Freshwater Biology

Freshwater Biology (2015) **60,** 526–536 doi:10.1111/fwb.12481

Diet-switching by omnivorous freshwater shrimp diminishes differences in nutrient recycling rates and body stoichiometry across a food quality gradient

MARCIA N. SNYDER*, GASTON E. SMALL† AND CATHERINE M. PRINGLE*

*Odum School of Ecology, University of Georgia, Athens, GA, U.S.A.

†Department of Biology, University of St. Thomas, Minneapolis, MN, U.S.A.

SUMMARY

1. In tropical streams, omnivorous shrimp may be important nutrient recyclers, because they have a lower body P demand than fish. However, little is known about the controls on nutrient recycling by freshwater shrimp.

2. Across a series of lowland tropical streams that range in dissolved P, we describe: (i) shrimp body stoichiometry in relation to stream P level; (ii) rates of P excretion by shrimp; (iii) shrimp trophic level using natural stable isotope values ($\delta^{15}N$); and (iv) the importance of shrimp as nutrient recyclers.

3. Shrimp body elemental composition varied across the study streams, with higher shrimp %C and %N in low-P streams. P content of shrimp did not change despite large differences in P content of their likely food resources. Also, shrimp P recycling rates did not increase in high-P streams with P-enriched food resources.

4. Stable isotope results combined with change in body nutrient content (%N and %C) suggest that shrimp show different diet choices over the P gradient, feeding at a higher trophic level in low-P streams. This dietary shift may partially compensate for the lower P content in a given food resource in the low-P streams. However, P recycling rates were more variable than predicted based on diet choice and stream P level, suggesting that other physiological or behavioural mechanisms are involved.

5. In comparison to fish species in these same study sites, shrimp species recycle P at lower rates per unit body mass than the majority of fish species, despite their lower body P content. Diet switching may be an important strategy for omnivorous shrimp in correcting the stoichiometric imbalance between food resource and consumer biomass.

Keywords: Costa Rica, excretion, freshwater, *Macrobrachium*, stable isotope

Introduction

Consumers can play important roles in nutrient cycling in stream ecosystems through excretion of excess nutrients (Schindler & Eby, 1997; Elser & Urabe, 1999; McIntyre *et al*., 2007), and understanding controls on excretion rates is a central question in ecological stoichiometry (Sterner & Elser, 2002). Excretion rates depend on both dietary supply and physiological demand for metabolic maintenance and production of new biomass. For example, P (phosphorus) excretion rates tend to be high in fish with low body P content (Vanni *et al*., 2002), and especially for fish with low body P content that feed on high-P diets (e.g. insects or other fish; Small *et al*., 2011).

Consumers could respond to increased dietary availability of a limiting nutrient (typically P) by increased growth rates (Acharya *et al*., 2006; Shimizu & Urabe, 2008), decreased feeding rates (Frost *et al*., 2006), increased P storage (i.e. deviation from strict homoeostasis) (Small & Pringle, 2010; Tsoi, Hadwen & Fellows,

Correspondence: Marcia N. Snyder, Odum School of Ecology, University of Georgia, 140 E. Green Street, Athens, GA 30602-2202, U.S.A.
E-mail: snydermn@gmail.com

2011) or increased P excretion rates (James *et al.*, 2007; Small *et al.*, 2011). For omnivores, dietary shifts in response to nutrient content of food items are also possible. Omnivores may use diet switching to minimise the difference between consumer and prey elemental stoichiometry across heterogeneous environments (Denno & Fagan, 2003), therefore dampening any differences in P recycling. Omnivory is pervasive in many ecosystems (Thompson *et al.*, 2007) and is more common among fish macroconsumers in tropical than temperate freshwater ecosystems (González-Bergonzoni *et al.*, 2012). Omnivory can have complex effects on ecosystem structure and function (Pringle & Hamazaki, 1998; Usio & Townsend, 2002), yet it is often not accounted for in studies of consumer nutrient recycling.

Although many studies of consumer-driven nutrient recycling in streams have focused on fish (e.g. Vanni, 2002; Pilati & Vanni, 2007; McIntyre *et al.*, 2008), invertebrates may also play important roles in nutrient recycling (e.g. Christian, Crump & Berg, 2008; Atkinson *et al.*, 2010). Omnivorous shrimp are common macroconsumers in many tropical streams (De Grave, Cai & Anker, 2008) and can be a significant portion of the consumer biomass (Mantel & Dudgeon, 2004; Cross *et al.*, 2008). Due to their high densities, they are important contributors to nutrient recycling in streams in Puerto Rico (Benstead *et al.*, 2010). Even in streams where shrimp occur in lower densities, they may be disproportionately important nutrient recyclers because, as invertebrates, their body P content is lower than that of fish, suggesting that they require less P for growth and thus may be expected to recycle P at a higher rate.

This study builds upon previous work exploring the effects of stream P levels on consumer–resource stoichiometry and nutrient recycling, using a series of lowland tropical streams that naturally vary in P due to differential groundwater inputs. In these high-P streams, detritus, epilithon and aquatic insects are elevated in P content by twofold or more relative to low-P streams (Small & Pringle, 2010), and these differences drive P excretion rates for fish that consume these resources (Small *et al.*, 2011). Despite the differences in P content of potential food resources, shrimp growth rates do not vary across these streams (Snyder, 2012), suggesting that shrimp may be excreting excess dietary P rather than using it for growth.

In this study, we tested the hypothesis that dietary plasticity in shrimp is an important determinant in the relationship between food quality, body nutrient demand and excretion rates. Specifically, if omnivorous shrimp maintain the same proportion of food resources in their diets regardless of P content of individual food items, then shrimp P excretion rates should be elevated in streams with P-enriched food resources. However, if shrimps adjust their dietary selection based on P content of individual food resources (i.e. to maintain a constant overall dietary P content), then we would expect no differences in P recycling rates. To examine this hypothesis, we (i) compared body elemental composition of two shrimp species (*Macrobrachium carcinus* and *Macrobrachium olfersi*) across high- and low-P sites; (ii) compared rates of shrimp P excretion with previously published estimates of fish in these streams; and (iii) inferred trophic position of these shrimp based on natural abundance stable isotope values.

