

Exploring Metabolic and Stoichiometric Controls for Nutrient Excretion: Body Size Has Primacy in a Tropical Stream Fish Community

PRISCILA OLIVEIRA-CUNHA (✉ priscilacunhaoli@gmail.com)

Universidade do Estado do Rio de Janeiro

PETER B. MCINTYRE

Cornell University

VINICIUS NERES-LIMA

Universidade do Estado do Rio de Janeiro

ADRIANO CALIMAN

Universidade Federal do Rio Grande do Norte

BEATRIZ MOREIRA-FERREIRA

Universidade do Estado do Rio de Janeiro

EUGENIA ZANDONÀ

Universidade do Estado do Rio de Janeiro

Article

Keywords: ecological stoichiometry, metabolic ecology, animals, nitrogen, phosphorus, freshwater.

Posted Date: April 22nd, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1560054/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Discussions of the factors regulating nutrient recycling by consumers have focused on predictions from Ecological Stoichiometry (ES) and the Metabolic Theory of Ecology (MTE). ES posits that imbalances between the composition of an animal's body tissues and its diet should determine its nutrient excretion rates, whereas the MTE predicts that excretion should directly reflect metabolic activity arising from body size and temperature. Each framework has been supported by data, but they are rarely tested together. In this study, we measured excretion rates of nitrogen (NH_4), phosphorus (SRP) and N:P excretion ratio, body N:P stoichiometry, body size, and temperature for 12 species of fish from an Atlantic rainforest stream in Brazil. We fitted 8 competing models reflecting different combinations of ES (body N:P, armor classification, diet group) and MTE (body size, temperature) variables. For both N and P excretion, as well as excreted N:P ratio, only body size was included in the best model, and interspecific differences in size-scaling were greater for N than for P. Fitted size scaling coefficients were lower than the MTE prediction of 0.75 for N (0.59, 95% CI = 0.45, 0.73), but not P (0.56, 95% CI = 0.40, 0.77). There was only weak evidence that body armor in 3 of 12 species led to more retention of P, and there was no discernable effect of diet group, body N:P, or water temperature. We conclude that differences in nutrient excretion among species within a shared environment primarily reflect contrasts in metabolic rates arising from body size, rather than disparities between consumer and resource stoichiometry. Our findings align with those from other ecosystems and synthesis across aquatic taxa, expanding support for the MTE as the primary framework for predicting nutrient excretion rates.

Introduction

Consumer nutrient recycling of nitrogen (N) and phosphorus (P) can have a significant impact to their ecosystems [1]. Through their ingestion, elimination and transport of nutrients between habitats, consumers can have a direct influence on the nutrient cycling process and can act as sinks or sources of nutrients depending on context [1, 2, 3].

Two distinct conceptual frameworks have been offered to understand and predict differences among consumers in nutrient recycling rates: Ecological Stoichiometry (ES) and the Metabolic Theory of Ecology (MTE). ES is based on the premise that consumer body composition is homeostatic, even when the nutrient content of the diet varies widely [2, 4]. Therefore, ES predicts that the rates and ratios of excreted nutrients reflect imbalances between the makeup of the diet versus body tissues [4]. For example, individuals with a nutrient-rich diet should excrete more nutrients than counterparts with a nutrient-poor diet. By extension, individuals with high dietary N:P should excrete more N than those with a low N:P diet. Differences in body composition should create similarly disparities in excretion, such as the high P demand for growing bones [2, 5, 6] reducing the P excretion rates of vertebrates.

The MTE posits that metabolic rates are the primary determinant of all ecological processes, from individual to ecosystem scales [7, 8]. Metabolic rates are affected by body size and ambient temperature [7]. The relation between metabolism and body size is a power function with a scaling coefficient of $\frac{3}{4}$ [7, 9], which means that smaller animals excrete disproportionately more nutrients per unit body mass than larger animals [2, 10, 11]. Allgeier et al. (2015) [12] found evidence of $\frac{3}{4}$ -power scaling for N and P excretion of fish and macroinvertebrates in marine ecosystems, whereas Vanni and McIntyre (2016) [13] found lower scaling

coefficients for both nutrients across all types of aquatic animals. Temperature also holds a central place in the MTE because it mediates the rates of chemical reactions [7, 14]; metabolic rates increase exponentially with temperature, hence nutrient excretion rates should also be positively related to temperature.

Both ES and MTE are compelling frameworks because they are rooted in fundamental principles, yet they emphasize completely different predictors of nutrient recycling due to their contrasting emphases on elemental mass balance versus energetics [12, 13, 15]. Species-rich ecosystems provide an interesting arena in which to compare ES- and MTE-based predictors of nutrient excretion rates because species vary widely in diet, tissue composition, and body size [12, 16]. Direct comparisons of the explanatory power of ES and MTE for aquatic animal excretion rates have included large numbers of coastal marine species [12] as well as a literature synthesis across a host of aquatic vertebrates and invertebrates [13]. Both of those studies concluded that MTE has primacy over ES because body size was more important than dietary or consumer nutrient content. However, the 5–8 orders of magnitude range in body sizes tested in each study may have obscured the comparatively modest range of stoichiometric variation [17]. Thus, it is possible that the influence of ES on nutrient excretion by consumers might become more apparent when focusing on one taxonomic group in a single ecosystem.

