

# Role of the fish *Astyanax aeneus* (Characidae) as a keystone nutrient recycler in low-nutrient Neotropical streams

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**Abstract.** Nutrient recycling by animals is a potentially important biogeochemical process in both terrestrial and aquatic ecosystems. Stoichiometric traits of individual species may result in some taxa playing disproportionately important roles in the recycling of nutrients relative to their biomass, acting as keystone nutrient recyclers. We examined factors controlling the relative contribution of 12 Neotropical fish species to nutrient recycling in four streams spanning a range of phosphorus (P) levels. In high-P conditions (135 µg/L soluble reactive phosphorus, SRP), most species fed on P-enriched diets and P excretion rates were high across species. In low-P conditions (3 µg/L SRP), aquatic food resources were depleted in P, and species with higher body P content showed low rates of P recycling. However, fishes that were subsidized by terrestrial inputs were decoupled from aquatic P availability and therefore excreted P at disproportionately high rates. One of these species, *Astyanax aeneus* (Characidae), represented 12% of the total population and 18% of the total biomass of the fish assemblage in our focal low-P study stream but had P excretion rates >10-fold higher than other abundant fishes. As a result, we estimated that P excretion by *A. aeneus* accounted for 90% of the P recycled by this fish assemblage and also supplied ~90% of the stream P demand in this P-limited ecosystem. Nitrogen excretion rates showed little variation among species, and the contribution of a given species to ecosystem N recycling was largely dependent upon the total biomass of that species. Because of the high variability in P excretion rates among fish species, ecosystem-level P recycling could be particularly sensitive to changes in fish community structure in P-limited systems.

**Key words:** *Astyanax aeneus*; *Atherinidae*; *Characidae*; *Cichlidae*; ecological stoichiometry; fish; La Selva Biological Station, Costa Rica; nutrient cycling; phosphorus; *Poeciliidae*; streams.

## INTRODUCTION

Ecologists have identified a number of species that can have disproportionately large effects on certain ecosystems, relative to their biomass (keystone species, sensu Power et al. 1996). However, most research in this area has focused on species' roles in trophic interactions (e.g., Paine 1966) rather than in recycling nutrients (but see Molvar et al. 1993, Knapp et al. 1999). Within aquatic ecosystems, nutrient recycling by animals can constitute an important biogeochemical flux and supply nutrients that limit primary productivity (e.g., Meyer et al. 1983, Grimm 1988, Vanni et al. 2002, McIntyre et al. 2008). In most of the cases in which excretion by a given species is an important component of ecosystem nutrient dynamics, these effects are explained by high biomass levels rather than by high mass-specific excretion rates (e.g., Caraco et al. 1997, Hall et al. 2003, McIntyre et al. 2007). However, stoichiometric variables unique to individual species, such as diet nutrient content and

body elemental composition, may result in some taxa playing disproportionately important roles in the recycling or retention of nutrients, thereby affecting ecosystem nutrient availability (e.g., Elser et al. 1988).

Aquatic ecosystems are commonly limited by phosphorus (P) and nitrogen (N) (Elser et al. 2007), but the stoichiometric variables controlling rates of P and N excretion by different species could lead to differential importance in the roles that individual species play in the cycling of these two elements. Phosphorus recycling rates are known to vary widely among species due to differences in body elemental composition. Vertebrate species with high body P content (e.g., due to bones and scales) excrete P at lower rates compared to species with lower levels of body P (Vanni et al. 2002). Similarly, diets higher in P can contribute to higher levels of P excretion for a given species (e.g., Pilati and Vanni 2007). A fish assemblage that varies in diet and body elemental composition could include species with a wide range of P excretion rates, with the potential for a species represented at relatively low biomass levels to account for a high proportion of ecosystem-level P recycling. In contrast to P, N recycling rates tend to be

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TABLE 1. Physical and chemical characteristics of the four streams used in this study, at La Selva Biological Station, lowland Costa Rica.

Stream	Discharge (m <sup>3</sup> /s)	Conductance ( $\mu$ S/cm at 25°C)	pH	Temperature (°C)	DIN:SRP (molar)	NO <sub>3</sub> -N ( $\mu$ g/L)	NH <sub>4</sub> -N ( $\mu$ g/L)	SRP ( $\mu$ g/L)
Arboleda	0.17	257	6.2	25.6	2.4	126	20	135
Sura-30	0.61	157	6.1	25.4	4.8	163	18	83
Sabalo	0.28	47	6.0	25.5	21.3	137	29	8
Sura-60	0.19	20	5.6	24.8	173.7	199	21	3

Notes: Values are means of monthly samples ( $n = 20$ ) collected from January 2006 to August 2007. Abbreviations are: DIN, dissolved inorganic nitrogen; SRP, soluble reactive phosphorus.

similar among similar-sized species, due to smaller variation both among N content of different food resources and in N demand by different consumer species (Vanni et al. 2002). The contribution of individual species to total N recycling is therefore largely dependent on the total biomass of each species (McIntyre et al. 2007). Thus, as a result of highly variable mass-specific P excretion rates among species, ecosystem-level P recycling (more so than N recycling) has the potential to be influenced by species identity.

Phosphorus excretion rates for homeostatic consumers reflect a balance between P supply in food resources and the P demand by the consumer for growth and maintenance (Sterner 1990, Elser and Urabe 1999), so that species with a low body P demand feeding on a high-P diet should recycle excess P at a high rate. In addition to the type of food resources consumed, the P content of a given food resource may vary according to levels of dissolved phosphorus in a lake or stream. Algae, detritus, and several invertebrate taxa have increased P content in high-P aquatic environments (e.g., Stelzer and Lamberti 2001, Cross et al. 2003, Elser et al. 2005), so that fishes in a high-P system may ingest more P, potentially resulting in higher P excretion rates across the fish assemblage. In low-P systems in which food resources are generally lower in P content, fish species that maintain high P excretion rates through a combination of low P demand and a relatively high P supply in their food resources (e.g., by feeding on terrestrial insects, which are independent of stream P levels) could become disproportionately important in supplying the limiting nutrient to the ecosystem.