Methods

Study site

This study was conducted at La Selva Biological Station (LSBS) (10°26′N 84°01′W), a 1600 hectare forested reserve located on the Caribbean slope of Costa Rica at the intersection of the coastal plain with the Cordillera Central. The long-term annual precipitation mean is 3962 mm (Sanford *et al.*, 1994). The geomorphology of the region results in heterogeneous stream chemistry because regional solute-rich ground water surfaces where the foothills transition to the lowlands (*c.* 60 m elevation) (Pringle, 1991;). Regional ground water has high levels of solutes (P, Cl, Na, Mg, HCO_3, Ca) and is 13–29 times more concentrated than local surface water (Genereux & Pringle, 1997). We sampled shrimp excretion in three streams (Salto, Sabalo and Sura) ranging from 1st to 4th order and used published data from an additional three streams (Arboleda, Piper and Saltito) for resource P content (Table 1, Fig. 1). Stream temperature is relatively stable (24–26 °C) across all study streams. Stream sites used in this study are part of a long-term study of monthly physicochemical observations going back to 1997 (Pringle & Triska, 1991; Triska *et al.*, 2006). Some of the long-term study sites are located at different points along the same stream, and these sites are denoted by a number indicating the approximate elevation (in metres above sea level) at that site. Sites are categorised as high solute or low solute based on a soluble reactive P (SRP) threshold of 40 $\mu g\ L^{-1}$, reflecting the relative contribution of high-solute regional groundwater inputs (Table 1, Fig. 1).

Fifteen species of amphidromous shrimp have been identified in Costa Rica, with six species occurring at

Table 1 Physical and chemical properties of the stream reaches used in this study to examine body stoichiometry, $\delta^{15}N$ stable isotope and P excretion rates by shrimp at La Selva Biological Station, Costa Rica

	Stream order	SRP ($\mu g\ L^{-1}$)	NO_3-N ($\mu g\ L^{-1}$)	NH_4-N ($\mu g\ L^{-1}$)	Temperature (C)	Discharge ($m^3\ s^{-1}$)	Conductance ($\mu S\ cm^{-1}$)	pH	Shrimp excretion	Shrimp $\delta^{15}N$	Food resource $\delta^{15}N$	Food resource P	Fish excretion
Piper	1	2.3	188	40	25	0.03	22	5.5				x	
Sura-60	3	3	199	21	24.8	0.19	20	5.6	x	x	x	x	x
Saltito-100	1	3.1	163	37	24.3	0.03	19	5.7				x	
Sabalo	4	8	137	29	25.5	0.28	47	6	x				
Salto-60	3	10	180	20	24.7	0.45	32	5.9		x			
Saltito-60	2	33	98	19	25	0.11	110	6.1				x	
Sura-30	3	83	163	18	25.4	0.61	157	6.1	x	x	x	x	
Salto-30	3	92.2	227	19	25.1	0.17	98	6	x	x	x	x	
Arboleda	2	135	126	20	25.6	0.17	257	6.2				x	

Values are means of monthly samples collected from January–April 2009. SRP is soluble reactive P. Also included are the sites where Small & Pringle (2010) measured basal food resource and aquatic insect stoichiometry and $\delta^{15}N$ stable isotopes (part of the long-term monitoring biochemistry sites) and the focal site used to compare fish (from Small *et al.*, 2011) and shrimp excretion values.

LSBS (Obregon, 1986). Our study focuses on *Macrobrachium olfersi* and *Macrobrachium carcinus* because they are the most common shrimp in streams at LSBS (Snyder, 2012). *Macrobrachium* spp. are omnivorous and consume algae, detritus, insects, molluscs, fish and other shrimps (Pringle & Hamazaki, 1998; Kilham & Pringle, 2000; March & Pringle, 2003; García-Guerrero *et al.*, 2013).

Body nutrient and stable isotope analysis

We analysed shrimp elemental composition (C, N, and P) and natural stable isotope values of N ($\delta^{15}N$) in shrimps collected in the Salto and Sura streams (Fig. 1). Elemental ratios (C:N, C:P and N:P) were measured in moles. As part of a long-term stream monitoring project, the upper reaches of both of these streams are well characterised by low-solute levels and low concentrations of soluble reactive P (SRP *c.* 2 $\mu g\ L^{-1}$), whereas the lower reaches receive inputs of high-solute ground water and have SRP concentrations > 80 $\mu g\ L^{-1}$. For body nutrient composition and stable isotope analysis, shrimp (*M. olfersi* and *M. carcinus*) were sampled from ten reaches along the Salto and Sura, during June–August 2008. We used a stratified random design, selecting stream reaches that were either high or low P and near or far from the mainstem river. Stream physiochemistry is measured at three sites in each stream; however, the shrimp sampling was more extensive at five sites per stream. Shrimp were collected using modified minnow traps baited with cat food.

Samples were kept frozen until they could be freeze-dried or oven-dried at 50 °C for 48 h and then homogenised. The digestive tract was extracted and excluded from nutrient and stable isotope analysis. Shrimp C and N samples were analysed on a Carlo Erba NA 1500 CHN analyzer (Carlo Erba, Milan). For P analysis, samples were acid-digested (Aqua Regia double acid; Jones, Wolf & Mills, 1991) and then analysed spectrophotometrically using the ascorbic acid method. Ground pine needles (US National Institute of Standards and Technology, 1575a) and poplar leaves (Analytical Chemistry Laboratory, University of Georgia) were used as external standards for P and N analyses.

Samples for stable isotope analysis were oven-dried for 24 h at 80 °C or freeze-dried for 48 h before being ground and weighed into tin combustion capsules. For natural abundance of $\delta^{15}N$, stable isotope samples were analysed with a mass spectrometer, and isotopic composition was quantified relative to standard reference materials and reported as parts per thousand difference from the standard (precision ± 0.15%). All samples were