In this study, we compared the predictive power of the ES and MTE frameworks using nutrient excretion rates from 12 species in the fish community of a Neotropical stream. Fishes play a significant role in nutrient cycling by virtue of storing large quantities of phosphorus in their tissues [2, 3], transporting nutrients between habitats [2, 18], varying widely in dietary and body nutrient content [19, 20], and being abundant in many freshwater ecosystems [21, 22]. Our study species ranged in body size from 0.02 to 22.0 g wet mass, and we used substantial seasonal variation in water temperature to test its effects on excretion rates. These fish species also differed sharply in the nutrient content of their diet (from algivory/detritivory to piscivory) and their body tissues (3 of 12 species are armored catfish, which are renowned for high body P). By collecting all data from a single site with consistent methods and background environmental conditions (flow, nutrient levels), our survey of excretion rates was designed to offer a rigorous comparison of the influence of ES and MTE variables.

We expected to find support for both the ES and MTE frameworks, and we adopted a model-selection approach to jointly testing their influence. We made the following four predictions. 1) Fish from higher trophic positions should excrete more N and P than like-sized fish from lower trophic positions due to the general increase in dietary nutrient content with trophic level [22, 23]. 2) Armored catfish should excrete less P than like-sized fishes due to their high P demand for building their boney armor. 3) Nutrient excretion rates should increase with water temperature, reflecting higher resting metabolic rates. 4) The relationship between body size and nutrient excretion should be allometric with a scaling coefficient of ~ 0.75 , in accordance with the MTE.

Results

Nitrogen excretion rates ranged from 1.8 to 2667.3 $\mu\text{g NH}_4\text{-N ind}^{-1}\text{h}^{-1}$, while phosphorus excretions rates ranged from 0.015 to 117.8 $\mu\text{g P ind}^{-1}\text{h}^{-1}$. The smallest species (*P. harpagos* and *M. microlepsis*) had the

lowest average N and P excretion rates per capita, while larger species (*R. quelen* and *Rineloricaria* sp.) had the highest N and P excretion rates (Table 1). However, excreted rates scaled allometrically with body mass, as indicated by scaling coefficients smaller than 1.

Table 1
Mean excretion rate of NH₄-N and SRP-P of all studied fish species (µg ind⁻¹h⁻¹).

Species	N excretion rate		P excretion rate	
	n	Mean ± SD	n	Mean ± SD
<i>A. leptos</i>	6	98 ± 40	5	8.6 ± 6.7
<i>A. multispinis</i>	8	130 ± 113	3	4.1 ± 3.8
<i>B. ornaticeps</i>	19	197 ± 169	8	9.7 ± 13.2
<i>C. vidali</i>	21	109 ± 67	13	6.6 ± 5.8
<i>M. microlepis</i>	15	54 ± 31	10	3.9 ± 4.5
<i>Pharpagos</i>	24	40 ± 22	12	2.3 ± 2.4
<i>Plateristriga</i>	22	115 ± 97	12	12.4 ± 8.8
<i>R. quelen</i>	18	494 ± 753	9	24.9 ± 40.0
<i>Rineloricaria sp</i>	20	197 ± 149	14	25.6 ± 26.5
<i>S. barbatus</i>	23	138 ± 94	15	13.9 ± 15.2
<i>S. marmoratus</i>	12	135 ± 124	6	9.5 ± 3.3
<i>Trichomycterus sp</i>	5	78 ± 56	4	11.1 ± 8.9

The simplest model—using only body size as a predictor—best explained N and P excretion rates as well as excreted N:P ratio (Model 8, Table 2). Testing the individual coefficients, only body size was significantly ($p < 0.05$) related to N and P excretion rates. No predictor had a significant effect on excreted N:P (Table 3, Fig. 1). Moreover, the full MTE model, including both body size and temperature (Model 3, Table 2), performed poorly compared to the model with body size alone (Model 8, Table 2).

Table 2

Comparisons among all statistical models fitted to N and P excretion rates, and excreted N:P ratio, for fish in a Brazilian stream. Each predictor is associated with either the Ecological Stoichiometry (ES) or Metabolic Theory of Ecology (MTE) framework. We compared models representing each framework, or both together (ES + MTE); bold font indicates the most parsimonious model based on AICc.

Model	Variables	N excretion			P excretion			N:P excretion		
		df	AICc	Δ AICc	df	AICc	Δ AICc	df	AICc	Δ AICc
1. ES + MTE	Body size, temperature, diet and Body N:P	17	128	17	17	168	23	17	191	26
1b. ES + MTE	Body size, temperature, diet and Armor	14	120	10	14	158	12	14	182	18
2.ES	Diet and Body N:P	9	236	126	9	237	92	9	210	45
2b. ES	Diet and Armor	7	226	116	7	226	80	7	205	41
3.MTE	Temperature and Body size	10	116	6	10	154	9	10	172	7
4. ES + MTE	Temperature and Body N:P	10	240	130	10	235	89	10	210	46
4b. ES + MTE	Temperature and Armor	7	233	123	7	227	82	7	202	38
5. ES + MTE	Temperature and diet	9	235	125	9	232	86	9	208	43
6. ES + MTE	Body size and Body N:P	10	116	6	10	155	9	10	173	8
6b. ES + MTE	Body size and Armor	7	111	1	7	147	2	7	167	2
7. ES + MTE	Body size and diet	9	114	3	9	148	3	9	171	6
8.MTE	Body size	6	110	0	6	145	0	6	165	0

Table 3
Fitted parameters of the most parsimonious model for N and P excretion rates, and excreted N:P ratio. The * indicates a statistically significant relationship.

