, which represent trade-offs between growth and reproduction, evolve in response to predation pressure (Reznick & Endler 1982). Guppies that have evolved in the presence of predators (HP phenotype) grow faster, reproduce earlier and reproduce smaller but more numerous individuals than guppies that have evolved in the absence of predators (LP guppies) (Reznick & Endler 1982). High-predation populations are thus skewed towards smaller individuals, while LP populations are skewed towards

larger individuals (Reznick, Butler & Rodd 2001). Life-history differences between phenotypes have been shown to be genetic and to evolve rapidly when guppies are transplanted from sites with HP pressure to sites with LP pressure in the same river (Reznick, Bryga & Endler 1990; Reznick *et al.* 1997). Recent studies suggest that guppies from LP and HP sites differ in their diets. Guppies from HP sites consume more invertebrates and are more selective in their choice of prey than guppies from LP sites, which tend to consume algae and detritus (Bassar *et al.* 2010; Zandonà *et al.* 2011). Other traits such as morphology, behaviour and male colouration also correlate with the presence or absence of predators, but their heritability in the context of guppy predation phenotype is still under study (Magurran 2005).

Many aspects of divergent life-history phenotype in guppies should be correlated with comparable divergences in organismal stoichiometry. For example, fast-growing fish have lower nitrogen to phosphorus (N:P) ratios than slow-growing fish, although drivers behind this pattern are not well understood (Tanner, Brazner & Brady 2000). Trade-offs between growth and reproduction potentially correlate with the ratio of carbon to nitrogen (C : N) because they modulate the allocation of lipids between somatic and reproductive tissues (Reznick 1983; Aksnes, Gjerde & Roald 1986; Sweeting, Polunin & Jennings 2006). Fish and other animals that specialize in feeding on low-quality diets such as detritus or algae typically have lower %P or %N content and higher %C content than carnivores that consume high-quality diets (Frost *et al.* 2006; McIntyre & Flecker 2010).

Based on these patterns, we would expect guppies from HP and LP sites to have distinct organismal stoichiometries that are driven by elemental investments in growth, life-history trade-offs or by differences in dietary quality between the two phenotypes. However, within this system, other factors can also influence stoichiometry. Predation pressure causes the evolution of life-history phenotype in rivers that differ widely in nutrient concentrations, light availability and algal productivity, which are all factors that can influence the stoichiometry of fish (Reznick & Bryga 1996; Reznick, Rodd & Cardenas 1996; Reznick, Butler & Rodd 2001; Dickman *et al.* 2008). As such, this system represents a unique opportunity to assess the relative influence of organismal traits, environmental conditions and their interactions on organismal stoichiometry.

Here, we characterize the organismal stoichiometry in guppies from HP and LP sites located in six different streams on the island of Trinidad. We examine organismal stoichiometry against the background of several factors including stream identity and predation regime (which selects for life-history phenotype), body size and sex. We predict that if life-history trade-offs drive organismal stoichiometry, predation regime will be the most important predictor of guppy elemental composition. If organismal stoichiometry varies primarily with other environmental factors such as nutrient availability, then stream identity will be the strongest predictor of guppy organismal stoichiometry. If other traits such as sex are more

important in driving organismal stoichiometry, then all populations will have similar elemental composition, regardless of predation pressure or the stream of origin. These factors may not be mutually exclusive influences and our approach allows us to gauge interactions among multiple factors. Finally, we assess whether dietary quality influences organismal stoichiometry in guppies by first assessing whether guppy stoichiometry is correlated with dietary stoichiometry and then by comparing the degree of elemental imbalance between guppies and their diets in different sites.

Materials and methods

SITE DESCRIPTION AND SAMPLE COLLECTION

Guppies were collected from six streams in the Northern Range Mountains on the island of Trinidad (Fig. 1). In each stream, HP and LP populations were separated by barrier waterfalls that restrict the distribution of predators from HP environments below the barrier to LP environment above the barrier (Magurran 2005). Predation regime of the population was confirmed by presence or absence of piscine predators such as the pike cichlid (*Crenicichla* sp) and the wolfish (*Hoplias malabaricus*). The populations sampled were also deliberately chosen because their populations had been sampled previously and because their life-history characteristics were well known (Reznick *et al.* 1996; Reznick, Rodd & Cardenas 1996; Grether *et al.* 2001; Reznick, Butler & Rodd 2001; Zandonà *et al.* 2011). Because these were field collections from wild populations, it was not possible to separate the environmental and genetic components of life-history phenotype. However, previous studies had repeatedly shown that a random sample of individuals from populations defined by the presence or absence of predators displayed consistent divergence of life-history phenotype (Reznick & Endler 1982; Reznick *et al.* 1996; Zandonà *et al.* 2011). In an analysis of all available guppy life-history data at the time, the presence or absence of predators predicted 70–80% of the variability in guppy life-history phenotype (Strauss 1990). As such, the influence of any environmental plasticity on distinguishing life-history phenotypes was likely much smaller than the known genetic influence, and predation regime was by

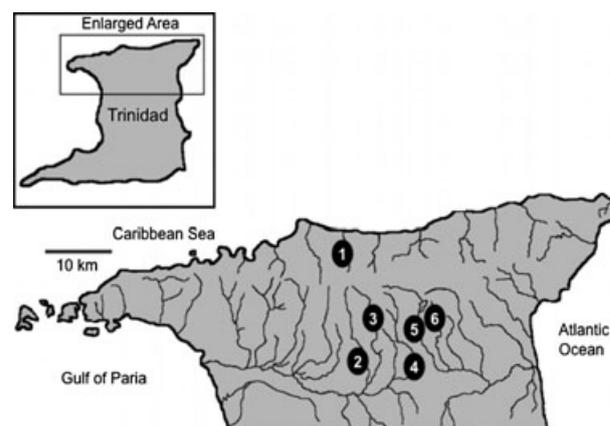


Fig. 1. Map showing location of sites from this study. Sampling took place in streams in the Northern Range Mountains of Trinidad (location shown in inset). 1 is the Marianne River, 2 Arima River, 3 Guanapo River, 4 Aripo River, 5 Quare River and 6 Turure River.

far the most important determinant of life-history phenotype in this species.