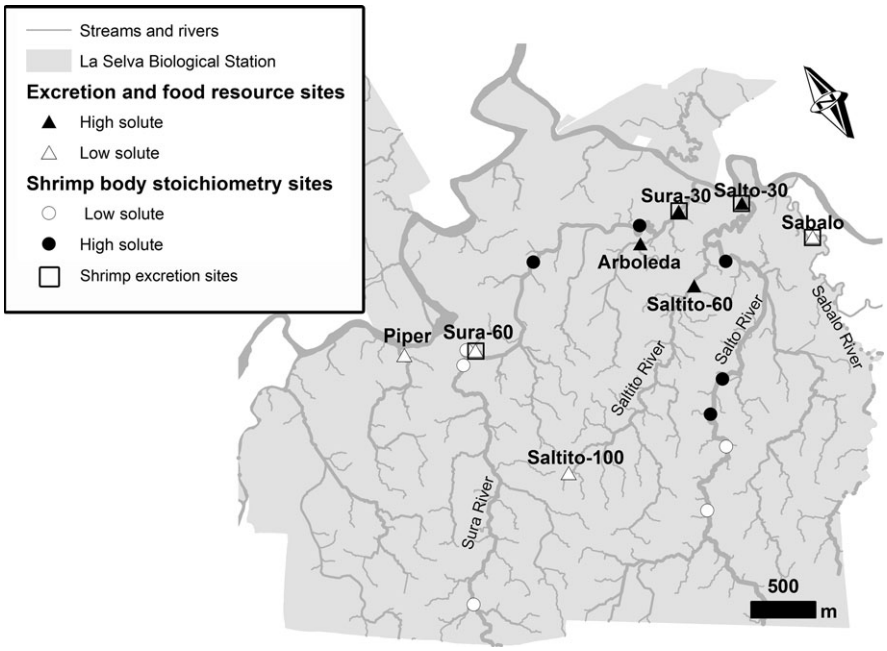

Fig. 1 Study sites at La Selva Biological Station. Long-term monitoring stream biochemistry sites where food resource P content was measured by Small & Pringle (2010) (n = 7) are represented by triangles. Sites where shrimp body nutrient content was measured are represented by circles (n = 10). The subset of sites where shrimp excretion was measured (n = 4) are indicated with squares. Closed and open shapes indicate high- and low-P sites, respectively.

analysed at the University of Georgia's Odum School of Ecology Analytical Chemistry Laboratory.

Elemental composition of potential food resources (leaf litter, periphyton and aquatic insects) was measured in 2006 for a subset of these study sites (Sura-60, Sura-30, and Salto-30), reported in Small & Pringle (2010). Here, we use these data to estimate food resource stoichiometry, and we also use additional unpublished data on $\delta^{15}N$ values for samples.

Excretion

We quantified P excretion rates for *M. olfersi* and *M. carcinus* across four study sites (Sura-30, Sura-60, Salto-30 and Sabalo) during April and May of 2010 (Table 1, Fig. 1). The study sites overlap with the sites from Small & Pringle (2010) and Small *et al.* (2011). Shrimp were collected using modified minnow traps that were placed in the afternoon and collected the next morning because both species are nocturnally active. Shrimp collection methods could have influenced excretion rates because time to gut clearance in *Macrobrachium* spp. occurs from eight to 24 h post-feeding (Carvalho, Collins & De Bonis, 2011). Traps were baited, but shrimp were prevented from consuming the bait by a physical barrier. Shrimp were immediately placed into individual plastic bags (1 individual per bag) containing 180–250 mL of water for small individuals (< 18 mm CL) and 500–1000 mL of water for larger individuals (>18 mm CL). Water for all excretion trials was collected from a low-P site (Sura-60) and filtered to remove larger particles using Whatman grade number 1 filter paper (11 μm pore size). Water samples were collected from each incubation chamber immediately after the shrimp had been placed and again after a one-hour incubation, and then filtered through a 0.45-μm Millipore filter (Millipore, Billerica, MA, U.S.A.) to remove faeces and other particulate matter. Shrimp showing signs of stress were not included in the analysis since stress can impact excretion rates in short-term incubations (Whiles *et al.*, 2009). Following previous studies of shrimp excretion (Benstead *et al.*, 2010), we used a one-hour incubation period to minimise handling stress and avoid starvation effects. Water samples were immediately frozen and transported on ice to the University of Georgia's Analytical Chemistry Laboratory for analysis. Water samples were analysed for total dissolved P (TDP) using the molybdate blue–ascorbic acid reaction after digestion by acid-persulfate oxidation (APHA, 1998). We measured excretion rates for 116 individual shrimp. Shrimp carapace lengths were measured in the field, and we identified the sex of each individual. Not all species were present in all four streams. Per capita P excretion rates were calculated as the increase in TDP compared to the sample taken at the beginning of the 1-hour incubation. For each stream reach for which we measured shrimp excretion rates, background nutrient concentrations were measured from filtered water samples (0.45-μm Millipore filters) for soluble reactive P (ascorbic acid method), NO_3–N (cadmium reduction method) and NH_4–N (phenate method; APHA, 1998).

Statistical analysis

All statistical analyses were performed in R (R Development Core Team, 2011). To test whether shrimp body element content and ratios change relative to site P level, we used individual linear mixed models for each response variable (%P, %N, %C, C:N, C:P and N:P) with stream P level and body size as the predictor variables. We used stream P level (high or low) as a surrogate for food resource P content because previous studies (Small & Pringle, 2010) found that as stream P levels increased stream basal resources and aquatic insect P levels increased. Stream reach P level was considered as a factor in the models, either high (>40 μg L^{-1}) or low (<40 μg L^{-1}). Shrimp body size was included as a continuous factor in the mixed model to account for ontogenetic effects. Body size (carapace length) and species identity were highly correlated (> 75%), and therefore, species identity was omitted from the mixed models and *M. olfersi* and *M. carcinus* were tested jointly. Percentage values were scaled by dividing by 100 and arcsine-transformed. Site was included as a random effect in the mixed models to account for variance in the residuals among sites. Ratios were log-transformed to meet assumptions of normality and equal variance. Pseudo R^2 values were calculated based on Wald's statistic (Vonesh, Chinchili & Pu, 1996).

We used $\delta^{15}N$ stable isotope data to inform our models about relative trophic level. To test for changes in shrimp diet (relative consumption rates of higher trophic levels), we examined the effect of stream SRP on shrimp $\delta^{15}N$ level with a one-way ANOVA jointly for *M. olfersi* and *M. carcinus*. To test for changes in $\delta^{15}N$ level of shrimp food resources, we used one-way ANOVAs to examine effect of stream SRP on CPOM, periphyton and insects. Stream SRP was considered as a factor (high or low) in the ANOVAs.

We used linear regression models to rank the relative support for different hypotheses examining what factors control shrimp total P excretion. Models were ranked with Akaike's information criterion corrected for small sample size (AIC_c); additionally, a psuedo R^2 value was calculated to assess model fit. Models were built to test whether body size (dry mass), SRP level (high or low), stream site and sex were important predictors of the log of P excretion rate. Stream SRP level was used as a proxy for food resource P level.

Twenty-eight of the 104 individual shrimp (12 were excluded because they showed signs of stress) included in the analysis for total P excretion had final values of total P equal to or less then the quantity of total P in the chamber at the start time (i.e. P excretion was not detected). Statistical models included a minimum detection value of 0.1 μg L^{-1} for those samples with rates < 0 before log transformation to ensure a positive excretion rate. We used 0.1 μg L^{-1} as the minimum detection value because it was close to the detection limit of a sample.