	Fixed effects	Estimate	SE	df	t-value	p
N excretion	Intercept	1.982	0.047	8.716	42.000	< 0.001
	Log ₁₀ Body size (g)	0.597	0.053	8.223	11.230	< 0.001*
P excretion	Intercept	0.768	0.055	14.151	14.087	< 0.001
	Log ₁₀ Body size (g)	0.563	0.079	11.200	7.113	< 0.001*
N:P excretion	Intercept	1.576	0.075	11.936	21.100	< 0.001
	Log ₁₀ Body size (g)	-0.004	0.101	31.149	-0.040	0.968

The second-best model for explaining excretion rates and excreted N:P (all $\Delta AICc < 2$) used body size and armor classification (Model 6b), thereby integrating both MTE and ES frameworks (Table 2). However, armor classification never had a significant effect in excretion rates ($p > 0.05$), despite armored fish having higher body demands for P in order to grow (Fig. 2a, b). The full MTE model (Model 3) performed far better than the full ES model (Models 2 and 2b) (Table 2).

Interestingly, models classifying fish species as armored or not (Models 1b, 2b, 4b and 6b) were always more informative than models using body N:P ratio (1, 2, 4 and 6). Armored and non-armored species differed significantly in their body nutrient composition, particularly for P (Fig. 2). However, there were no significant differences in N and P excretion rates based on armor classification (Figure S1 in the Supplementary Information).

The overall scaling coefficient between N excretion rates with body mass was 0.59 (95% CI: 0.45–0.73), which was significantly less than 0.75 (Table 3). The variance explained by body mass (marginal R^2) was 57%, while the variance explained by the entire model (conditional R^2), including both fixed (body mass) and random (species) effects was 64%, indicating that differences between species explained some of the variation in N excretion rates. Species-specific scaling coefficients for N excretion varied from 0.57 (*M. microlepis*) to 0.64 (*S. marmoratus*) (Table 4). For P excretion, the overall scaling coefficient was 0.56 (95% CI: 0.44–0.77), which was not statistically different from the prediction under the MTE (Table 3). The marginal R^2 and conditional R^2 were identical (37%), indicating that accounting for species identity did not increase the explanatory power of the model. Indeed, the species-specific scaling coefficients for P excretion were all comparable (0.55–0.56) (Table 6).

Table 4
Intercept and coefficient values from the relation between body size (g) and the excretion of N and P of all fish species.

Species	N excretion		P excretion	
	Intercept	Log ₁₀ Body size	Intercept	Log ₁₀ Body size
<i>Acentronichthys leptos</i>	2.00	0.59	0.77	0.56
<i>Ancistrus multispinis</i>	1.92	0.61	0.77	0.56
<i>Bryconamericus ornaticeps</i>	2.06	0.57	0.77	0.56
<i>Characidium vidali</i>	2.06	0.59	0.77	0.56
<i>Mimagoniates microlepis</i>	2.11	0.57	0.77	0.57
<i>Phalloceros harpagos</i>	2.11	0.57	0.77	0.56
<i>Pimelodella lateristriga</i>	1.89	0.62	0.77	0.57
<i>Rhamdia quelen</i>	2.04	0.60	0.76	0.55
<i>Rineloricaria sp</i>	1.99	0.60	0.77	0.57
<i>Scleromystax barbatus</i>	1.88	0.62	0.77	0.56
<i>Synbranchus marmoratus</i>	1.72	0.64	0.77	0.56
<i>Trichomycterus sp</i>	2.00	0.59	0.77	0.56

Table 5

List of species, their feeding groups, body size measures (as estimated dry weight) and if they show a boney armor.

Order	Family	Species	Average body size (g)	Body size range (g)	Feeding group	Armored fish
Siluriformes	Callichthyidae	<i>Scleromystax barbatus</i>	0.91	0.31–2.05	Omnivore	Yes
Siluriformes	Heptopteridae	<i>Pimelodella lateristriga</i>	1.4	0.18–4.16	Invertivore	No
Siluriformes	Heptopteridae	<i>Rhamdia quelen</i>	2.59	0.19–22.01	Piscivore	No
Siluriformes	Heptoteridae	<i>Acentronichthys leptos</i>	0.42	0.39–0.45	Invertivore	No
Siluriformes	Loricariidae	<i>Ancistrus multyinis</i>	2.59	0.93–6.62	Detritivore	Yes
Siluriformes	Loricariidae	<i>Rineloricaria sp</i>	1.69	0.16–7.48	Omnivore	Yes
Siluriformes	Trichomycteridae	<i>Trichomycterus sp</i>	0.97	0.26–2.19	Invertivore	No
Characiformes	Characidae	<i>Bryconamericus ornaticeps</i>	1.48	0.14–5.30	Invertivore	No
Characiformes	Characidae	<i>Mimagoniates microlepis</i>	0.19	0.03–0.49	Invertivore	No
Characiformes	Crenuchidae	<i>Characidium vidali</i>	0.44	0.13–0.84	Invertivore	No
Ciprinodontiformes	Poeciliidae	<i>Phalloceros harpagos</i>	0.11	0.02–0.22	Omnivore	No
Synbranchiformes	Synbranchidae	<i>Synbranchus marmoratus</i>	2.19	0.42–10.01	Piscivore	No

Table 6

Models used to compare the Ecological Stoichiometry (ES) and Metabolic Theory of Ecology (MTE) frameworks for predicting fish excretion rates of N and P, and excreted N:P ratio, in a Brazilian stream. Below each variable we indicate which framework it relates to.