Streams were also chosen to capture a wide range of conditions across which distinctive guppy life histories are found. The Northern Range is divided into three slopes: East, South and North. East Slope rivers drain into the Oropouche River, which flows into the Atlantic Ocean, while South Slope rivers drain into the Caroni River, which flows into the Gulf of Paria. North Slope rivers comprise a series of independent drainages most of which flow into the Caribbean Sea (Magurran 2005). Rivers from the South and East Slopes contain predators derived from South American fauna, whereas North Slope fishes are derived from Antillean and Caribbean ancestors (Reznick, Rodd & Cardenas 1996). Populations of guppies from different Slopes are genetically distinct, although the exact boundaries of genetically distinct populations are debated (Magurran 2005; Suk & Neff 2009; Willing *et al.* 2010). In general guppies from each slope represent a different lineage, but guppies from the Turure River (East Slope) derive from the Caroni (South Slope) lineage (Suk & Neff 2009). A few rivers in Trinidad have limestone complexes (or 'tufta') that correlate with increased adsorption of P from the water column, potentially inducing P limitation in stream algae (Wetzel 2001; Day & Chenoweth 2004). In this study, guppy predation pairs were sampled from the Guanapo and Arima Rivers (Caroni lineage and drainage), the Aripo River (Caroni lineage and drainage, moderate limestone complexes), the Marianne River (North Slope lineage and drainage), the Quare River (Oropouche lineage and drainage, moderate limestone complexes) and the Turure River (Caroni lineage, Oropouche drainage, extensive limestone complexes in the LP site, but not in the HP site).

At each site basic environmental variables were collected from three representative pools. Environmental variables were quantified using standard methods (Appendix S1, Supporting information) and included dissolved nutrient concentrations (phosphorus, ammonium and total dissolved inorganic nitrogen), light availability (estimated as per cent open canopy) and algal standing stocks measured as chlorophyll-*a*. All site characteristics are summarized in Appendix S1 (Supporting information).

From each community, ~20 guppies were collected and analysed for stoichiometric composition (C, N and P) using standard methods (Appendix S1, Supporting information). To estimate quality of guppy diets, samples of epilithon (a rock-bound matrix of detritus and algae) and invertebrates, which together comprise the bulk of guppy diets (Zandonà *et al.* 2011), were collected from each site and analysed for elemental composition and stoichiometry using standard methods as described in Appendix S1 (Supporting information). All single-element composition variables were expressed as % of dry weights and all elemental ratios as molar ratios.

CALCULATION OF NUTRITIONAL IMBALANCES AND THRESHOLD ELEMENTAL RATIOS

To examine whether diet generated stoichiometric constraints on guppy populations, we first assessed whether intraspecific variability in guppy stoichiometry was correlated to variability in the stoichiometry of the diet. We then assessed whether guppies were imbalanced with their diets and whether the degree of imbalance differed systematically between LP and HP populations. We limited this analysis to C : P because P supply varied significantly between streams and because fish in general are thought to be more vulnerable to P limitation than N limitation (Boersma *et al.* 2008).

The stoichiometry of guppy diets was estimated based on: (i) per cent composition of guppy diets as reported in previous studies (Zandonà *et al.* 2011) and (ii) the elemental composition of epilithon and invertebrates sampled directly from each site (see Appendix S1 for details, Supporting information).

Correlations between guppy stoichiometry and dietary stoichiometry were assessed using linear regression. Such correlations are used to determine the degree of elemental homeostasis, which is the degree to which animals maintain their stoichiometry in the face of variable dietary stoichiometry. We computed the strength of elemental homeostasis from these regressions based on (Persson *et al.* 2010) (Appendix S1, Supporting information).

Imbalances between guppies and their diets were estimated in two ways: first, as the arithmetic difference between guppy C : P and diet C : P:

$$\text{Imbalance}_{\text{C:P}} = \text{Guppy}_{\text{C:P}} - \text{Diet}_{\text{C:P}} \quad \text{eqn 1}$$

While this provides a qualitative estimate of nutritional imbalance, it also overestimates imbalance because animals typically require a higher C : P in their diet relative to their body composition as they lose some C in respiration (Sterner & Elser 2002). Estimates of nutritional imbalance that incorporate animal physiology, such as Threshold Elemental Ratios (TER), provide more realistic estimates of nutritional thresholds. Threshold elemental ratios are thresholds where nutritional limitation shifts between two elements (Sterner & Elser 2002).