Results

Shrimp body content stoichiometry and natural stable isotope values

Macrobrachium olfersi and *M. carcinus* had similar body nutrient content (Table 2). Significant relationships were found between stream SRP level and shrimp body nutrient composition (Fig. 2). Both shrimp %N ($F_{1,44} = 2.60$, $P = 0.03$) and %C ($F_{1,44} = -3.80$, $P = 0.0004$) were higher in low-P streams, but %P was not significantly different. The mean %N for shrimp in high-P stream reaches was 9.5 (± 0.139 SE), compared to 10.0 (± 0.224 SE) in low-P stream reaches. The mean %C in high- and low-P stream reaches was 36.7 ± 0.402 and 38.4 ± 0.661, respectively. Three measures of body elemental content changed ontogenetically; as shrimp body size increased, body nutrient content decreased significantly in %C ($F_{1,44} = -3.80$, $P = 0.0004$), %N ($F_{1,44} = -3.30$, $P = 0.002$) and %P ($F_{1,44} = -3.15$, $P = 0.003$).

Table 2 Shrimp body nutrient content (percentage of dry mass), molar ratios and $\delta^{15}N$ stable isotope values for the two shrimp species examined in this study

Species	%C	%N	%P	C:N	C:P	N:P	$\delta^{15}N$
High-P sites							
Macrobrachium carcinus	35.8 (29.7–39.0)	9.2 (6.91–10.6)	1.04 (0.79–1.3)	4.55 (4.3–5.0)	89.9 (57.7–122.3)	19.9 (11.5–28.3)	7.6 (6.3–9.3)
Macrobrachium olfersi	37.6 (31.8–40.3)	9.8 (8.5–10.8)	1.2 (0.70–2.0)	4.5 (4.1–5.0)	86.1 (52.5–137.7)	19.2 (10.9–31.1)	7.6 (7.0–8.3)
Low-P sites							
M. carcinus	36.4 (32.2–39.6)	9.4 (7.7–10.8)	1.1 (0.79–1.8)	4.5 (4.3–4.9)	86.9 (49.1–127.0)	19.4 (10.3–29.0)	8.1 (7.3–9.4)
M. olfersi	40.5 (38.5–44.9)	10.5 (9.7–12.7)	1.3 (1.1–1.4)	4.5 (4.1–4.8)	81.4 (73.1–93.7)	18.2 (15.8–21.5)	8.3 (7.5–8.9)

The shrimp were sampled in five low-phosphorus and five high-phosphorus stream sites from two rivers, the Sura and Salto Rivers at La Selva Biological Station. Mean values are reported with the range in parentheses.

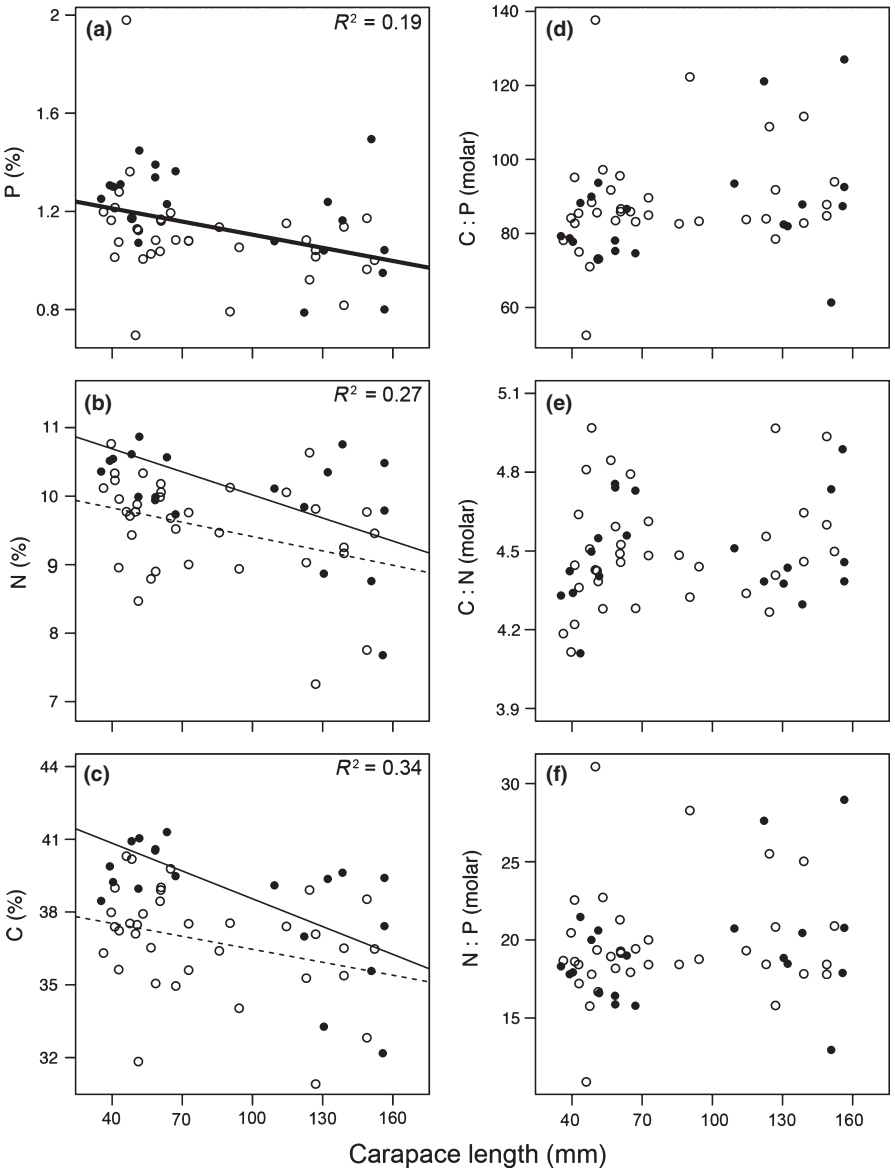

Fig. 2 Relationships between body size (carapace length) and body stoichiometry (%C, %N and %P, and C:N, C:P and N:P molar ratios) for two shrimp species (*Macrobrachium olfersi* and *Macrobrachium carcinus*) from ten stream sites in the Salto and Sura Rivers at La Selva Biological Station. Closed circles represent low-P reaches, and open circles represent high-P reaches. Only significant ($P < 0.05$) stream P level regressions are shown, with dotted lines for high-P reaches and solid lines for low-P reaches; where the mixed model did not show stream P level to be significant, but body size was significant, only a single solid line is shown. Data from the two shrimp species were combined.