MTE		ES			
log ₁₀ Body size (g)	Temperature (°C)	Body N:P	Armor	Feeding group	
X	X	X	-	X	
X	X	-	X	X	
-	-	X	-	X	
-	-	-	X	X	
X	X	-	-	-	
-	X	X	-	-	
-	X	-	X	-	
-	X	-	-	X	
X	-	X	-	-	
X	-	-	X	-	
X	-	-	-	X	
X	-	-	-	-	

Discussion

Metabolic Theory of Ecology (MTE) and Ecological Stoichiometry (ES) are two common frameworks used to predict energy and nutrient budgets at various biological levels of organization [4, 7]. By comparing both theories, we found that the ES variables (diet, body N:P, “armor”) were outperformed by body size, which indicates that the ES framework has relatively little predictable effect on nutrient excretion compared to the role of body size. Also, we saw that the scaling coefficients for the relation between N excretion and body size were lower than the 0.75 coefficient predicted by MTE.

Similarly to previous studies, our results show that body size is a key control on excretion of N and P by fish [12, 24, 25, 26]. Bigger fish excreted more nutrients per capita when compared to smaller fish, however they excreted less nutrients per mass. This result was expected since MTE states that there is an allometric relation between metabolism and body size, described by $\frac{3}{4}$ -power scaling [7]. In fact, Allgeier et al. (2015) [12] found quantitative support for $\frac{3}{4}$ -power scaling of nutrient excretion rates with body mass using data from marine fish and invertebrates. In our study, the lower scaling coefficients for both N and P were closer to a $\frac{2}{3}$ factor than $\frac{3}{4}$, perhaps echoing debates in metabolic ecology about the most appropriate scaling factor [27, 28, 29]. Our results are similar to Vanni and McIntyre (2016) [13], who found scaling coefficients

comparable to ours (0.68 for N; 0.56 for P). The ecological significance of these low scaling coefficients is that size-based increases in nutrient excretion are smaller than would be expected.

The reasons for these lower-than-expected scaling coefficients are uncertain because the ingestion and assimilation of nutrients should be directly related to metabolic rates, hence it is reasonable to expect that release of nutrients in wastes would be as well. One possibility is that focusing on dissolved wastes while excluding solid wastes could create a bias in studies like ours [13]. Alternatively, biochemical mechanisms have received little attention. For instance, Delong et al. (2010) [30] found a gradient of size-scaling coefficients from 1.0 to 0.75 in a survey of metabolism across prokaryotes, unicellular eukaryotes and metazoans, and argued that the differences reflected the number of membrane-bound sites where ATP synthesis and proton pumping occur, as well as differential constraints on resource supply and vascular systems. Although MTE refers to metabolism instead of excretion, it is reasonable to assume the theory also applies to any biological rate that is derived from metabolism [13]. Therefore, it could be that our lower than $\frac{3}{4}$ power-scaling is related to fish growth and ingestion rates or to ontogenetic diet shifts.

A novel aspect of our study was the range of temperature variation during our excretion incubations (9.9 to 25.7° C), and we were surprised to find no evidence that temperature affected nutrient excretion rates of fish. Both the MTE framework and many previous studies [13, 31–35] have suggested that temperature should have discernable effects. This is a surprising result since fish are poikilotherms, which means their body temperature is determined by the external temperature of the water they inhabit and should have direct influence on metabolic rates, feeding rates and activity levels [36]. It could be that in the tropics, as the rate of diel temperature change is usually slow [12, 37], fish can acclimate and perform metabolic compensation [36]. Consequently, because of fish acclimation, we see no apparent changes in nutrient excretion rates.

As for the effects of ES variables, diet and body stoichiometry, our results revealed counterintuitive patterns. Many studies have demonstrated that diet can directly influence fish nutrient excretion rates [38, 39]. The nutritional quality of the diet of aquatic consumers progressively increases from detritivores, to omnivores, to invertivores and, finally, to piscivores [23]. Therefore, according to ES, it is expected that piscivores present the highest nutrient excretion rates compared to detritivores, for example. However, similarly to other comparisons of MTE and ES variables [12, 13], our results do not reflect this pattern. Vanni and McIntyre (2016) [13] attribute the lack of a diet effect to the absence of information on growth, ingestion, and egestion data, and Allgeier et al. (2015) [12] question how useful diet is for predicting nutrient excretion rates. Clearly, we need future studies to investigate the importance of diet by measuring growth, ingestion, excretion and egestion rates across a range of feeding and taxonomic groups.

As expected, armored catfish species presented up to 3x more P in their body composition than the other fish species. Therefore, according to ES predictions, it was expected that they would excrete less P because of their higher P demand for building their bony plates. However, armored and non-armored fish did not differ in their P excretion rates. One possible explanation for this deviation from our prediction is that we sampled primarily adult fishes whose bony skeletons have already been formed, such that further assimilation of dietary P reflects only tissue maintenance. Perhaps if we had sampled individuals in different life stages, we would see growing individuals with a higher P demand and consequent low P excretion.