The TER model formulated by (Frost *et al.* 2006) was used in this study:

$$\text{TER}_{\text{C:P}} = (\text{AE}_\text{P} / \text{GGE}_\text{C}) * \text{Guppy}_{\text{C:P}} \quad \text{eqn 2}$$

Where $\text{TER}_{\text{C:P}}$ is the threshold elemental ratio, AE_P is the assimilation efficiency of phosphorus, and GGE_C is gross growth efficiency of carbon. Assimilation efficiency of P (AE_P) is assumed to be ~0.8 (Frost *et al.* 2006). Independent laboratory studies suggest that guppy GGE_C is ~15% (C. Jørgensen, unpublished data and CM Dalton, unpublished data). The imbalance between guppies and their diets is estimated by subtracting $\text{TER}_{\text{C:P}}$ from dietary C : P.

$$\text{Imbalance}_{\text{TER}} = \text{TER}_{\text{C:P}} - \text{Diet}_{\text{C:P}} \quad \text{eqn 3}$$

Positive imbalance values indicate increased potential for C limitation, whereas negative imbalances indicate increased potential for P limitation (see Appendix S1 for model details, Supporting information). Note that these estimates do not take into account differences in the metabolic availability of different C pools within the animal and assume that all body C is metabolically active (Raubenheimer & Simpson 2004).

STATISTICAL ANALYSES

The goal of this analysis was to assess the relative contributions of streams, predation regime (which selects for life-history phenotype), sex and size to the elemental composition of guppies. Factors contributing to variability in guppy elemental composition stoichiometry across sites were assessed using a general linear model (GLM). The interaction of stream \times predation indicated whether local site

conditions affected organismal stoichiometry more than the effects of stream identity or predation. Other interactions in the model (e.g. predation \times size and predation \times sex) were found to be insignificant in preliminary model runs and were removed to preserve degrees of freedom. Juveniles were removed from this analysis because we were unable to identify their sex and because they comprised a small proportion of the data (<8% of the sample size). The data were almost evenly split between LP and HP fish (52 and 48% of the samples, respectively) and were slightly biased towards females (55% of the sample) compared with males (45%). Females from our samples were on average slightly larger than males (20 mm compared with 17 mm, $F_{1,200} = 57.4$, $P < 0.001$), but there were no significant differences in the size distribution between guppies from HP and LP sites ($P = 0.98$).

Individual GLMs were constructed for %P, %C, %N as well as N : P, C : N and C : P. All variables were modelled as fixed effects, including stream, because stream was a fixed component of the analysis that was repeated across all variables tested (Sokal & Rohlf 1995). Data and residuals were tested for normality and heterogeneity of variance and data were transformed where appropriate. Tukey's *post hoc* HSD analysis ($\alpha = 0.05$) was applied to test the significance of least mean squares where appropriate. The contribution of each predictive variable to the overall explained variance was calculated as eta squared (η^2), defined as the sums of squares of the variable divided by the sums of squares of the overall model. Eta squared was preferred over partial η^2 (in which sum of squares of each variable is divided by the sum of squares of the error) because η^2 was easier to interpret, although results from both of these methods were similar.

The 'stream' variable is the combined effect of a variety of abiotic factors, including differences in algal standing stocks, light availability and nutrient concentrations (Appendix S1, Supporting information). We assessed whether guppy stoichiometry was associated with particular environmental variables by correlating guppy stoichiometry from each site to nutrient concentrations, light availability (estimated as % open canopy) or algal standing stocks (summarized in Appendix S1, Supporting information), using Spearman's rank correlation coefficient (ρ , at $\alpha = 0.05$). All statistical testing was performed using either the 'fit model' or the 'multivariate' platform in JMP (version 8 for Macintosh computers).

Results

GENERAL PATTERNS IN ELEMENTAL COMPOSITION

Guppy populations exhibited a wide range of elemental variability (Fig. 2a), with % P ranging from 2.05 to 5.4% (Fig. 2a), % N ranging from 6.8 to ~11.5% (Fig. 2b) and % C ranging from 33 to 48% (Fig. 2c). Per cent P was the most variable element in guppies (overall coefficient of variability (CV) = 19.7%), followed by % N (CV = 9.8%) and then % C (CV = 7.5%).

CORRELATES OF ORGANISMAL STOICHIOMETRY

The GLM analysis attributed variability in guppy elemental composition and stoichiometry to variety of factors including stream, predation regime, size and sex. In the overall model, stream and the interaction of stream \times predation regime contributed more to elemental composition and organismal stoichiometry than predation regime, size or sex (Table 1). Body size was a weak predictor of stoichiometry (Fig. 3, Table 1). The interaction between stream \times body size occurred because body size was significantly correlated with %P, N : P and C : P in 2 of the 12 sites (Arima HP ($r^2 = 0.2$, $F_{1,19} = 6.7$, $P < 0.02$) and Quare HP ($r^2 = 0.27$, $F_{1,15} = 9.8$, $P = 0.007$), but not in any of other sites (Fig. 3). The inclusion or removal of juveniles from these GLMs did not improve the predictive power of body size indicating that the statistical analysis was not biased by the removal of small juvenile guppies from the analysis (data not shown).