The $\delta^{15}N$ values for *M. carcinus* and *M. olfersi*, pooled across all sites (Sura and Salto Rivers), were 7.83 (± 0.14 SE) and 7.84 (± 0.10 SE), respectively. Shrimp $\delta^{15}N$ level was significantly lower (-0.61) in high-P stream reaches ($F_{1,57} = 15.62$, $P = 0.0002$). The $\delta^{15}N$ values for CPOM, periphyton and aquatic insects were 3.13 (± 0.13), 5.42 (± 0.28) and 4.37 (± 0.22 SE), respectively. No differences in $\delta^{15}N$ of CPOM and insects were found across study sites ranging in SRP (Sura-30, Sura-60 and Salto-30). Periphyton $\delta^{15}N$ was higher in high-P stream reaches ($F_{1,22} = 10.88$, $P = 0.0032$). Assuming that isotopic fractionation is similar for shrimp among streams, the observation that baseline food resources for shrimp are similar or lower in low-P streams suggests that the higher $\delta^{15}N$ values measured in shrimp in these streams is probably due to feeding on a different combination of foods.

Effects of stream SRP on P excretion rate

P excretion rates across all sites ranged from 0.42 to 1.18 and 0.09 and 1.92 μg TDP g^{-1} DM^{-1} h^{-1} μg for *M. olfersi and M. carcinus, respectively* (Table 3). We tested eight different linear regression models to assess support for hypotheses about which factors would best predict shrimp total P excretion. Two of the explanatory factors, species identity and body mass, were highly correlated ($r^2 = 0.76$, $P < 0.00005$), so we included only body mass in our models. The best-supported model (pseudo $R^2 = 0.17$, $w_i = 0.96$) includes both body mass and site as

Table 3 Mass-specific P excretion rates (mean with SE in parenthesis) for three shrimp species in four streams ranging in dissolved P levels from 3 to 92 μg SRP L^{-1}

	Salto (92 μg L^{-1} SRP)	Sura-30 (83 μg L^{-1} SRP)	Sabalo (8 μg L^{-1} SRP)	Sura-60 (3 μg L^{-1} SRP)
Species				
M. carcinus	0.09 (0.03)	1.92 (0.86)	–	0.13 (0.04)
M. olfersi	0.42 (0.21)	1.18 (0.20)	1.06 (0.22)	0.44 (0.09)

P excretion rates are in μg TDP g^{-1} DM^{-1} h^{-1}. Minimum detection limit values are included in these means.

important predictor factors of P excretion rate and is 96 times more likely than the next best-supported model (Table 4). According to the best-supported model, body size is positively correlated with P excretion rate (Fig. 3). Stream SRP level was not included in the best-supported models, which indicates little evidence that stream SRP influences P excretion rates.

Estimation of contribution of shrimp to reach-scale P recycling

To test stoichiometric predictions of how body P influences P recycling rates among stream macroconsumers, we compared shrimp rates from this study with fish excretion rates from previous studies (Small *et al.*, 2011) at the same low-P site (Sura-60). In this reach, shrimp excreted P at a similar or lower rate per unit biomass (μg TDP g WM^{-1} h^{-1}) than the majority of the fish species (Table 5). The exception is the two species, *Astatheros alfari* and *Archocentrus septemfasciatus*, in the Cichlidae family, which had the highest body %P (4.3–4.7) of the fish measured and the lowest mass-specific P excretion rates (0.01 and 0.03 μg TDP g WM^{-1} h^{-1}. Overall, shrimp are excreting at rates (0.11–0.32 μg TDP g WM^{-1} h^{-1}) lower than would be predicted solely by their body %P because fish body %P ranged from 3.6 to 4.7%P (Small *et al.*, 2011), while shrimp body %P ranged from 1.02 to 1.04% P. Comparing P excretion rate per individual, excretion rates for *M. olfersi* (0.71 μg TDP $shrimp^{-1}$ h^{-1}) and *M. carcinus* (1.5 μg TDP $shrimp^{-1}$ h^{-1}) were similar to two fish, *Archocentrus septemfasciatus* and *Astatheros alfari*, with the lowest excretion rates 0.3 and 0.2 μg TDP $fish^{-1}$ h^{-1}. In Sura-60, we estimated overall shrimp biomass to be 4.38 g WM m^{-2}, which constitutes 12% of combined fish and shrimp biomass. We estimated total shrimp excretion in this reach to be 0.36 μg P m^{-2} h^{-1}, <1% of the

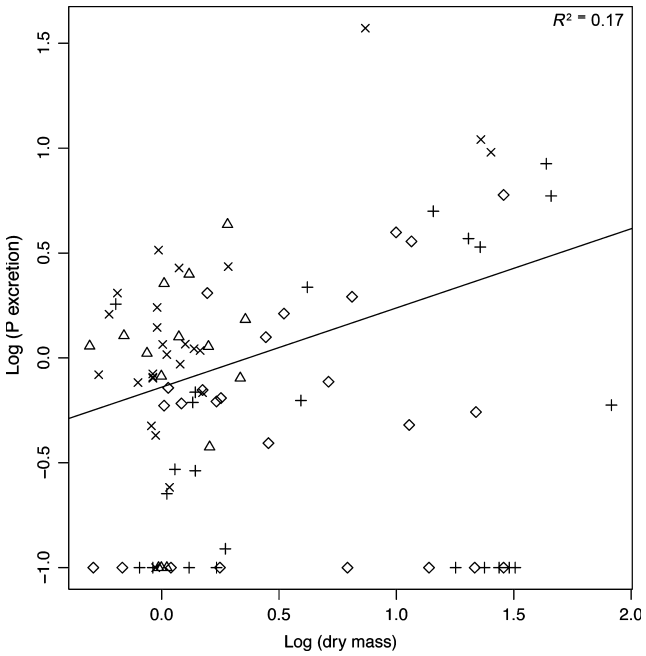

Fig. 3 Log–log relationship between P excretion and dry mass of individual shrimp of two species (*Macrobrachium carcinus* and *Macrobrachium olfersi*) in four study streams with different levels of soluble reactive P (SRP) at La Selva Biological Station. Excretion rate was measured as μg TDP $shrimp^{-1}$ h^{-1}, and biomass was measured in grams. The symbols refer to sites: triangles = Sabalo, plusses = Salto-5, crosses = Sura-30, diamonds = Sura-60.