Surprisingly, differentiating between armored and non-armored fish proved to be more efficient than using actual data on body NP for our model selection. This classification captures the major differences in body composition among our study species, so relying on a simple classification of armor investment by fish could be a sufficient proxy for differences in body stoichiometry. Given that directly measuring body composition is both time-consuming and requires specialized lab facilities, the use of such proxies is appealing in lieu of systematic characterization of body P and stoichiometry across aquatic animals.

Our work built upon the previous studies of Vanni and McIntyre (2016) [13] and Allgeier et. al (2015) [12] to integrate the MTE and ES frameworks for predicting animals' nutrient excretion rates, and all three studies found that body size was by far the strongest influence on nutrient excretion. However, these prior studies tested a much wider range of body sizes (1µg to 500g dry mass [13], 0.04 to 2,597g [12]) than body N:P ratio. That disparity could yield a bias in favor of detecting the influence of MTE variables, so we designed our study to focus on a single taxon with a more limited range of body size (0.021 to 22.01g dry mass) yet similarly variable body stoichiometry. However, we still found that body size is the key control on N and P excretion rates.

Conceptual integration of MTE and ES in this study and others revealed that body size is the key control on nutrient recycling by aquatic animals. Even though our study included a wide range of temperatures, body stoichiometry, and diets, these factors had little detectable influence. While our statistical models provide a useful way to estimate nutrient excretion rates in our study system, they are not a replacement for collecting field data. The mass balance constraints embodied in the ES framework are a fundamental constraint on nutrient recycling, hence researchers seeking accurate estimates of nutrient excretion by aquatic animals should gather direct measurements to verify the applicability of predictive models to their focal species or ecosystem.

Methods

Study site and species

The study was conducted at Rio Guapiaçu, (22°26'08.1"S, 42°45'34.2"W), a fourth order stream located in the hydrographic complex Guapiaçu-Macacu, inside the Reserva Ecológica de Guapiaçu (REGUA), in Cachoeiras de Macacu, RJ, Brazil. The hydrographic complex supplies water to approximately 2.5 million people in five cities [40]. All fish were sampled from an approximate 100 m long reach containing a mixture of substrates (bedrock, leaf litter and sand patches) and habitat types (run and pool).

We focused on the 12 numerically-dominant fish species in the community, representing 8 families and 4 orders (Table 5). Information on the feeding groups (detritivore, omnivore, invertivore and piscivore) was obtained on published literature [41–44] and Fishbase (www.fishbase.se), and confirmed by isotope analysis (Figure S2 in the Supplementary Information).

Nutrient recycling trials

We measured individual excretion rates ($\mu\text{g ind}^{-1} \text{h}^{-1}$) of nitrogen and phosphorus. Nitrogen was analyzed as ammonium ($\text{NH}_4^+\text{-N}$, hereafter N) using fluorometry (Aquafluor, Turner Designs, Sunnyvale, CA, USA) following Holmes et al. (1999) [45] as modified by Taylor et al. (2007) [46]. Phosphorus was analyzed as soluble reactive phosphorus (PO_4^{3-}P , hereafter P) using the molybdenum blue method [47] with an autoanalyzer (Lachat, Zellweger Analytics, Milwaukee, WI, USA).

Fish were collected through backpack electrofishing (LR-24, Smith Root, Vancouver, WA, USA) and placed in a holding chamber in the river for ~ 15 minutes to acclimate. To begin a trial, one fish was placed in a ziplock bag or translucent plastic box containing a known volume (400–5000 mL, depending on fish size) of fresh stream water that was pre-filtered (200 μm) to remove particles. Plastic bags or boxes were placed at the river margin to maintain temperature and minimize fish stress [37].

After a 60 minute incubation, we collected a water sample using a 60 mL syringe, and filtered it (GF/F, 0.70 μm pore size, Whatman, Maidstone, Kent, UK) into a new high density polyethylene bottle, and frozen until analysis. Supplemental samples from the stream were collected on each day to correct for background nutrient concentrations.

Each fish was measured (standard length) following the incubation, and most were released unharmed. We estimated its wet mass using a species-specific length-mass regression relationship from previous work, and converted to estimated dry mass using a dry:wet ratio of 0.23 derived as an average of observations from this fauna. A subset of 3–5 individuals per species were sacrificed to measure body chemistry (%C, %N, %P).

Excretion trials were conducted during three seasons (summer, fall and winter) between 2016 and 2018, and included both daytime (9am to 4pm) and nighttime work (8pm to 12am). Water temperature was measured once daily with a thermometer on the day of most excretion trials. For the days in which temperature measurements were not available, we estimated it using simple linear regression of air and water temperatures (see details in Supplementary Information). Temperatures varied widely (9.9–25.7 $^{\circ}\text{C}$; Table S1 in Supplementary Information), and our estimation approach for missing temperature data captured the seasonal patterns well.

Statistical analyses

To compare the predictive power on nutrient excretion rates between ES and MTE frameworks, we tested models considering all variables from both frameworks (Model 1), various combinations of variables from both frameworks (Models 4, 5, 6 and 7) and variables from just one framework (2, 3 and 8) (Table 6). We also compared whether classifying species as “armored”, which sidesteps the need to quantify body stoichiometry, captures as much explanatory power as using Body N:P (Models 1b, 2b, 4b and 6b).