To better understand how species-specific nutrient recycling rates are affected by ecosystem nutrient availability, we measured N and P excretion rates for fish assemblages in four lowland Neotropical streams that had a wide range in dissolved P levels due to natural inputs of solute-rich groundwater. Across this P gradient, algae and aquatic invertebrates increase in P content by fivefold and twofold, respectively (Small and Pringle 2010), so that most fishes in high-P streams ingest more P per unit of food consumed compared to fishes in low-P streams. We predicted that P excretion rates for fish species that feed primarily on aquatic insects and algae will reflect stream dissolved P levels, whereas excretion rates of species that feed on terrestrial insects will be decoupled from stream nutrient levels.

## METHODS

### Study sites

This study took place at La Selva Biological Station in the lowlands of Costa Rica's Caribbean Slope. Some streams at La Selva receive natural inputs of solute-rich groundwater (Pringle et al. 1993), resulting in high soluble reactive phosphorus (SRP) concentrations, whereas other streams do not receive solute-rich groundwater and are P limited (Pringle and Triska 1991). We selected four third- to fourth-order streams that range in SRP from 2 to 135  $\mu$ g/L (Table 1). These sites are a subset of streams monitored as part of a long-term study on the physicochemistry of La Selva streams (Pringle and Triska 1991, Triska et al. 2006) for which continuous monthly data are available since 1997. Daily and seasonal stream temperature has low variation, ranging from 24°C to 26°C.

The P content of aquatically derived food resources increases across the natural P gradient in our study streams. Comparing the low-P Sura-60 and the high-P Arboleda, the P content increases more than twofold for epilithon (0.18–0.47% P by dry mass), fivefold for filamentous algae (0.06–0.30% P), and twofold for aquatic invertebrates (0.5–1.1%; Small and Pringle 2010). Terrestrial invertebrates, collected from pan traps along the study streams, have a mean P content of 0.5% that is independent of stream chemistry (G. E. Small, unpublished data).

Forty-three species of fishes have been documented in the rivers and streams of La Selva Biological Station (Bussing 1994). Our study streams contain a subset of these species; Burcham (1988) documented 19 species in the Sura-30 and 26 species in the Sabalo. In our study, we measured N and P excretion rates for 12 fish species (within four different families) that are sufficiently abundant in our study sites to be collected by seine (Table 2).

### Quantification of nutrient recycling rates by fishes

We quantified N and P excretion rates for these 12 fish species across our study streams during June and July 2007. Fishes were collected by seine and were placed immediately into plastic bags (1 individual/bag) containing 250 mL of water for smaller individuals (<3 g wet mass) or 500–1000 mL of water for larger individuals. Water for all excretion trials was collected

TABLE 2. Number of individuals of each fish species in the four study streams for which N and P excretion rates were measured.

Family and species	Arboleda	Sura-30	Sabalo	Sura-60
Atherinidae				
<i>Melaniris hubbsi</i>	...	20	13	20
Characidae				
<i>Astyanax aeneus</i>	15	16	14	12
Cichlidae				
<i>Archocentrus septemfasciatus</i>	3	5	22	18
<i>Astatheros alfari</i>	16	20	19	23
<i>Hypsophrys nicaraguensis</i>	...	10	9	...
<i>Neotroplus nematopus</i>	1	9	11	...
Poeciliidae				
<i>Alfaro cultratus</i>	19	23	20	20
<i>Brachyraphis parismina</i>	...	18	20	...
<i>Neoheterandria umbratilis</i>	...	20	19	...
<i>Phallichthys amates</i>	2	11	19	...
<i>Poecilia gillii</i>	...	13	20	...
<i>Priapichthys annectens</i>	2	15	...	16

Note: Ellipses indicate that the species was absent (or not collected) from that stream.

from the low-P Sura-60 and filtered to remove suspended particles using Whatman Grade Number 1 filter paper (11  $\mu\text{m}$  pore size). During incubations, bags were kept in shallow water along the stream margins to maintain constant temperature and to minimize stress. Any individuals showing visible signs of stress during the incubation were not used in the study. After 1 h, water samples were collected from each bag and filtered through a 0.45- $\mu\text{m}$  Millipore filter (Millipore, Billerica, Massachusetts, USA) to remove feces and other particles. Water samples were also collected from five control bags (incubated for 1 h with no fish) for every round of measurements. Water samples were immediately frozen and transported on ice to the University of Georgia's Analytical Chemistry Laboratory for analysis. Soluble reactive phosphorus was measured using the ascorbic acid method, and  $\text{NH}_4$  was measured using the phenate-hypochlorite method (APHA 1998).

We measured excretion rates for 531 individual fish. Not all species were present in all four streams, and the number of excretion replicates varied among species roughly in proportion to species abundances (Table 2). Per capita N and P excretion rates were calculated as the increase in  $\text{NH}_4\text{-N}$  and SRP, relative to controls, during the 1-h incubation.

Fish total lengths were measured in the field to the nearest 0.1 mm, and wet mass was estimated using regressions established for each species from individuals collected for body nutrient content. Collected individuals were weighed, guts were removed and preserved in formalin for later analysis, and specimens were then dried and ground to a powder. Samples of ground animals were analyzed for carbon (C) and N with a Carlo Erba NA 1500 CHN analyzer (Carlo Erba, Milan, Italy). For P analysis, samples were acid-digested (aqua regia double acid; Jones et al. 1991) and analyzed spectrophotometrically (ascorbic acid method). Gut

contents were examined using a dissecting microscope: food items were classified as terrestrial insect, aquatic insect, terrestrial plant, or aquatic plant. Material in guts was spread over a grid of 1-mm squares, and diet proportion was estimated based on the relative area covered by each food category.

Body nutrient composition and gut contents were measured for 235 individuals (a subset of the 531 fish used for excretion measurements; typically five individuals of each species in each stream). Body N:P and diet for each species did not vary significantly across body sizes and between streams.

#### Statistical analyses

Of 531 fish excretion measurements, 96 measurements of  $\text{NH}_4$  and 124 measurements of SRP were equal to or slightly less than mean values from corresponding control bags. These points were excluded from the statistical models presented below. We also compared statistical models with all points included, based on assigning a nutrient increase in these samples of 0.1  $\mu\text{g/L}$ , essentially a minimum detection limit for excreted nutrients, to ensure that all values were positive before log transformation. These near-zero values were also included in the calculation of reach-scale P recycling for the low-P focal stream.