Table 4 Linear regression models used to predict phosphorus excretion rates (μg TDP g DM^{-1} h^{-1}), showing values for number of parameters (k), Akaike's information criterion with the adjustment for small sample size (AIC_c), change in AIC_c (ΔAIC_c), Akaike weights (w_i), log-likelihood (LL) for each of the models

Parameters	k	AIC_c	ΔAIC_c	w_i	LL	R^2
Mass, site	6	156.17	0	0.96	−71.58	0.17
Site	5	164.68	8.51	0.01	−76.98	0.07
Stream P, site	5	164.68	8.51	0.01	−76.98	0.02
Null	2	167.99	11.81	0	−81.92	
Mass	3	168.32	12.15	0	−81.02	0.009
Stream P	3	169.53	13.35	0	−81.62	−0.004
Sex	4	172.05	15.87	0	−81.79	−0.019

The adjusted R^2 for the best-supported model is 0.17. Both *Macrobrachium carcinus* and *Macrobrachium olfersi* were included in these models. Mass refers to the weight (g DM) of the individual shrimp. Site refers to which of the four study stream reaches. Stream P is soluble reactive P in μg L^{-1}. Sex refers to whether an individual shrimp was male or female. Null is the model run without any parameters.

Table 5 Estimated contribution to stream P recycling for the two common shrimp species and the five most abundant fish species in a stream reach (Sura-60) with low SRP (soluble reactive P) at La Selva Biological Station

	Density (individuals m^{-2})	Mean size (g WM)	Density (g WM m^{-2})	Mean P excretion rate (μg TDP g WM^{-1} h^{-1})	Mean P excretion rate (μg TDP $shrimp^{-1}$ h^{-1})	P excreted (μg TDP m^{-2} h^{-1})
Shrimp species						
Macrobrachium olfersi	0.26	1.89	0.49	0.32	0.71	0.18
Macrobrachium carcinus	0.12	32.4	3.89	0.11	1.5	0.18
	Density (individuals m^{-2})	Mean size (g WM)	Density (g WM m^{-2})	Mean P excretion rate (μg SRP g WM^{-1} h^{-1})	Mean P excretion rate (μg SRP $fish^{-1}$ h^{-1})	P excreted (μg SRP m^{-2} h^{-1})
Fish species						
Astyanax aeneus	0.5	10.6	5.5	7.4	81	40.5
Astatheros alfari	0.5	36.6	17.1	0.01	0.3	0.1
Archocentrus septemfasciatus	0.6	5.9	3.7	0.03	0.2	0.1
Priapicthys annectens	2.5	1.8	4.3	0.3	2.2	1.1
Alfaro cultratus	0.4	0.9	0.3	11.3	8.6	3.4

Fish P excretion rates are from Small *et al.* (2011) except for mean P excretion rate (μg TDP g WM^{-1} h^{-1}) which was calculated by dividing P excreted with the fish density. Shrimp mass values were converted from dry mass to wet mass using a conversion factor from Ricciardi & Bourget (1998).

estimated P flux from fish excretion previously measured for this same reach (45.2 μg P m^{-2} h^{-1}).

Discussion

Our results suggest that omnivorous shrimp may be adjusting their diets and maintaining relatively constant bulk diet %P across sites. Despite large differences in P content of basal food resources (up to 2.4-fold) across streams, we did not observe differences in shrimp P excretion rates across streams. Instead, shrimp $\delta^{15}N$ values and %N were elevated both in low-P stream sites, suggesting that shrimp are feeding less on detritus, and more in insects or periphyton, which are high in P. The lack of change in shrimp body %P is also consistent with a diet switching strategy. This result is consistent with other studies that have found food choice to be an important determinant of nutrient recycling rates (Pilati & Vanni, 2007).

The stoichiometric benefits of diet switching can be examined using a threshold elemental ratio ($TER_{C:P}$) approach (Frost *et al.*, 2006), which describes the theoretical optimally balanced diet for a consumer, given the stoichiometric demands of new biomass production and baseline metabolism. Based on estimates of other crustaceans by Frost *et al.* (2006), we estimate a $TER_{C:P}$ of 200 for shrimp (*M. carcinus* and *M. olfersi*). In the low-P sites, CPOM C:P is *c.* 2000, and periphyton and insect C:P are both ~200 (Small & Pringle, 2010), indicating that shrimp would need to feed exclusively on the latter two resources to achieve an optimal diet. In the high-P streams, CPOM C:P is *c.* 800, and periphyton and insect C:P are both *c.* 100 (Small & Pringle, 2010), suggesting that an optimal shrimp diet could include up to 14% CPOM. This predicted shift from a lower P resource (CPOM) to higher P resources (insects and periphyton) in low-P streams is consistent with the observed increase in shrimp $\delta^{15}N$ in these streams, and also with the observed lack of variation across sites in body P content and in P excretion rates.

Although shrimp P excretion rates did not differ significantly across streams, rates were highly variable (i.e. the best model explained only 17% of variation). Differences in diets of individual shrimp, food limitation and higher P assimilation rates, differences in individual growth rates, and differences in P content among different insect taxa could have contributed to this variability. The timing of shrimp capture and incubation stress also could have contributed to variation in observed excretion rates. Depending on the exact time of capture during the night, shrimp could have gone without food for anywhere from 2 to 14 h. The stress of incubation and starvation could have influenced individual shrimp differently, resulting in increased variability in measured excretion rates.

The appearance of both plasticity and strict homoeostasis in body nutrient stoichiometry suggests that decapod crustaceans can respond in multiple ways to nutrient loading. %N and %C of shrimp increased as stream P decreased but their body %P did not change. This differed from smaller macroinvertebrates, which showed twofold increases in body %P in the high-solute

Arboleda-30 relative to the low-solute Sura-60 (Small & Pringle, 2010). Previous studies have found both plasticity and homoeostasis in decapod crustacean stoichiometry. Atyid shrimp differed in body N:P and C:P across a nutrient-loading gradient in urban streams (Tsoi *et al*., 2011), but crayfish in nutrient-enriched artificial streams did not show changes in body elemental composition (Evans-White & Lamberti, 2006). Research elucidating the driving factors of the variable response of shrimp body stoichiometry would be useful in understanding stoichiometric responses of decapod crustacean to nutrient loading.

We found that shrimp body elemental content changed ontogenetically, while other studies on Atyid shrimp have found that only %P decreased as shrimp grew and that C:P and C:N ratios changed with body mass. We found that shrimp body elemental content changed ontogenetically; %C, %N and %P decreased as shrimp grew, but elemental ratios were unchanged. Benstead *et al.* (2010) also found differences in body stoichiometry in inter-species comparisons of atyid shrimp (*Atya* and *Xiphocaris*), which suggests that shrimp in different families and even potentially different genera have different relationships between ontogeny and body stoichiometry.