Our fixed predictor variables were body size ($\log_{10}[\text{g dry mass}]$), temperature ($^{\circ}\text{C}$), body N:P (molar), armor classification (presence of well-developed scutes or not) and dietary group (as a proxy for dietary nutrient content). We used linear mixed-effects models (LMEM) with species as a random factor. A question of interest is whether changes in excretion rate in relation to the fixed predictor variables are related to the species identity. Consequently, we ran the LMEM where both the intercept and slope varied among species factor [48].

To select the most parsimonious statistical model to explain nutrient excretion, we compared AICc (Akaike Criterion for small sample sizes) values. Models with the lowest AICc value were considered best. All models that differed by < 2 were considered equivalent (i.e., $\Delta\text{AICc} < 2$), and we preferred the one with the fewest variables to minimize data requirements for broader application of the fitted model. The LMEM were generated using the package lme4 [48] and lmerTest [49] in statistical program R version 4.1.1 [50].

Ethical Approval

Statement

All methods were performed in accordance with the relevant guidelines and regulations. All fish in this research were collected following Brazilian laws under the permits numbers 39170 and 64907-1, authorized by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio/MMA).

Declarations

Acknowledgements

We would like to thank the staff of Reserva Ecológica de Guapiaçu (REGUA) for their logistic support during our field expeditions. We would also like to thank Bruna Silva, Jefferson Ribeiro Amaral, Bruno Gorini, Fernanda Motta and Juliana Argento for assisting with field work. The research was supported by Prociência-UERJ, Fundação Carlos Chagas Filho de Amparo a Pesquisa do Estado do Rio de Janeiro (FAPERJ) (E-26/203.213./2017), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (308261/2017-8) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (001) to EZ. POC received a PhD scholarship (88882.182443/2018-01) and a sandwich PhD scholarship (88881.188575/2018-01) from CAPES. BMF received a master (88882.182426/2018-01) and PhD scholarship (88887.339168/2019-00) from CAPES. VNL received post-doctoral scholarship from FAPERJ (E-26/202.493/2019) and Teaching Support Scholarship from UERJ (PAPD/2019).

Author contributions

Conceptualization: POC, EZ, PBM; Methodology: POC, EZ, BMF; Formal analysis and investigation: VNL, EZ, POC; Writing - original draft preparation: POC, EZ; Writing - review and editing: PBM, AC, VNL, BMF; Funding acquisition: EZ; Resources: EZ, PM; Supervision: EZ, PBM, AC. All authors read and approved the final manuscript.

Data Availability Statement

The data that support the findings of this study will be made available as Supplementary Information files that will be freely accessible on nature.com upon publication.

Additional Information

The author(s) declare no competing interests.

References

1. Atkinson, C. L., Capps, K. A., Rugenski, A. T. & Vanni, M. J. Consumer-driven nutrient dynamics in freshwater ecosystems: from individuals to ecosystems. *Biol. Rev.*, **92**: 2003–2023 (2016).
2. Vanni, M. J. Nutrient cycling by animals in freshwater ecosystems. *Annu. Rev. Ecol. Syst.*, **33**:341–370 (2002).
3. Vanni, M.J., Boros, G. & McIntyre, P.B. When are fish sources vs. sinks of nutrients in lake ecosystems? *Ecol.*, **94(10)**:2195–206 (2013).
4. Sterner, R. W. & Elser, J. J. *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton University Press, Princeton, New Jersey, USA (2002).
5. Lovell, T. *Nutrition and feeding of fish*. Vol. 260. New York: Van Nostrand Reinhold (1989).
6. Hood, J. M., Vanni, M. J. & Flecker, A. S. Nutrient recycling by two phosphorus-rich grazing catfish: the potential for phosphorus-limitation of fish growth. *Oecologia* **146**, 247–257 (2005).
7. Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M. & West, G. B. Toward a metabolic theory of ecology. *Ecol.*, **85**: 1771–1789 (2004).
8. Schramski, J. R., Dell, A. I., Grady, J. M., Sibly, R. M. & Brown, J. H. Metabolic theory predicts whole-ecosystem properties. *Proc. Nat. Acad. Sci. USA*, **112(8)**:2617–2622 (2015).
9. West, G. B., Brown, J. H. & Enquist, B. J. A general model for the origin of allometric scaling laws in biology. *Science*, **276**:122–126 (1997).
10. Alves, J. M., Caliman, A., Guariento, R. D., Figueiredo-Barros, M. P., Carneiro, L. S., Farjalla, V. F., Bozelli, R. L. & Esteves, F. A. Stoichiometry of benthic invertebrate nutrient recycling: interspecific variation and the role of body mass. *Aquatic Ecology*, **44**:421–430 (2010).
11. Hall, R. O. J., Koch, B. J., Marshall, M. C., Taylor, B. W. & Tronstad, L. M. *How body size mediates the role of animals in nutrient cycling in aquatic ecosystems* (Cambridge University Press, New York), 286–305 (Hildrew, A.G., Edmonds-Brown, R., & Raffaelli, D., 2007).
12. Allgeier, J.E., Wenger, S.J., Rosemond, A.D., Schindler, D.E. & Layman, C.A. Metabolic theory and taxonomic identity predict nutrient recycling in a diverse food web. *Proc. Nat. Acad. Sci. USA*, **112**: 2640–2647 (2015).
13. Vanni, M. J. & McIntyre, P. B. Predicting nutrient excretion of aquatic animals with metabolic ecology and ecological stoichiometry: a global synthesis. *Ecol.*, **97**: 3460–3471 (2016).
14. Burel, C., Person-Le Ruyet, J., Gaumet, F., Le Roux, A., Sévère, A. & Boeuf, G. Effects of temperature on growth and metabolism in juvenile turbot. *J. Fish Biol.*, **49**: 678–692 (1996).
15. Allen, A. P. & Gillooly, J. F. Towards an integration of ecological stoichiometry and the metabolic theory of ecology to better understand nutrient cycling. *Ecol Lett*, **12(5)**, 369–384 (2009).
16. McIntyre, P. B., Jones, L.E., Flecker, A. S., & Vanni, M. J. Fish extinctions alter nutrient recycling in tropical freshwaters. *Proc. Nat. Acad. Sci. USA*, **104**:4461–4466 (2007).
17. Barneche, D.R. & Allen, A.P. Embracing general theory and taxon-level idiosyncrasies to explain nutrient recycling. *Proc. Nat. Acad. Sci. USA*, **112**: 6248–6249 (2015).
18. Glaholt Jr, S. P. & Vanni, M. J. Ecological responses to simulated benthic-derived nutrient subsidies mediated by omnivorous fish. *Freshw. Biol.*, **50**, 1864–1881 (2005).