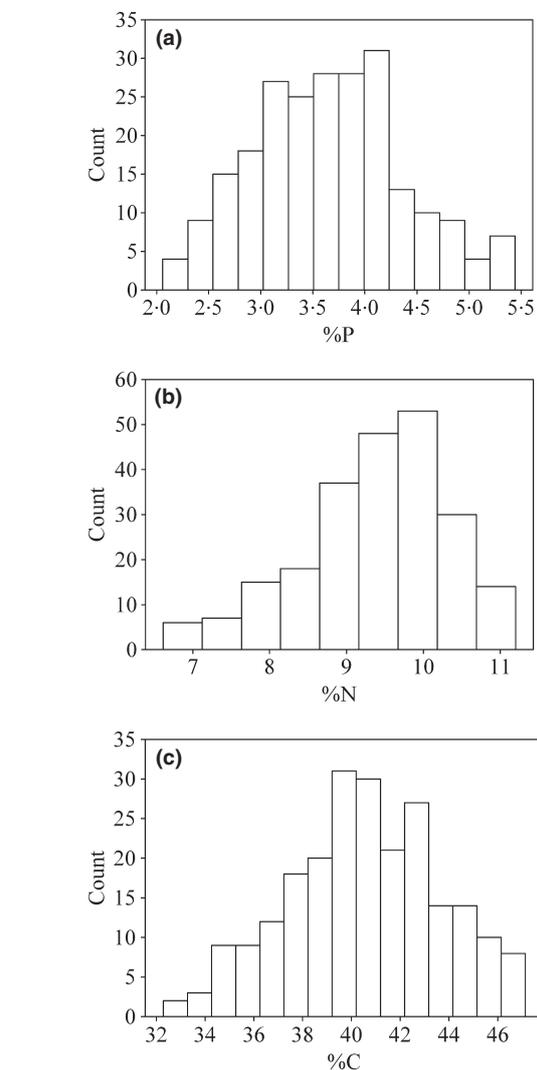


Fig. 2. The distribution of %P (a), %N (b) and %C (c) in all guppies sampled in this study. The x-axis indicates % range of each bin, while y-axis indicates numbers of samples in each bin.

Sex was also an insignificant predictor of organismal stoichiometry, but it significantly interacted with stream (Table 1). The significant interaction between sex \times stream was driven by sites such as the Guanapo LP where males had significantly different elemental composition than females (Appendix S2, Supporting information). These interactions between size \times stream as well as between sex \times stream made

Table 1. General linear model (GLM) analysis and variance decomposition for guppy elemental composition and C : N : P stoichiometry. The global model included size as covariate and sex as a main effect and was conducted on male and female guppies. Size was removed as a covariate in the sex-specific GLMs because it did not contribute significantly to the overall variance explained in the model. Variance decomposition (η^2) was estimated only for variables in the sex-specific model as described in the text

Data	Effect types	Model variables	DF	%P	%N	%C	N : P	C : N	C : P	
All data (GLM)	Main	Stream	5.00	5.96†	28.73†	4.72†	13.35†	43.69†	6.87†	
		Predation	1.00	0.05	0.38	1.83	0.07	7.33†	1.02	
		Sex	1.00	0.01	0.07	0.01	0.37	0.03	0.07	
	Covariate	Size	1.00	2.36#	0.66	0.05	2.44#	0.78	1.80#	
		Interactions	Stream × predation	5.00	9.64†	9.07†	4.38†	10.36†	20.95†	10.09†
	Stream × size		5.00	2.48*	1.76	1.33	3.02*	1.76	2.26*	
	Stream × sex		5.00	2.59*	2.26*	1.73#	3.23*	1.33	2.46*	
	Adjusted r^2				0.30	0.42	0.18	0.39	0.55	0.31
	Error MS			178	0.35	0.40	7.7	1.05	0.04	0.006
Females (GLM)	Main	Stream	5.00	3.86†	13.68†	3.38†	65.56†	37.96†	4.93†	
		Predation	1.00	1.67	0.15	3.84*	1.37	12.51*	4.17*	
	Interactions	Stream × predation	5.00	6.46†	2.79†	3.01*	51.51†	11.06†	8.39†	
	Adjusted r^2			0.32	0.35	0.20	0.42	0.62	0.37	
	Error MS			102.0	0.34	0.4	7.2	1.1	0.04	0.005
Males (GLM)	Main	Stream	5.00	1.78†	17.10†	1.20	5.27†	9.43†	1.78†	
		Predation	1.00	1.07	0.26	0.19	0.76	0.33	0.35	
	Interactions	Stream × predation	5.00	4.85†	6.99†	2.41*	4.88†	11.58†	5.45†	
	Adjusted r^2			0.23	0.48	0.09	0.31	0.46	0.23	
Error MS			94.00	0.34	0.41	8.6	1.2	0.05	0.006	
Females (η^2)	Main	Stream		0.100	0.342	0.105	0.207	0.556	0.120	
		Predation		0.009	0.001	0.024	0.004	0.037	0.020	
	Interactions	Stream × predation		0.167	0.070	0.096	0.163	0.162	0.204	
Males (η^2)	Main	Stream		0.064	0.413	0.052	0.173	0.240	0.064	
		Predation		0.008	0.001	0.002	0.005	0.002	0.003	
	Interactions	Stream × predation		0.177	0.169	0.104	0.160	0.294	0.201	

* $P < 0.05$; † $P < 0.001$; # P between 0.05 and 0.10.

the overall GLM difficult to interpret. Conducting sex-specific GLMs allowed us to scrutinize the interactions between stream × predation regime without the complicating effect of interactions between sex and stream and also removed any potential for the effects of body size and sex to be confounded with each other through the greater body size of females.

Stream and the interaction of stream × predation regime were stronger predictors of organismal stoichiometry than predation in almost all sex-specific models (Table 1, Table 2, Fig. 4). Body size and the interaction of body size × stream were not significant predictors of any of the elements or elemental ratios and were removed from the sex-specific models (P in all models > 0.4 , F ratio of size in all models < 1). The relative contribution of each predictive factor to the variance explained in the model depended on the element or elemental ratio. For example, the interaction between stream × predation regime drove most of the variance explained in %P in both males and females (Table 1, Fig. 4a,b). Stream explained most of the variance in %N in both males and females, although the interaction between stream × predation regime accounted for a substantial proportion of the variance explained by the model (Table 1, Table 2). Post hoc analysis on least square means of all elements and ratios is reported in Appendix S2 (Supporting information).