Many studies examining consumer nutrient recycling focus solely on one community (e.g. aquatic insects or fish); however, each community assemblage may have variable and interacting effects within a site. Data from this study provide a unique opportunity for direct comparisons to previously measured excretion rates for the fish assemblage in one of the study reaches (Small *et al*., 2011). The lower mass-specific P excretion rates for shrimp were surprising, given the lower P demand of invertebrates. Shrimp %P was *c.* 1% (Table 2), compared to 3.6–5.0% for fish (Small *et al*., 2011). This discrepancy could be the result of fish in this reach feeding on higher P diets (most were primarily insectivores), lower feeding rates by shrimp or faster rates of biomass production (i.e. P sequestration) by shrimp relative to fish. Our results suggest that the diet of omnivorous consumers can strongly influence excretion rates and are not congruent with simple predictions based solely on resource stoichiometry, body size, body nutrient ratios and growth rates.

Shrimp P excretion at Sura-60 was small when compared with the contribution to P demand through fish excretion, in part due to the high contribution of a single fish species that accounts for 90% of total P recycled (Small *et al*., 2011). In streams in Puerto Rico, where shrimp density can be much higher than in LSBS, shrimp excretion constitutes an important nutrient flux (Benstead *et al*., 2010). Differences in shrimp biomass among streams in Puerto Rico can lead to variation in P flux from excretion ranging over nearly two orders of magnitude. Our study found per-shrimp P excretion rates within the range reported by Benstead *et al*., yet our reach-scale estimates were only 36% of the magnitude of the lowest estimate from Puerto Rico. Even though *Macrobrachium* may not be play an important direct role in P recycling in these streams, it may still play an important indirect role in stream nutrient dynamics through controlling standing stocks of periphyton and CPOM (Pringle & Hamazaki, 1998).

In summary, despite large differences in food resource P content across streams, shrimp did not show differences in P excretion rates, probably due in part to dietary shifts towards higher P food resources in low-P streams. Despite low body P content relative to fish, shrimp displayed lower mass-specific P excretion rates, suggesting differences in diet, feeding rate or growth rate are important factors in regulating the relative contribution of consumer nutrient recycling to aquatic systems. Our findings suggest that while shrimp are not significant contributors to stream P demand that shrimp could play a larger role as nutrient recyclers of N relative to fish. In contrast to other invertebrates, shrimp showed no difference in body %P, but did differ in body %C and %N across these streams. Our findings highlight the importance of diet choice, body nutrient content and behavioural traits in influencing the relationship between consumer and food resource stoichiometry, and nutrient recycling rates.

Acknowledgments

We are grateful to M. Hidalgo for help with fieldwork and to Amy Rosemond, Mary Freeman, Alan Covich, Robert Cooper and the Pringle lab for helpful feedback that improved this manuscript. This study was supported by the National Science Foundation through the Long-Term Studies Environmental Biology program (DEB 9528434, DEB 0075339, DEB 0545463) and by the Organization for Tropical Studies through the David and Deborah Clark and the Christiane and Christopher Tyson Fellowships.

References

Acharya K., Bukaveckas P.A., Jack J.D., Kyle M. & Elser J.J. (2006) Consumer growth linked to diet and RNA-P stoichiometry: response of *Bosmina* to variation in riverine

food resources. *Limnology and Oceanography*, **51**, 1859–1869.

Anderson D.R., Burnham K.P. & Thompson W.L. (2000) Null hypothesis testing: problems, prevalence, and an alternative. *Journal of Wildlife Management*, **64**, 912–923.

APHA (1998) *Standard Methods for the Examination of Water and Wastewater*, 20th edn. American Public Health Association, Washington, DC.

Atkinson C.L., Opsahl S.P., Covich A.P., Golladay S.W. & Conner L.M. (2010) Stable isotopic signatures, tissue stoichiometry, and nutrient cycling (C and N) of native and invasive freshwater bivalves. *Journal of the North American Benthological Society*, **29**, 496–505.

Benstead J.P., Cross W.F., March J.G., McDowell W.H., Ramírez 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.

Burnham K.P. & Anderson D.R. (2002) *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*, 2nd edn. Springer, New York, NY.

Carvalho D.A., Collins P.A. & De Bonis C.J. (2011) Gut Evacuation time of *Macrobrachium borellii* (Caridea: Palaemonidae) feeding on three types of prey from the littoral-benthic community. *Journal of Crustacean Biology*, **31**, 630–634.

Christian A.D., Crump B.G. & Berg D.J. (2008) Nutrient release and ecological stoichiometry of freshwater mussels (Mollusca: Unionidae) in 2 small, regionally distinct streams. *Journal of the North American Benthological Society*, **27**, 440–450.

Covich A.P., Crowl T.A., Johnson S.L. & Pyron M. (1996) Distribution and abundance of tropical freshwater shrimp along a stream corridor: response to disturbance. *Biotropica*, **28**, 484–492.

Cross W.F., Covich A.P., Crowl T.A., Benstead J.P. & Ramírez A. (2008) Secondary production, longevity and resource consumption rates of freshwater shrimps in two tropical streams with contrasting geomorphology and food web structure. *Freshwater Biology*, **53**, 2504–2519.

De Grave S., Cai Y. & Anker A. (2008) Global diversity of shrimps (Crustacea: Decapoda: Caridea) in freshwater. *Hydrobiologia*, **595**, 287–293.

Denno R.F. & Fagan W.F. (2003) Might nitrogen limitation promote omnivory among carnivorous arthropods? *Ecology*, **84**, 2522–2531.

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

Evans-White M.A. & Lamberti G.A. (2006) Stoichiometry of consumer-driven nutrient recycling across nutrient regimes in streams. *Ecology Letters*, **9**, 1186–1197.

Frost P.C., Benstead J.P., Cross W.F., Hillebrand H., Larson J.H., Xenopoulos M.A. *et al.* (2006) Threshold elemental ratios of carbon and phosphorous in aquatic consumers. *Ecology Letters*, **9**, 774–779.

García-Guerrero M.U., Becerril-Morales F., Vega-Villasante F. & Espinosa-Chaurand L.D. (2013) Los langostinos del género *Macrobrachium* con importancia económica y pesquera en América Latina: conocimiento actual, rol ecológico y conservación. *Latin American Journal of Aquatic Research*, **41**, 651–675.

Genereux D. & Pringle C.M. (1997) Chemical mixing model of streamflow generation at La Selva Biological Station, Costa Rica. *Journal of Hydrology*, **199**, 319–330.