We used multiple regression to quantify relationships between rates and ratios of nutrient recycling and body mass, fish body nutrient content, proportion of diet made up of insects, and stream SRP, with individual fish as the unit of observation. For the 271 individuals for which we directly measured diet and body nutrient content, we used these data in the model. For the other individuals, we used a mean value for that species in each stream to estimate diet and body nutrient content. Excretion rates (N and P) and ratios (N:P), as well as wet mass, were log-transformed. Species identity per se

was not included in the model, because our goal was to produce a general model that uses species attributes (body elemental composition and diet) to predict nutrient recycling rates.

To evaluate the importance of the three stoichiometric variables (body nutrient content, percentage of insects in diet, and stream SRP) on excretion rates, we selected models using the corrected Akaike information criterion ( $AIC_c$ ; Burnham and Anderson 2002). We then calculated standardized regression coefficients ( $\beta_{std}$ ) to compare the relative importance of body nutrient composition, diet, and stream identity in controlling rates of nutrient recycling (Neter et al. 1996). The standardized regression coefficient is defined as the change in the dependent variable (in terms of standard deviation) resulting from a change of 1 SD in the corresponding independent variable. All statistical analyses were conducted in SAS using PROC GLM (SAS Institute 2001).

#### *Contribution of fish excretion to nutrient recycling in the low-P focal stream*

In March 2008, population sizes were estimated for the five most abundant fish species (*Alfaro cultratus* [Poeciliidae], *Archocentrus septemfasciatus* [Cichlidae], *Astatheros alfari* [Cichlidae], *Astyanax aeneus* [Characidae], and *Priapichthys annectens* [Poeciliidae]) in our focal low-P study stream, the Sura-60. Fish were collected by electrofishing along a 50-m reach. Each individual was marked, and population estimates were calculated based on the number of recaptures using the program NOREMARK (G. C. White, unpublished software). We estimated total N and P recycling rates for these five species based on the mean per capita excretion rate of the actual size distribution and then extrapolated to the estimated total population of each species in the reach. Per capita N and P excretion rates were based on mass relationships calculated for each species from this stream (Appendices A and B).

These five most abundant species make up an estimated 85% of the total fish assemblage in this reach (87 out of 102 captured individuals). Eight other species were captured: *Anguilla rostrata* (Anguillidae), *Awaous tajasica* (Gobiidae), *Brycon guatemalensis* (Characidae), *Gymnotus cyllindricus* (Gymnotidae), *Melaniris hubbsi* (Atherinidae), *Parachromis dovii* (Cichlidae), *Rhamdia guatemalensis* (Heptapteridae), and *Rivulus isthmensis* (Aplocheilidae). We have insufficient data to accurately estimate population densities of these species (most were represented by a single individual), although densities are likely  $<0.05$  individuals/m<sup>2</sup>, if we assume capture probabilities similar to the abundant species. Based on their low densities, we assume that each of these taxa were minor contributors to total N and P recycling by the fish assemblage.

In March 2006, we measured stream nutrient demand in our focal low-P study stream, Sura-60, using a short-term addition of  $NH_4^+$  and  $PO_4^{3-}$ . The reactive solutes

were injected along with a conservative tracer (rhodamine WT) for 6 h to measure plateau concentrations at three downstream stations (at 260, 360, and 460 m). At plateau,  $NH_4^+$ -N was elevated to 55  $\mu\text{g/L}$  and SRP was elevated to 63  $\mu\text{g/L}$ . The rate of decline in dilution-corrected nutrient concentration over distance was converted into an areal uptake rate ( $U$ ) by multiplying by stream velocity, mean depth, and background nutrient concentration (Stream Solute Workshop 1990). We calculated 95% confidence intervals for  $U$  using confidence intervals for the slope of the uptake regression.

## RESULTS

### *Fish nutrient content and diet*

Across the 12 species, body N content was essentially constant, with species means ranging from 10.1% to 10.2% N by dry mass. Species means for body P content ranged from 3.6% to 5.0% P by dry mass, and as a result, mean body N:P molar ratios ranged from 5.0 to 6.7 (Table 3). Within individual species, fish elemental composition did not vary significantly with body size or across streams. Species in the family Cichlidae had the highest body P content, while the families Atherinidae and Poeciliidae were intermediate. The characid, *Astyanax aeneus*, had the lowest body P content among species in this study.

The 12 fish species ranged in diet from herbivory to insectivory, with most species consuming food items from multiple categories (Table 3). Individual species did not show differences in diets among streams. Aquatic insects constituted the majority of the diets for the atherinid *Melaniris hubbsi* and for the cichlids *Archocentrus septemfasciatus*, *Astatheros alfari*, and *Hypsophrys nicaraguensis*. The characid *Astyanax aeneus* and poeciliids *Alfaro cultratus* and *Brachyrhaphis parismina* fed primarily on terrestrial insects. The diet of *Priapichthys annectens* (Poeciliidae) was evenly divided between terrestrial and aquatic insects. The cichlid *Neotroplus nematopus* and poeciliids *Neoheterandria umbratilis*, *Phallichthys amates*, and *Poecilia gillii* fed primarily or exclusively on aquatic plants (filamentous algae or diatoms). Terrestrial plant material was only a minor contribution to the diets of any fish species in our study (Table 3).

### *Relative importance of nutrient supply and demand in affecting excretion rates*

The best model (based on lowest  $AIC_c$  values) for per capita N excretion ( $n = 448$ ) includes the percentage of insects in diet in addition to mass, although adding stream SRP yielded a nearly identical  $AIC_c$  value (Appendix C). However, the addition of one or both of these stoichiometric variables results in a minimal improvement of the model's explanatory power ( $r^2 = 0.40$ ) compared to a model with mass alone ( $r^2 = 0.39$ ; Fig. 1). Excretion rates for N increase as the proportion of insects in a fish's diet increases (Table 4).

TABLE 3. Body N and P content (percentage dry mass), N:P ratios, and diets (based on gut contents) for the 12 fish species examined in this study.