19. McIntyre, P.B. & Flecker, A.S. Ecological Stoichiometry as an integrative framework in stream fish ecology. *Amer. Fish. Soc. Symp.*, **73**:539-558 (2010).
20. Pough, F. H., Janis, C. M. & Heiser, J. B. *Vertebrate Life*. Prentice-Hall, Upper Saddle River, NJ (2005).
21. Griffiths, D. The direct contribution of fish to lake phosphorus cycles. *Ecol. Freshw. Fish* **15**:86–95 (2006).
22. McIntyre, P. B., Flecker, A. S., Vanni, M. J., Hood, J. M., Taylor, B. W. & Thomas, S. A. Fish distributions and nutrient cycling in streams: can fish create biogeochemical hotspots? *Ecol.*, **89**(8), 2008, pp. 2335–2346 (2008).
23. Cross, W. F., Benstead, J. P., Rosemond, A. D. & Wallace, J. B. Consumer-resource stoichiometry in detritus-based streams. *Ecol. Lett.*, **6**:721–732 (2003).
24. Schindler, D. E. & Eby, L. A. Stoichiometry of fishes and their prey: implications for nutrient recycling. *Ecol.*, **78**(6), pp. 1816–1831 (1997).
25. Vanni, M. J., Flecker, A. S., Hood, J. M. & Headworth, J. L. Stoichiometry of nutrient recycling by vertebrates in a tropical stream: linking species identity and ecosystem processes. *Ecol. Lett.*, **5**, 285–293 (2002).
26. Fritschie, K. J. & Olden, J. D. Disentangling the influences of mean body size and size structure on ecosystem functioning: an example of nutrient recycling by a non-native crayfish. *Ecol. Evol.*, **6**: 159–169 (2016).
27. Dodds, P.S., Rothman, D.H. & Weitz J.S. Re-examination of the “3/4-law” of metabolism. *J. Theor. Biol.* **209**:9–27 (2001).
28. White, C.R. & Seymour, R.S. Mammalian basal metabolic rate is proportional to body mass^{2/3}. *Proc. Natl Acad. Sci. USA*, **100**:4046–4049 (2003).
29. Capellini, I., Venditti C. & Barton R.A. Phylogeny and metabolic scaling in mammals. *Ecol.*, **91**:2783–2793 (2010).
30. DeLong, J.P., Okie, J.G., Moses, M.E., Sibly, R.M. & Brown, J.H. Shifts in metabolic scaling, production, and efficiency across major evolutionary transitions of life. *Proc. Natl. Acad. Sci.*, **107**, 12941– 12945 (2010).
31. Tátrai, I. Influence of temperature, rate of feeding and body weight on nitrogen metabolism of bream, *Abramis brama* L. *Comp. Biochem. Physiol.*, **83A**, 543–547 (1986).
32. Tsui, T. K. N., Randall, D. J., Chew, S. F., Jin, Y., Wilson, J. M. & Ip, Y. K. Accumulation of ammonia in the body and NH₃ volatilization from alkaline regions of the body surface during ammonia loading and exposure to air in the weather loach *Misgurnus anguillicaudatus*. *J. Exp. Biol.*, **205**, 651–659 (2002).
33. Zakés, Z., Szczepkowski, M., Demska-Zakés, K. & Jesiotowski, M. Oxygen consumption and ammonia excretion by juvenile pike, *Esox lucius* L. *Arch. Pol. Fish.*, **15**, 79–92 (2007).
34. Liu, F., Yang, S. & Chen, H. Effect of temperature, stocking density and fish size on the ammonia excretion in palmetto bass (*Morone saxatilis* x *M. chrysops*). *Aquac. Res.*, **40**, 450–455 (2009).
35. Currie, S., Bagatto, B., DeMille, M., Learner, A., LeBlanc, D., Marka, C., Ong, K., Parker, J., Templeman, N., Tuftd, B. L. & Wright, P. A. Metabolism, nitrogen excretion, and heat shock proteins in the central mudminnow (*Umbra limi*), a facultative air-breathing fish living in a variable environment. *Can. J. Zoo*, **88**, 43–58 (2010).