In females, stream, predation regime and the interaction of stream × predation regime were all significant predictors of % C, C : P and C : N, but the effects of predation regime were weaker than the effects of stream or the interaction of stream × predation (Table 1, Table 2, Fig. 4). The weakness of this effect was also highlighted by the fact that the interactions of predation × sex did not appear to be significant in early trials of the GLM model (see Material and methods). When averaged across different streams, females from LP sites had significantly higher % C (~41.8%) than females from HP sites (~40%) (Table 2, Appendix S2, Supporting information). Females from LP sites had higher C : P than females from HP sites (C : P ~31.5 and ~28.8, respectively, Appendix S2, Supporting information). Females from LP sites also had higher C : N ratios than females from HP sites (~5.3 compared with ~5, Appendix S2, Supporting information).

Correlates of guppy elemental composition and stoichiometry were further assessed by testing the strength of correlations between environmental predictors and guppy least square elemental composition and stoichiometry from each site. The only significant relationship was observed in females, where %N was negatively and significantly correlated with ammonium concentration ($\rho = -0.52$, $P < 0.001$, Appendix S3, Supporting information).

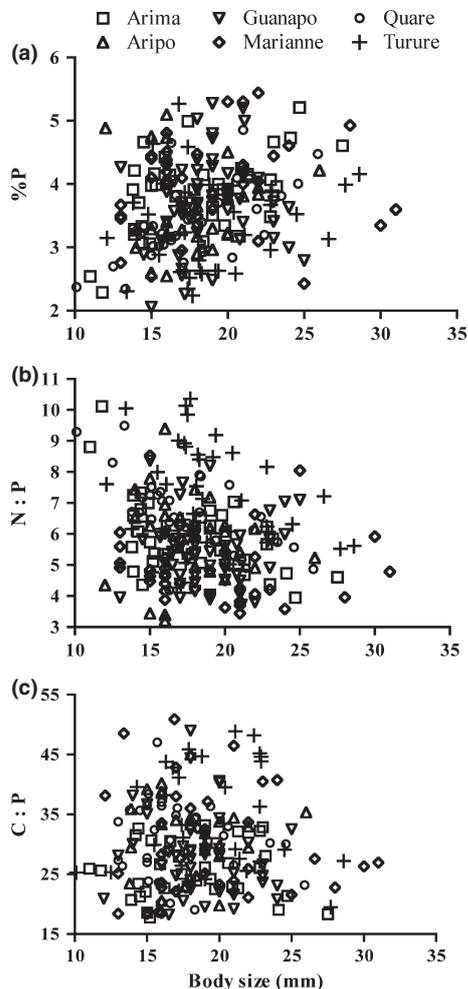


Fig. 3. The relationship between body size (mm) and %P (a), N : P (b) and C : P (c) in guppies.

DIETARY STOICHIOMETRY AND ELEMENTAL IMBALANCE

Invertebrate and epilithon quality (C : P ratio) varied widely between streams (Appendix S4, Supporting information). Constructed dietary C : P, calculated by applying known proportions of invertebrates and epilithon in guppy guts to their respective C : P ratios, varied widely between streams, but overall dietary quality was higher in HP (average C : P \sim 165) than LP sites (average C : P \sim 350) (Appendix S4, Supporting information). There was no significant correlation between the dietary C : P and body C : P in either LP or HP guppies, although the strength of the correlation between guppies and their diets was stronger in LP sites compared with HP sites ($r^2 = 0.14$ and $P = 0.44$ for LP, and $r^2 = 0.06$ and $P = 0.50$ for HP, Fig. 5a). In addition, no significant correlations were observed between guppy stoichiometry and the stoichiometry of invertebrates ($r^2 = 0.10$, $P = 0.54$ for LP, and $r^2 = 0.05$, $P = 0.70$ for HP, Fig. 5b) or epilithon ($r^2 = 0.2$, $P = 0.32$ for LP, and $r^2 = 0.05$, $P = 0.45$ for HP, Fig. 5c). Analysing guppies from LP and HP sites together did not improve the strength of these correlations or change the outcome of the statistical analysis.

Arithmetic imbalances between guppies and their diets calculated by subtracting dietary C : P from guppy C : P (Imbalance_{C : P}) revealed that guppies from LP sites were generally more imbalanced with their diets than guppies from HP sites in the same stream (Fig. 6a). Elemental imbalances, calculated by subtracting guppy TER_{C : P} from dietary C : P, were generally more negative in LP populations compared with HP populations (suggesting P limitation). Positive imbalances (suggesting C limitation) were observed in HP populations from the South Slope (Arima, Aripo and Guanapo Rivers) (Fig. 6b).

Discussion

GENERAL PATTERNS AND THE EFFECT OF LOCAL ENVIRONMENTAL CONDITIONS ON ORGANISMAL STOICHIOMETRY

Guppies display remarkably wide intraspecific variability in their elemental composition and stoichiometry (Fig. 2, Table 2). Intraspecific variability in fish composition is thought to be narrower than interspecific variability (Sterner & George 2000), but the range of elemental composition in guppies spans much of the range of elemental variability reported across freshwater fish taxa. The CV of %P in guppies, for example, is almost 70% of the CV of P observed across a 100 species of freshwater fish (19.7% compared with 29.8%) (McIntyre & Flecker 2010). Differences in %P between the 12 guppy populations studied here vary by 1.6%, a third of the range of %P known to occur in freshwater fish (between 1 and 6%, Fig. 4) (McIntyre & Flecker 2010). As such, intraspecific variability in elemental composition in guppies is neither narrow nor homogenous.