González-Bergonzoni I., Meerhoff M., Davidson T.A., Teixeira-de Mello F., Baattrup-Pedersen A. & Jeppesen E. (2012) Meta-analysis shows a consistent and strong latitudinal pattern in fish omnivory across ecosystems. *Ecosystems*, **15**, 492–503.

Huggins R. (1989) On the statistical analysis of capture experiments. *Biometrika*, **76**, 133–140.

James L.A.H., Xenopoulos M.A., Wilson H.F. & Frost P.C. (2007) Land use controls nutrient excretion by stream invertebrates along a gradient of agriculture. *Journal of the North American Benthological Society*, **26**, 523–531.

Jones J.B. Jr, Wolf B. & Mills H.A. (1991) *Plant Analysis Handbook I: Methods of Plant Analysis and Interpretation*. Micro-Macro Publishing, Athens, GA.

Kilham S.S. & Pringle C.M. (2000) Food webs in two neotropical stream systems as revealed by stable isotope ratios. *Verhandlungen Internationale Vereinugung für Theoretische und Angewandte Limnologie*, **27**, 1768–1775.

Mantel S.K. & Dudgeon D. (2004) Growth and production of a tropical predatory shrimp, *Macrobrachium hainanense* (Palaemonidae), in two Hong Kong streams. *Freshwater Biology*, **49**, 1320–1336.

March J.G. & Pringle C.M. (2003) Food web structure and basal resource utilization along a tropical island stream continuum, Puerto Rico. *Biotropica*, **35**, 84–93.

McIntyre P.B., Flecker A.S., Vanni M.J., Hood J.M., Taylor B.W. & Thomas S.A. (2008) Fish distributions and nutrient cycling in streams: can fish create biogeochemical hotspots. *Ecology*, **89**, 2335–2346.

McIntyre P.B., Jones L.E., Flecker A.S. & Vanni M.J. (2007) Fish extinctions alter nutrient recycling in tropical freshwaters. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 4461–4466.

Obregon F.C. (1986) *Revision de conocimiento de los camarones de agua dulce de Costa Rica*. Masters thesis, Universidad de Costa Rica, San Jose.

Otis D.L., Burnham K.P., White G.C. & Anderson D.R. (1978) Statistical inference from capture data on closed animal populations. *Wildlife Monographs*, **62**, 3–135.

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

Pringle C.M. (1991) Geothermally modified waters surface at La Selva Biological Station, Costa Rica: volcanic processes introduce chemical discontinuities into lowland tropical streams. *Biotropica*, **23**, 523–529.

Pringle C.M. & Hamazaki T. (1998) The role of omnivory in a neotropical stream: separating diurnal and nocturnal effects. *Ecology*, **79**, 269–280.

Pringle C.M. & Triska F.J. (1991) Effects of geothermal groundwater on nutrient dynamics of a lowland Costa Rican stream. *Ecology*, **72**, 951–965.

R Development Core Team (2011) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Ricciardi A. & Bourget E. (1998) Weight-to-weight conversion factors for marine benthic macroinvertebrates. *Marine Ecology Progress Series*, **163**, 245–251.

Sanford R.L., Paaby P., Luvall J.C. & Phillips E. (1994) Climate, geomorphology, and aquatic systems. In: *La Selva: Ecology and Natural History of a Neotropical Rainforest* (Eds L.A. McDade, K.S. Bawa, H.A. Hespenheide & G.S. Hartshorn), pp. 19–33. University of Chicago Press, Chicago, IL.

Schindler D.E. & Eby L.A. (1997) Stoichiometry of fishes and their prey: implications for nutrient recycling. *Ecology*, **78**, 1816–1831.

Shimizu Y. & Urabe J. (2008) Regulation of phosphorus stoichiometry and growth rate of consumers: theoretical and experimental analyses with *Daphnia*. *Oecologia*, **155**, 21–31.

Small G.E. & Pringle C.M. (2010) Deviation from strict homeostasis across multiple trophic levels in an invertebrate consumer assemblage exposed to high chronic phosphorus enrichment in a Neotropical stream. *Oecologia*, **162**, 581–590.

Small G.E., Pringle C.M., Pyron M. & Duff J.H. (2011) Role of the fish *Astyanax aeneus* (Characidae) as a keystone nutrient recycler in low-nutrient Neotropical streams. *Ecology*, **92**, 386–397.

Snyder M.N. (2012) *Abundance, distribution, energy flow and nutrient dynamics of freshwater shrimps in lowland Costa Rica*. Dissertation. University of Georgia, Athens, GA.

Sterner R.W. & Elser J.J. (2002) *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. Princeton University Press, Princeton, NJ.

Thompson R.M., Hemberg M., Starzomski B.M. & Shurin J.B. (2007) Trophic levels and trophic tangles: the prevalence of omnivory in real food webs. *Ecology*, **88**, 612–617.

Triska F., Pringle C.M., Duff J.H., Avanzino R.J. & Zellweger G. (2006) Soluble reactive phosphorus (SRP) transport and retention in tropical, rain forest streams draining a volcanic landscape in Costa Rica: in situ SRP amendment to streams and laboratory studies. *Biogeochemistry*, **81**, 145–157.

Tsoi W.Y., Hadwen W.L. & Fellows C.S. (2011) Spatial and temporal variation in the ecological stoichiometry of aquatic organisms in an urban catchment. *Journal of the North American Benthological Society*, **30**, 533–545.

Usio N. & Townsend C.R. (2002) Functional significance of crayfish in stream food webs: roles of omnivory, substrate heterogeneity and sex. *Oikos*, **98**, 512–522.

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

Vanni M.J., Flecker A.S., Hood J.M. & Headworth J.L. (2002) Stoichiometry of nutrient recycling by vertebrates in a tropical stream: linking species identity and ecosystem processes. *Ecology Letters*, **5**, 285–293.

Vonesh E.F., Chinchili V.M. & Pu K. (1996) Goodness-of-fit in generalized nonlinear mixed-effect models. *Biometrics*, **52**, 572–587.

Whiles M.R., Huryn A.D., Taylor B.W. & Reeve J.D. (2009) Influence of handling stress and fasting on estimates of ammonium excretion by tadpoles and fish: recommendations for designing excretion experiments. *Limnology and Oceanography Methods*, **7**, 1–7.

White G.C. & Burnham K.P. (1999) Program mark: survival estimation from populations of marked animals. *Bird Study*, **46**(Suppl.), 120–138.

Yip P.S.F., Huggins R.M. & Lin D. (1996) Inference for capture-recapture experiments in continuous time with variable capture rates. *Biometrika*, **83**, 477–483.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Estimation of contribution of shrimp to reach-scale P recycling.

(Manuscript accepted 13 September 2014)