Species	% N	% P	Body N:P, molar	Diet (percentage of gut contents by volume)			
				Aquatic insects	Terrestrial insects	Aquatic plants	Terrestrial plants
Atherinidae							
<i>Melaniris hubbsi</i>	10.6 (0.1)	3.8 (0.2)	6.0 (0.2)	62.1	19.1	18.6	0.2
Characidae							
<i>Astyanax aeneus</i>	10.6 (0.2)	3.6 (0.1)	6.7 (0.2)	20.8	65.4	10.6	3.2
Cichlidae							
<i>Archocentrus septemfasciatus</i>	10.5 (0.3)	4.3 (0.1)	5.4 (0.2)	80.2	15.5	4.4	0.0
<i>Astatheros alfari</i>	10.2 (0.1)	4.7 (0.3)	5.1 (0.2)	85.4	12.0	2.6	0.0
<i>Hypsophrys nicaraguensis</i>	10.1 (0.1)	5.0 (0.7)	5.0 (0.4)	60.7	10.1	29.2	0.0
<i>Neotroplus nematopus</i>	10.3 (0.1)	4.6 (0.3)	5.1 (0.3)	17.6	0.0	79.9	2.5
Poeciliidae							
<i>Alfaro cultratus</i>	10.5 (0.1)	3.6 (0.1)	6.6 (0.1)	31.6	66.0	2.2	0.2
<i>Brachyrhaphis parismina</i>	10.2 (0.2)	4.1 (0.2)	5.6 (0.3)	13.3	80.0	6.7	0.0
<i>Neoheterandria umbratilis</i>	10.5 (0.1)	4.5 (0.5)	5.8 (0.5)	8.1	16.7	75.2	0.0
<i>Phallichthys amates</i>	10.5 (0.1)	4.3 (0.2)	5.5 (0.3)	0.0	0.0	100.0	0.0
<i>Poecilia gillii</i>	10.6 (0.1)	3.6 (0.2)	6.6 (0.3)	5.3	1.8	92.9	0.0
<i>Priapichthys annectens</i>	10.3 (0.1)	3.8 (0.1)	6.2 (0.2)	49.8	50.2	0.0	0.0

Note: Reported values are means for individuals across the four streams, with SE in parentheses.

Fish taxa showed different responses in P excretion rates across the four study streams (Table 5), with more individuals (especially in the family Cichlidae) falling out along the minimum detection limit (MDL) in lower SRP streams (Fig. 2). For per capita P excretion rates ( $n = 423$ ; MDL points removed), the full model containing all three stoichiometric variables (body P percentage [%P], percentage of insects in diet, and stream SRP) was supported by AIC<sub>c</sub> and explained 33% of variance in the data (compared to 23% of variance explained by mass alone; Appendix C). Rates of P excretion were negatively related to body %P, and positively related to the percentage of insects in the diet and to stream SRP (Table 4). Based on  $\beta_{\text{std}}$  values, the model was most sensitive to changes in the percentage of insects in the diet of the three stoichiometric variables. However, with the MDL points included in the model, stream SRP became the most important of these three variables (Appendix D).

The best model for N:P excretion ratios explained only 12% of variance and therefore should be interpreted with caution. The AIC<sub>c</sub> supported the inclusion of all three candidate stoichiometric variables (but not body mass). Based on this model, excretion N:P was negatively related to body N:P, the percentage of insects in the diet, and stream SRP (Table 4). Based on  $\beta_{\text{std}}$  values, this model was most sensitive to changes in body N:P across our data set.

#### Contribution of fish excretion to nutrient recycling in a low-SRP stream

Estimated population densities for the five fish species that were most abundant in the Sura-60 ranged from 0.4 to 2.5 individuals/m<sup>2</sup>. Mean wet mass for individuals of

these abundant species ranged from 0.9 g to 36.6 g, and estimated total biomass ranged from 0.3 to 17.1 g wet mass/m<sup>2</sup> (Table 6). Mean P excretion rates for the two cichlids, *Archocentrus septemfasciatus* and *Astatheros alfari*, were essentially at the detection limit ( $\sim 0.2 \mu\text{g SRP}\cdot\text{individual}^{-1}\cdot\text{h}^{-1}$ ). In contrast, the characid *Astyanax aeneus* had the highest per capita P excretion rate among the five abundant fishes in this stream (81.0  $\mu\text{g SRP}\cdot\text{individual}^{-1}\cdot\text{h}^{-1}$ ), nearly 10-fold higher than the next highest of the most abundant species in this reach. As a result, *Astyanax aeneus* supplied an estimated 90% of the P recycled by the five abundant fish species (40.5 out of 45.2  $\mu\text{g SRP}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ ), while accounting for only 9% of the total population (9 out of 102 individuals collected in this reach during the population survey) and 19% of the total biomass of the fish assemblage (5.5 g wet mass/m<sup>2</sup> out of a total fish biomass of 29.4 g wet mass/m<sup>2</sup>; Table 6). By contrast, variation among N excretion rates for these five abundant species was largely due to differences in body size, so that estimated total N recycled by each species was proportionate to its total biomass (Table 6).

From the nutrient addition experiment in this low-P focal study stream, we estimated areal uptake rates ( $U$ ) of 755 (95% CI, 499-999)  $\mu\text{g NH}_4^+\cdot\text{m}^{-2}\cdot\text{h}^{-1}$  and 45 (17-74)  $\mu\text{g PO}_4^{3-}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ . Based on these measurements, fishes appear to be important nutrient recyclers in this low-nutrient stream. The fish assemblage in our study reach may supply 100% or more of stream  $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$  demand. Phosphorus excretion by *A. aeneus* supplied an estimated 90% of stream P demand, while P excretion by the other four abundant species in this reach supplied a combined 10% of stream P demand.