Ontogeny, body size or morphology typically explain a significant proportion of intraspecific organismal stoichiometry in fish (Pilati & Vanni 2007; Vrede *et al.* 2011). Our findings show that stream identity explains more of the variance in organismal stoichiometry than body size, sex or predation regime, which selects for life-history phenotype in this species. Furthermore, the significant interaction of predation regime \times stream in the GLM model indicates that local site conditions are more influential predictors of organismal stoichiometry than either streams or the suite of life-history traits associated with adaptation to predation (Table 1). In addition, guppies from streams where there are extensive limestone complexes, such as the Turure LP, have lower %P and higher N : P ratios than any of those from the Guanapo or Arima Rivers, even though guppies from these streams are all from the Caroni lineage (Fig. 4) (Suk & Neff 2009). This finding suggests that the overall biogeochemical setting generated by the effects of limestone, which influences P cycling and retention in aquatic ecosystems (Wetzel 2001), is more influential in shaping organismal stoichiometry than lineage.

We observe a significant negative correlation between female %N and dissolved ammonium concentrations, which suggests a link between guppy N content and

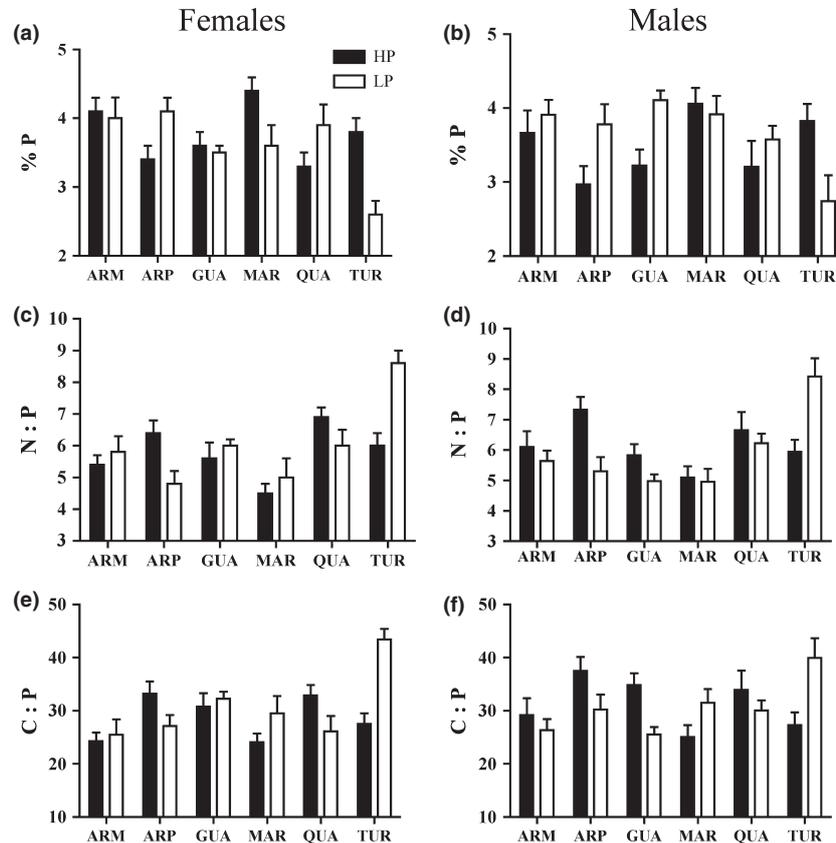


Fig. 4. Least square means (and standard errors) of % P, N : P and C : P in female guppies (panels a,c and e) and male guppies (panels b,d and f) from each site. High-predation localities are in black bars, and low-predation localities are in white bars. ARM is the Arima River, ARP is Aripo River, GUA is the Guanapo River, MAR is the Marianne River, QUA is the Quare River and TUR is the Turure River. Results from a *post hoc* Tukey's HSD test are reported in Appendix S2 (Supporting information).

nitrogen cycling (Appendix S3, Supporting information). Observations from mesocosm experiments suggest that guppies have a significant influence on N recycling in this system (Palkovacs *et al.* 2009; Bassar *et al.* 2010), and other studies have shown that N turnover is correlated with N content in a wide range of stream consumers, although these correlations do not include any fish taxa (Dodds *et al.* 2004). There are no other significant correlations between organismal stoichiometry and environmental variables, and the organismal stoichiometry of guppies is also not correlated with the stoichiometry of their diet (Fig. 5a). This observation agrees with other studies that have shown fish to be strictly homeostatic in their elemental composition, meaning that they regulate their elemental composition in face of variability in dietary stoichiometry (Sterner & George 2000). However, Persson *et al.* (2010) cautioned against concluding strict elemental homeostasis based on cases where there is an insignificant relationship between dietary and body stoichiometry because such correlations can be sensitive to noise in the data. The strength of elemental homeostasis as well as potential differences in homeostatic regulation between LP and HP locations need to be evaluated further with a large number of individuals from a variety of sites.

It is possible that organismal stoichiometry in guppies responds to other local site conditions that we did not consider here or that it represents a response to time-integrated local conditions while our measurements of environmental factors represent a snapshot in time. It is also possible that organismal stoichiometry is a response to the additive or interactive effects of several environmental factors. Regardless of the mechanism, our data suggest that wide intraspecific variability in guppy stoichiometry can occur on relatively small spatial scales. Clearly more work is needed to evaluate causes of intraspecific variability in organismal stoichiometry in guppies and other species, as well as assess the ecological consequences of this variability.