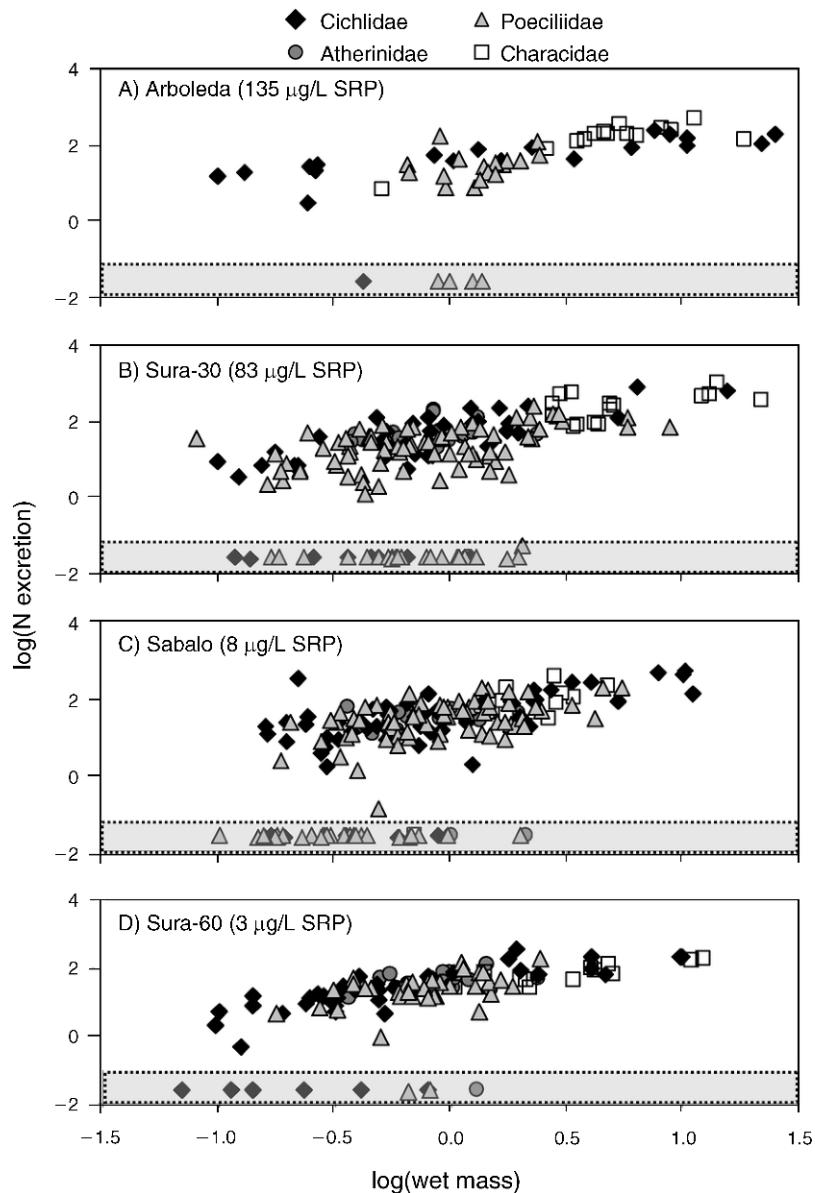


FIG. 1. Log-transformed per capita N excretion rate vs. log-transformed mass of individual fishes in the four study streams with different levels of soluble reactive phosphorus, SRP. The shaded area represents points along the minimum detection limit. Excretion rate was measured as  $\mu\text{g NH}_4^+\text{-fish}^{-1}\cdot\text{h}^{-1}$ ; biomass was measured in grams. The study took place at La Selva Biological Station in the lowlands of Costa Rica's Caribbean Slope.

Expressing nutrient excretion in volumetric units and accounting for stream velocity:  $(\text{per area excretion} \times \text{velocity}) / (\text{depth} \times \text{solute concentration})$  indicated that nutrient recycling by the entire fish assemblage in this stream (assuming constant conditions downstream) would be sufficient to turn over the pools of dissolved  $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$  in 1.3 km and 3.4 km, respectively. Phosphorus excretion by *A. aeneus* alone would be sufficient to turn over the dissolved P pool over 3.8 km, compared to a distance of 33 km that would be required for turnover due to the combined P excretion of the other four most abundant species.

## DISCUSSION

### *Role of Astyanax aeneus in ecosystem-level nutrient recycling*

Our calculations indicate that *Astyanax aeneus* acts as a keystone nutrient recycler in the low-SRP Sura-60 because it maintained a relatively high P excretion rate in an ecosystem in which other abundant fish species excreted P at much lower levels. Our results highlight the fact that the importance of species identity in ecosystem processes is context dependent. In higher-SRP streams, P excretion rates across the fish assemblage tended to be

TABLE 4. Results of multiple regression to predict per capita N and P excretion rates and N:P excretion ratios.

Parameter	Estimate	SE	df	<i>t</i>	<i>P</i>	$\beta_{\text{std}}$
log(N excretion rate)						
Intercept	1.432	0.045	1	31.55	<0.001	
log(wet body mass)	0.683	0.040	1	17.23	<0.001	0.587
Insects in diet	0.133	0.053	1	2.49	0.013	0.096
log(P excretion rate)						
Intercept	0.729	0.221	1	3.30	0.001	
log(wet body mass)	0.909	0.080	1	11.41	<0.001	0.586
Body %P	-0.151	0.048	1	-3.16	0.002	-0.161
Insects in diet	0.561	0.088	1	6.36	<0.001	0.308
Stream SRP	0.002	0.001	1	2.65	0.008	0.141
log(N:P excretion ratio)						
Intercept	3.135	0.343	1	9.15	<0.001	
Body N:P ratio	-2.092	0.447	1	-4.68	<0.001	-0.268
Insects in diet	-0.333	0.086	1	-3.86	0.001	-0.166
Stream SRP	-0.002	0.001	1	-2.66	0.008	-0.089

Notes:  $R^2$  values for these models were 0.40, 0.33, and 0.12 for per capita N and P excretion rates and N:P excretion ratios, respectively. All 12 fish species were included in these models. Minimum detection limit values were excluded from this analysis (see Appendix D for model results with the complete data set). Values in column 2 are parameter estimates from the multiple regression model. Insects in diet refers to the proportion (0–1) of insects found in the gut contents of a given fish. Body %P is the P content per unit dry mass in a given fish. Stream SRP is soluble reactive phosphorus, in  $\mu\text{g/L}$ . Body N:P is the molar ratio. The standardized regression coefficient ( $\beta_{\text{std}}$ ) quantifies the change in the independent variable (in SD) relative to a change of 1 SD in the dependent variable.

uniformly high as a result of P-enriched food resources; in low-SRP streams in which aquatically derived food resources were depleted in P, species that fed on aquatically derived food resources had much lower P excretion rates. However, several species whose diet was subsidized by terrestrial insects maintained relatively high P excretion rates, and of these species, only *A. aeneus* made up a significant (>1%) proportion of total fish biomass. *Astyanax aeneus* maintained a high P excretion rate that was >10-fold higher than other species that had similarly high levels of biomass. *Astyanax aeneus* represented 18% of the total biomass

of the five abundant fish species, yet contributed 90% of the P recycled by these species while also supplying an estimated 90% of stream P demand.

We suggest that the P recycled by the fish assemblage (and *A. aeneus* in particular) may be important in ecosystem functioning, given previous studies that have found that microbial respiration (Ramírez et al. 2003) and leaf decomposition (Rosemond et al. 2002) increase rapidly with slight increases in P availability in these streams. Nonetheless, these values should be interpreted with some caution, because we did not measure P excretion for all fish species (i.e., our estimates of total P

TABLE 5. Mass-specific P excretion rates (mean with SE in parentheses) for 12 fish species in four streams ranging in dissolved P levels from 3 to 135  $\mu\text{g SRP/L}$ .

Family and species	Arboleda (135 $\mu\text{g/L SRP}$ )	Sura-30 (83 $\mu\text{g/L SRP}$ )	Sabalo (8 $\mu\text{g/L SRP}$ )	Sura-60 (3 $\mu\text{g/L SRP}$ )
Atherinidae				
<i>Melaniris hubbsi</i>		23.9 (4.9)	18.8 (7.6)	22.0 (5.1)
Characidae				
<i>Astyanax aeneus</i>	11.1 (2.0)	30.3 (5.9)	21.7 (4.4)	11.8 (1.9)
Cichlidae				
<i>Archocentrus septemfasciatus</i>	5.4 (1.3)	2.9 (2.5)	3.1 (1.5)	0.2 (0.1)
<i>Astatheros alfari</i>	9.8 (3.3)	21.3 (9.6)	1.3 (0.3)	1.1 (0.6)
<i>Hypsophrys nicaraguensis</i>		1.7 (0.6)	11.1 (5.6)	
<i>Neotrophus nematopus</i>	3.4	4.0 (2.0)	5.7 (1.7)	
Poeciliidae				
<i>Alfaro cultratus</i>	16.1 (4.8)	70.1 (18.0)	12.2 (2.8)	9.0 (2.8)
<i>Brachyrhaphis parismina</i>		26.3 (9.2)	1.7 (0.5)	
<i>Neoheterandria umbratilis</i>		0.6 (0.2)	2.2 (1.4)	
<i>Phallichthys amates</i>	5.8 (0.8)	2.5 (1.8)	1.9 (0.6)	
<i>Poecilia gillii</i>		5.7 (1.6)	3.5 (1.0)	
<i>Priapichthys annectens</i>	0.1 (0.1)	1.9 (0.9)		0.7 (0.4)

Note: P excretion rates are in  $\mu\text{g soluble reactive phosphorus (SRP)}\cdot[\text{g wet mass}]^{-1}\cdot\text{h}^{-1}$ . Minimum detection limit values are included in these means (see *Methods: Statistical analyses* for explanation).

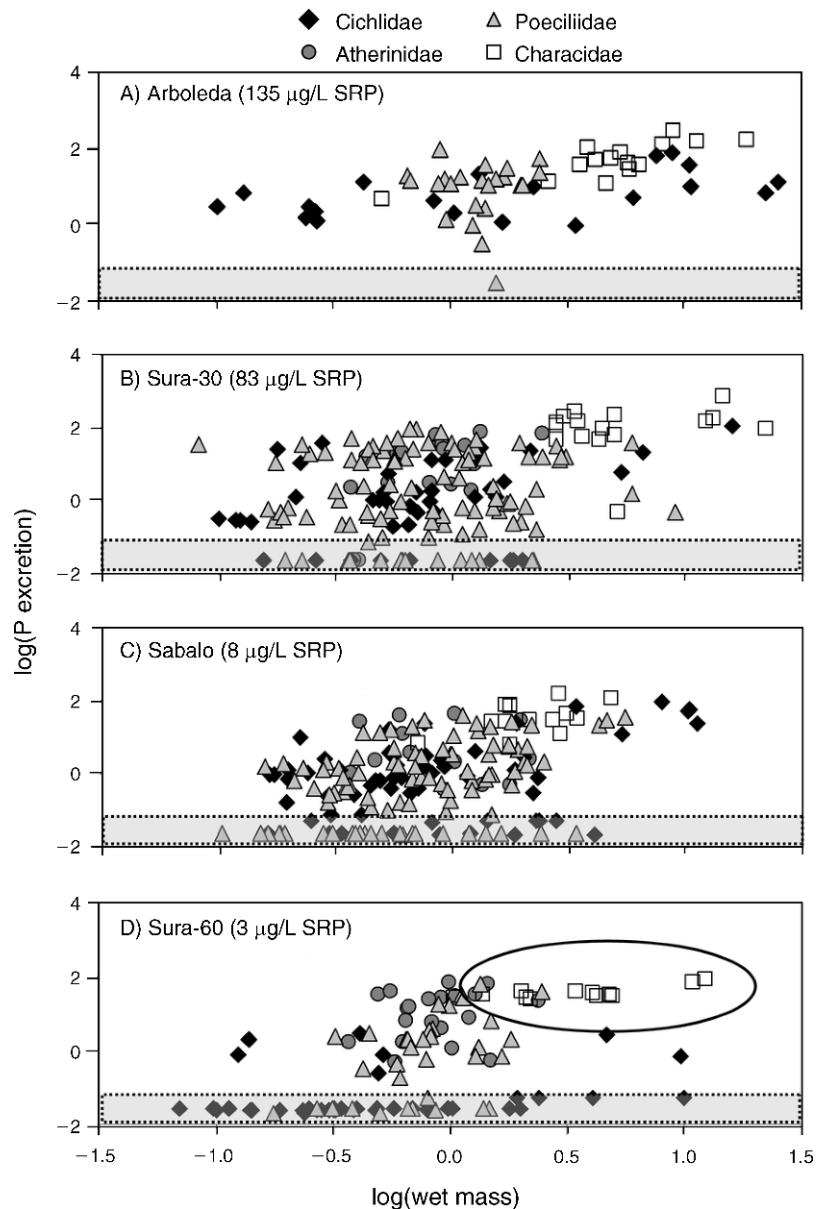


FIG. 2. Log-transformed per capita P excretion rates vs. log-transformed mass of individual fishes in the four study streams. The shaded area represents points along the minimum detection limit. Excretion rate was measured as  $\mu\text{g SRP}\cdot\text{fish}^{-1}\cdot\text{h}^{-1}$ ; biomass was measured in grams. Data points for *Astyanax aeneus* in the Sura-60 are enclosed to illustrate that this species maintained relatively high P excretion rates in the stream with low soluble reactive phosphorus (SRP).

excreted by the entire fish assemblage are conservative). Direct measurements of ambient nutrient excretion rates are subject to error from effects of fasting and stress; the hour-long incubation times used in our study are likely long enough to compensate for initial stress effects, but could cause underestimates of excretion rates due to fasting, especially for insectivores with faster gut passage times (Whiles et al. 2009). The higher excretion rates documented for insectivores are therefore likely conservative. We cannot rule out the possibility that placing fish from a high-solute (high-SRP) stream into low-

solute water (from the low-SRP stream) during the incubation could contribute to additional stress, but the fact that P excretion, but not N excretion, was higher in the high-SRP streams suggests that this effect was due to food nutrient content rather than physiological stress. Additionally, our estimates of stream N and P uptake are based on a single nutrient addition experiment (so we cannot estimate temporal variability in uptake rates), and estimates of stream N and P demand from our short-term nutrient addition most probably underestimated ambient uptake rates (Mulholland et al. 2002).