THE LINK BETWEEN LIFE-HISTORY PHENOTYPE AND ORGANISMAL STOICHIOMETRY IN FISH

Predation regime generates striking differences in guppy life-history traits such as growth and reproduction, and these differences are repeated across a wide range of environmental conditions (Reznick, Rodd & Cardenas 1996). Furthermore, adaptation to predation may generate differences in a suite of other organismal traits such as morphology and colour (Magurran 2005). Recent studies show that predation

Table 2. Least Square means (and standard error) of elemental composition and C : N : P stoichiometry from male and female guppies representing high- and low-predation regimes across study streams. Results from a post hoc Tukey's HSD test are reported in Appendix S2 (Supporting information)

Sex	Stream	Predation	%N	%C	C : N
Females	Arima	HP	9.7 (0.2)	37.6 (1.0)	4.5 (0.1)
		LP	10.4 (0.4)	38.8 (1.7)	4.4 (0.2)
	Aripo	HP	9.6 (0.3)	43.0 (1.4)	5.2 (0.1)
		LP	8.9 (0.3)	41.5 (1.2)	5.5 (0.1)
	Guanapo	HP	8.6 (0.3)	41.9 (1.4)	5.7 (0.2)
		LP	8.8 (0.2)	42.5 (0.8)	5.4 (0.1)
	Marianne	HP	8.6 (0.2)	38.7 (0.9)	5.3 (0.1)
		LP	7.8 (0.4)	45.4 (1.9)	6.2 (0.2)
	Quare	HP	10.0 (0.3)	40.5 (1.1)	4.7 (0.1)
		LP	10.2 (0.4)	38.2 (1.7)	4.4 (0.2)
	Turure	HP	10.2 (0.3)	39.9 (1.2)	4.6 (0.1)
		LP	10.2 (0.3)	44.2 (1.2)	5.1 (0.1)
Males	Arima	HP	10.1 (0.3)	41.2 (1.7)	4.8 (0.3)
		LP	9.8 (0.2)	39.1 (1.1)	4.7 (0.2)
	Aripo	HP	9.6 (0.2)	42.7 (1.1)	5.2 (0.2)
		LP	8.8 (0.3)	42.9 (1.4)	5.7 (0.2)
	Guanapo	HP	7.8 (0.2)	40.7 (1.1)	6.0 (0.2)
		LP	9.0 (0.1)	40.1 (0.7)	5.1 (0.1)
	Marianne	HP	9.0 (0.2)	38.0 (1.0)	4.9 (0.2)
		LP	8.2 (0.2)	42.8 (1.1)	5.8 (0.2)
	Quare	HP	9.6 (0.4)	42.0 (2.0)	5.1 (0.3)
		LP	9.7 (0.2)	40.0 (1.0)	4.8 (0.2)
	Turure	HP	10.2 (0.3)	40.0 (1.3)	4.7 (0.2)
		LP	10.3 (0.4)	41.8 (2.0)	4.7 (0.3)

HP, high-predation; LP, low-predation.

influences dietary specialization (Bassar *et al.* 2010; Zandonà *et al.* 2011). In contrast, predation regime predicts only a small component of the variability in female %C, C : N and C : P. An elevated C : N or C : P in females from LP sites implies that they store more lipids per unit protein or per unit bone than females from HP sites. This agrees with laboratory observations on this species (Reznick 1983; Sweeting, Polunin & Jennings 2006; Post *et al.* 2007). Differences in dietary quality between guppies from LP and HP guppies may also explain this pattern in organismal stoichiometry because some herbivorous organisms can increase lipid storage in response to excess carbon in their diet (Sterner & Hessen 1994). However, predation regime is a weak predictor of organismal stoichiometry, and its effect on stoichiometry is overwhelmed by the effect of local site conditions (Fig. 4).

Given the well-documented influence of predation on life-history and other organismal traits and the strong correlations between organismal traits and stoichiometry that are commonly observed in fish, it is surprising that predation does not generate strong patterns in guppy organismal stoichiometry. One possible explanation is that the pools of elements involved in life-history traits are too small to generate significant differences in organismal stoichiometry. For example, growth rate may generate significant differences in %P content because it modulates the concentrations of mRNA, which is P-rich (Vrede *et al.* 2004). In invertebrates, mRNA is a significant phosphorus pool in the body, but in

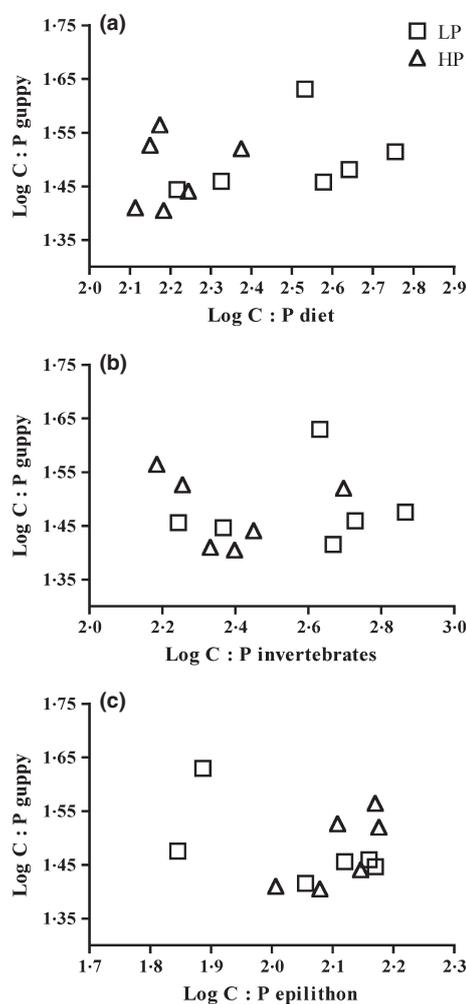


Fig. 5. Estimating the degree of homeostasis in guppy populations based on three scenarios: (a) a constructed diet based on weight-averaged gut contents, applied to the stoichiometry of invertebrates and epilithon collected from each site, (b) assuming that guppy diets were composed entirely of invertebrates and (c) assuming that guppy diets were composed entirely of epilithon. High-predation (HP) sites are in triangles, and low-predation (LP) sites are in squares.