TABLE 6. Estimated contribution to stream N and P recycling for five common fish species in Sura-60, the study stream with low soluble reactive phosphorus (SRP).

Species	Population density (no./m <sup>2</sup> )	Mean size (g wet mass)	Total biomass (g wet mass/m <sup>2</sup> )	Mean P excretion rate (μg SRP·fish <sup>-1</sup> ·h <sup>-1</sup> )	SRP excreted (μg SRP·m <sup>-2</sup> ·h <sup>-1</sup> )	Mean N excretion rate (μg NH <sub>4</sub> -N·fish <sup>-1</sup> ·h <sup>-1</sup> )	NH <sub>4</sub> excreted (μg NH <sub>4</sub> -N·m <sup>-2</sup> ·h <sup>-1</sup> )
<i>Astyanax aeneus</i>	0.5	10.6	5.5	81.0	40.5	195.7	97.9
<i>Astatheros alfari</i>	0.5	36.6	17.1	0.3	0.1	787.3	393.7
<i>Archocentrus septemfasciatus</i>	0.6	5.9	3.7	0.2	0.1	392.6	235.6
<i>Priapichthys annectens</i>	2.5	1.8	4.3	2.2	1.1	247.5	123.8
<i>Alfaro cultratus</i>	0.4	0.9	0.3	8.6	3.4	32.2	12.9

Notes: Total P excreted was 45.2 μg SRP·m<sup>-2</sup>·h<sup>-1</sup>; stream P demand was 45 (17–74, 95% CI) μg SRP·m<sup>-2</sup>·h<sup>-1</sup>; total N excreted was 863.9 μg NH<sub>4</sub>-N·m<sup>-2</sup>·h<sup>-1</sup>; and stream N demand was 755 (499–999) μg NH<sub>4</sub>-N·m<sup>-2</sup>·h<sup>-1</sup>. *Astyanax aeneus* constituted an estimated 12% of the total population and 18% of the total biomass of this fish assemblage, yet P excretion by this species accounted for 90% of P recycled by this fish assemblage.

The measured nutrient uptake rates are integrated over a 200-m reach, whereas the fish census represented a 50-m subset of this reach that tended to be good fish habitat, so reported fish densities (and therefore per area excretion rates) are likely to be higher in our study reach than for the entire stream. In spite of these qualifications, our data provide robust evidence that P excretion by *A. aeneus* is a large flux relative to the rest of the fish assemblage and to stream P demand.

The stoichiometric traits of a given fish species can be used to predict its potential role in nutrient recycling. Insectivorous (and piscivorous) fish species should excrete excess P at high rates, which increase with the P content of their food, since they are rarely P limited (Schindler and Eby 1997). Herbivorous and detritivorous fishes may be P limited (Hood et al. 2005), and P excretion rates should be low. Rates of P excretion by fishes feeding on aquatically derived food resources are affected by stream nutrient levels (via dietary P content). However, because terrestrial subsidies are independent of stream nutrient status, subsidized species may be more likely to play disproportionately important roles in stream P recycling. The effects of diet stoichiometry are compounded by a species' P demand (Vanni et al. 2002). In low-P La Selva streams, three species (*A. aeneus*, *Melanurus hubbsi*, and *Alfaro cultratus*) maintained high mass-specific P excretion rates (>9 μg SRP·[g biomass]<sup>-1</sup>·h<sup>-1</sup>) due to primarily insectivorous (P-rich) diets and relatively low body P content (N:P > 6.0). Of these three species, only *Astyanax aeneus* had sufficient abundance for their nutrient recycling to represent an appreciable ecosystem flux in our focal study stream (Sura-60), and as a result, this species dominated consumer-driven P recycling. While the importance of *A. aeneus* in nutrient recycling is highly context dependent, as is typical of keystone species (Power et al. 1996), we note that low-nutrient, forested streams are common across the range of this widespread taxon, and it is likely that *Astyanax* plays similarly important roles in many of these stream ecosystems.

#### Effect of stream P levels, diet, and body nutrient content on nutrient recycling rates

Nitrogen excretion rates of fishes in this study were minimally influenced by the stoichiometric variables that we measured. Fish body N content showed little variation among all individuals (coefficient of variation: 0.04) and was not related to N excretion rates. Of the stoichiometric variables, only percentage of insects in the diet was significantly related to N excretion rates. However, despite the fact that insects have an N content ~10-fold higher than epilithon (Small and Pringle 2010), adding this variable led to a minimal improvement in the amount of variance explained by the model. As with the data set analyzed by McIntyre et al. (2007), in our data set, ecosystem-level N excretion by the fish assemblage can be predicted with reasonable accuracy using only the total biomass of fish in the ecosystem.

In contrast to N excretion, P excretion rates were dependent upon dietary P supply and body P demand. Differences among these variables led to differences in P excretion rates among species and, for some species, intraspecific differences among the study streams. Our findings are consistent with the conclusions of Vanni et al. (2002) that fish body P content is an important factor controlling P excretion rates, with species with higher body P recycling P at a lower rate. The importance of body P content was high even though the range among species in our study (molar N:P ratio: 5.0–6.7) was much lower than that of the fish and tadpole assemblage measured by Vanni et al. (2002), in which body N:P ranged from 4 to 23.

Our analysis also indicates that dietary P content is as important as fish P demand in determining P excretion rates. Insectivorous fishes excrete P at higher rates than algivores, consistent with the fact that insects have twofold higher P content compared to epilithon across these streams (Small and Pringle 2010). Furthermore, stream SRP is an important predictor of P excretion across the entire data set. Aquatically derived food resources increase in P content greater than twofold with increasing SRP across these four study streams (Small and Pringle 2010).

The effect of stream SRP on P excretion rates varied by species. The insectivorous cichlids *Astatheros alfari* and *Archocentrus septemfasciatus* had ~20-fold decreases in mass-specific P excretion rates between the highest and the lowest P streams (Table 4). These two species have some of the lowest mean body N:P ratios of all species considered in this study (5.13 and 5.45, respectively). They also both feed primarily on aquatic insects, which are 50% lower in P content relative to the high-P streams (Small and Pringle 2010). This combination of lower dietary P supply coupled with high P demand explains the very low levels of P excretion measured for these species in the Sura-60. Interestingly, the poeciliids *Alfaro cultratus* and *Brachyrhaphis parismina* also showed decreases in P excretion rates in low-P streams, despite somewhat higher body N:P ratios and a greater reliance on terrestrial insects. The P excretion responses of the four species discussed above suggest that they eliminate excess P in high-P streams but not in low-P streams. In contrast, other species in the fish assemblage did not show changes in P excretion rates with stream SRP. Herbivorous species such as the cichlid *Neotroplus nematopus* and the poeciliids *Phallichthys amates*, *Neoheterandria umbratilis*, and *Poecilia gillii* maintained relatively low P excretion rates across all four streams (Table 4), despite documented three- to fivefold increases in the P content of epilithon and filamentous algae. If algalivorous fishes are P limited (Hood et al. 2005), the additional P in algae in high-SRP streams may result in increased growth rates, so that ingested P goes into biomass production rather than being excreted. Finally, *Astyanax aeneus* (Characidae) and *Melanirius hubbsi* (Atherinidae) maintained high P excretion rates across all four streams (Table 4). Both of these species are characterized by insectivorous diets and relatively low body P content. Due to their lower P demand and the high amount of P ingested in their food resources, these species eliminate excess P at high rates in all four streams, regardless of ambient P levels.

Nutrient recycling rates may not always be completely explained by the nutrient imbalance between consumer and food resource. Species with a high P demand feeding on low-P food resources could potentially compensate by ingesting more food, feeding selectively, or assimilating nutrients more efficiently. We did not measure rates of fish growth, ingestion, or assimilation; variation in these rates could decouple the relationship between diet P content and consumer P excretion rates. For example, food limitation could explain the low P excretion rates measured for *Priapichthys annectens*, an insectivore with a relatively high body N:P ratio. Besides excretion, fecal production is a significant nutrient flux (e.g., Grimm 1988, Rodehutsord et al. 2000, Hall et al. 2003) that we did not measure, although homeostatic regulation is thought to occur primarily through excretion rather than egestion (Rodehutsord et al. 2000, Anderson et al. 2005).

#### *When is species identity an important predictor of nutrient recycling?*

Species-specific differences in P excretion rates, due to diet and body P content, create the potential for a species to have a disproportionately important role in ecosystem P cycling relative to its biomass. Freshwater ecosystems are losing species at an unprecedented rate (Bunn and Arthington 2002, Dudgeon et al. 2006), and the loss of a dominant P recycler (e.g., through overharvesting or loss of critical habitat) could have a large effect on ecosystem processes in a P-limited stream. Because of the species-specific traits that control P recycling rates, compensation by remaining species would be unlikely to buffer the decline in ecosystem P recycling rates.

Similarities in N recycling rates among species suggest two mechanisms that may stabilize ecosystem-level N recycling against species losses. First, if rarity predicts extinction risk, then the numerically dominant species with the largest role in N recycling should be less prone to extinction. Secondly, because mass-specific rates of N excretion are fairly similar between species, there is a higher potential for species redundancy in this trait, so that if a dominant N recycler is extirpated, compensatory responses by other species would likely dampen ecosystem-level effects. The first of these mechanisms confers resistance and the second resilience, potentially decreasing the sensitivity of consumer-driven N recycling to changes in community structure.

In summary, our results show that terrestrial food subsidies can decouple aquatic consumers from aquatic P availability. Among the species that maintained relatively high P excretion rates in the low-P focal stream, most were relatively rare, representing <1% of total fish biomass. Only *Astyanax aeneus* was among the four high-biomass taxa. The other three common species had low P excretion rates in the low-P focal stream. As a result, *Astyanax* dominates ecosystem P recycling by the fish assemblage in this low-P Neotropical stream, accounting for 90% of P recycling by the fish assemblage, although it only represents 18% of total fish biomass in this reach. Because of the disproportionately large effect that *Astyanax* has on ecosystem P recycling relative to its biomass, our study contrasts with other studies that have demonstrated that fish can be important nutrient recyclers. For example, while McIntyre et al. (2007) found that another characid, *Prochilodus mariae*, was the major contributor to N and P recycling in a Venezuelan river, its importance was largely due to its large individual size and high total biomass in this river rather than especially high mass-specific excretion rates. The important role of *A. aeneus* in P recycling is due to its high P excretion rates in an ecosystem in which other abundant consumers minimize their loss of this element, illustrating that the importance of species identity on nutrient cycling is context-dependent (i.e., it depends on background nutrient levels). In low-P aquatic ecosystems, species that excrete

P at high rates due to a low body P demand and a diet composed of high-P food resources are most likely to play disproportionately important roles in P recycling. Our study suggests that shifts in community composition in aquatic ecosystems, through extirpations or invasions, could dramatically alter ecosystem-scale P recycling rates and significantly affect ecosystem functioning. Future manipulative experiments are needed to further elucidate how changes in aquatic communities will alter nutrient dynamics in stream ecosystems.

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#### APPENDIX A

Nitrogen excretion estimates by species and stream for the model  $\log_{10}(\text{NH}_4^+\text{-N excretion}) \sim \log_{10}(\text{wet mass})$  (*Ecological Archives* E092-034-A1).

#### APPENDIX B

Phosphorus excretion estimates by species and stream for the model:  $\log_{10}(\text{SRP excretion}) \sim \log_{10}(\text{wet mass})$  (*Ecological Archives* E092-034-A2).

#### APPENDIX C

Model selection criteria for N and P excretion rates and N:P excretion (*Ecological Archives* E092-034-A3).

#### APPENDIX D

Best statistical models (based on lowest corrected Akaike information criterion value) for N and P excretion rates and N:P excretion ratio when points at minimum detection limit are included (*Ecological Archives* E092-034-A4).