fish, it is a much smaller pool than structural components such as bone and scales (Gillooly *et al.* 2005). As such, links between life-history traits such as growth may be stronger in invertebrates than they are in fish. Another possibility is that organismal stoichiometry is constrained by environmental heterogeneity that modulates elemental supply. Other studies have suggested that organismal stoichiometry of animals is more likely to reflect environmental heterogeneity in the availability of an element when animals are limited by the supply of that element (Dickman *et al.* 2008; Small & Pringle 2010). Our findings suggest that some guppy populations may be limited by the supply of elements, especially P (Fig. 6). Even though several studies have investigated correlations between organismal traits on body stoichiometry in fish (Pilati & Vanni 2007; Vrede *et al.* 2011), almost none have assessed how these correlations are influenced by heterogeneous environmental conditions or by variability in

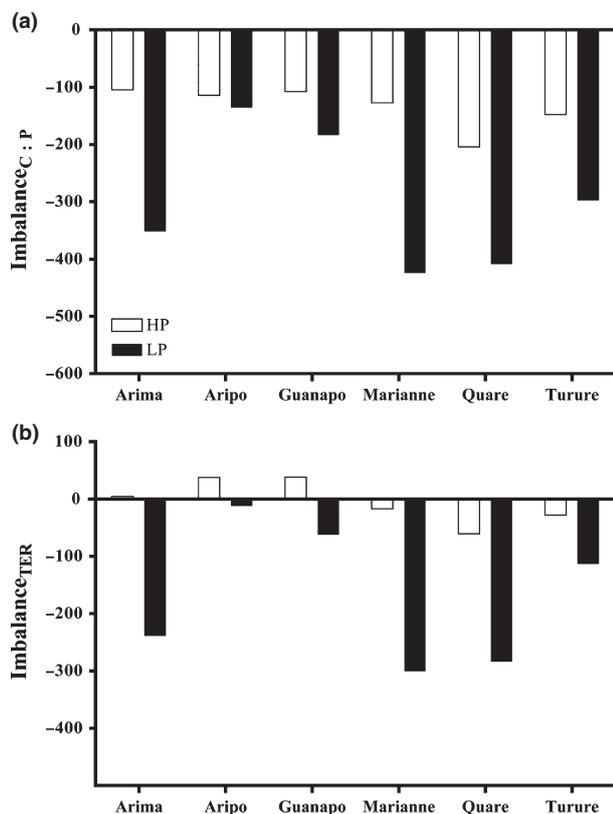


Fig. 6. Imbalances between guppy C : P and dietary C : P in high- and low-predation communities calculated using two methods: (a) $\text{Imbalance}_{C:P}$ refers to the arithmetic difference between the C : P of guppies and their diets and (b) $\text{Imbalance}_{\text{TER}}$ refers to the difference between the threshold elemental ratio ($\text{TER}_{C:P}$) of guppies from dietary C : P. Raw values of guppy C : P, $\text{TER}_{C:P}$ and dietary C : P are reported in Appendix S4 (Supporting information). High-predation (HP) sites are in white bars, and low-predation (LP) sites are in black bars.

elemental supply [but see (Hambäck *et al.* 2009)]. More studies need to examine the influence of organismal traits on organismal stoichiometry in the context of environmental heterogeneity and in a wide variety of taxa.

It is important to note that even though predation does not correlate strongly with organismal stoichiometry in guppies, it may generate intraspecific differences in elemental constraints on individuals because of differences in dietary quality between the two life-history phenotypes. Our analysis of elemental imbalance and TER suggests that because their herbivorous diets are generally poor in P content, guppies from LP sites are more likely to be limited by P than guppies from HP sites (Fig. 6). This difference in dietary quality and TER also suggests that guppies from LP sites are likely to recycle less P than guppies from HP sites. Elemental constraints have been shown to fundamentally change the outcome of population models in some species (Andersen, Elser & Hessen 2004) and to also influence their contribution to nutrient recycling (Sterner 1997). However, all models that address the interactions of predation-adapted life-history phenotypes and population dynamics in guppies are expressed only in

terms of energy (carbon or biomass). Expressing these models in terms of energy (C) and P simultaneously will likely further our understanding of how predation-adapted life-history phenotype and dietary elemental composition influence ecosystem function and population dynamics.

In conclusion, our study shows that the effects of body size, sex and life-history phenotype on organismal stoichiometry are generally weaker than the effects of local site conditions, which can generate striking spatial differences in intraspecific organismal stoichiometry. Clearly, future studies need to tease apart mechanisms underlying stoichiometric variation in the context of environmental heterogeneity. In addition, the ecological consequences of intraspecific variability in elemental composition are largely unknown, although few studies suggest that they can significantly influence demographic rates and population dynamics (Grover 2003; Nakazawa 2011). As such, a new challenge for ecological stoichiometry will be to understand the ecological and evolutionary consequences of intraspecific variability in organismal stoichiometry.

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Supporting Information

Additional supporting information can be found in the on line version of this article.

Appendix S1. Sampling of environmental variables, organismal stoichiometry and dietary stoichiometry.

Appendix S2. Least Square means of predation regime effect (a) and stream effect (b), and the results of post hoc Tukey's HSD analysis (c).

Appendix S3. Correlations between stoichiometry and environmental variables in females (a) and males (b).

Appendix S4. Average C : P ratios of guppies, guppy threshold elemental ratios of C : P (Guppy $TER_C : P$) and guppy diet C : P composition.

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