36. Dockray, J. J., Reid, S. D. & Wood, C. M. Effects of elevated summer temperatures and reduced pH on metabolism and growth of juvenile rainbow trout (*Oncorhynchus mykiss*) on unlimited ration. *Can. J. Fish. Aquat. Sci.* **53**, 2752–2763 (1996).
37. Oliveira-Cunha, P., Capps, K. A., Neres-Lima, V., Lourenço-Amorim, C., Tromboni, F., Moulton, T. P. & Zandonà, E. Effects of incubation conditions on nutrient mineralisation rates in fish and shrimp. *Freshw. Biol.*, **63**(9), 1107–1117 (2018).
38. Pilati, A. & Vanni, M. J. Ontogeny, diet shifts, and nutrient stoichiometry in fish. *Oikos*, **116**: 1663–1674 (2007).
39. Moody, E. K., Corman, J. R., Elser, J. J. & Sabo, J. L. Diet composition affects the rate and N:P ratio of fish excretion. *Fresh. Biol.*, **60**: 456–465 (2015).
40. Helder, C. Subsídios para gestão dos recursos hídricos das bacias hidrográficas dos rios Macacu, São João, Macaé e Macabu. Rio de Janeiro: Secretaria do Meio Ambiente (1999).
41. Mazzoni, R., Moraes, M., Rezende, C. F. & Miranda, J. C. Alimentação e padrões ecomorfológicos das espécies de peixes de riacho do alto rio Tocantins, Goiás, Brasil. *Iheringia. Série Zoologia*, **100**, 2 (2010).
42. Menezes, N. A., Weitzman, S. H., Weitzman, M. J., Oyakawa, O. T., Lima, F. C. T. & Castro, R. M. C. Peixes de água doce da Mata Atlântica. Museu de Zoologia, Universidade de São Paulo, 1ª edição. ISBN: 9788587735034 (2007).
43. Oyakawa, O. T., Akama, A., Mautari, K. C. & Nolasco, J. Peixes de Riachos da Mata Atlântica. Editora Neotropica, 1ª edição. Brasil. ISBN: 859904902x (2006).
44. Fogaça, F. N. O., Aranha, J. M. R. & Esper, M. D. L. P. Ictiofauna do rio do Quebra (Antonina, PR, Brasil): ocupação espacial e hábito alimentar. *Interciencia*, **28**(3), 168–173 (2003).
45. Holmes, R. M., Aminot, A., Kerouel, R., Hooker, B. A. & Peterson, B. J. A simple and precise method for measuring ammonium in marine and freshwater ecosystems. *Can. J. Fish. Aquat. Sci.*, **56**(10): 1801–1808, 10.1139/f99-128 (1999).
46. Taylor, B. W., Keep, C. F., Hall Jr., R. O. Koch, B. J., Tronstad, L. M., Flecker, A. S. & Ulseth, A. J. Improving the fluorometric ammonium method: matrix effects, background fluorescence, and standard additions. *J. North Am. Benthol. Soc.*, **26**:167–177 (2007).
47. Gotherman, H. L., Clymo, R. S. & Ohnstad, M. A. M. Methods for physical and chemical analysis of freshwater. Blackwell, Oxford (1978).
48. Bates, D., Mächler, M., Bolker, B. & Walker, S. Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.*, **67**(1), 1–48 (2015).
49. Kuznetsova, A., Brockhoff, P. B. & Christensen, R. H. B. “lmerTest Package: Tests in Linear Mixed Effects Models.” *J. Stat. Softw.*, **82**(13), 1–26 (2017).
50. R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

Figures

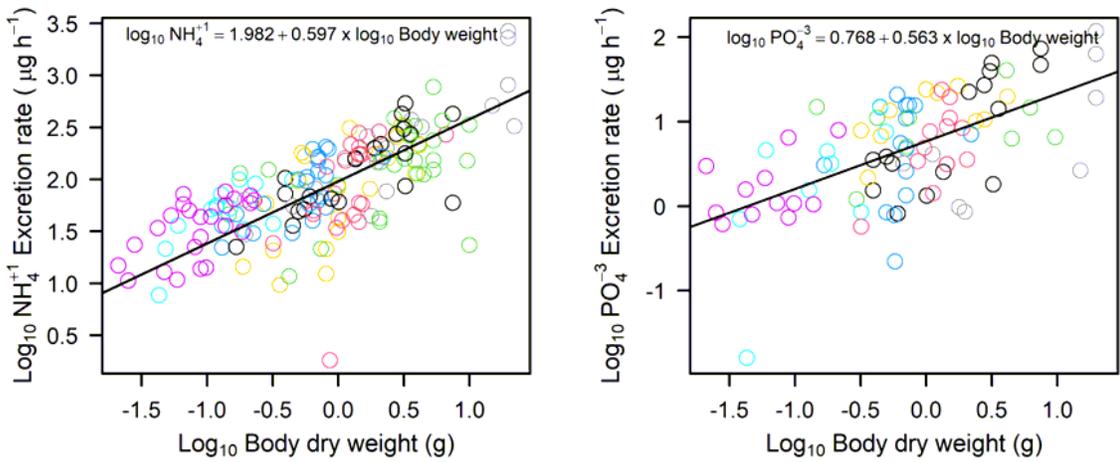


Figure 1

Fitted allometric relationships between fish body mass and excretion rates for N (a) and P (b) across 12 species in a Brazilian stream. Colors represent different species. Inset equations display the best-fitting parameter values.

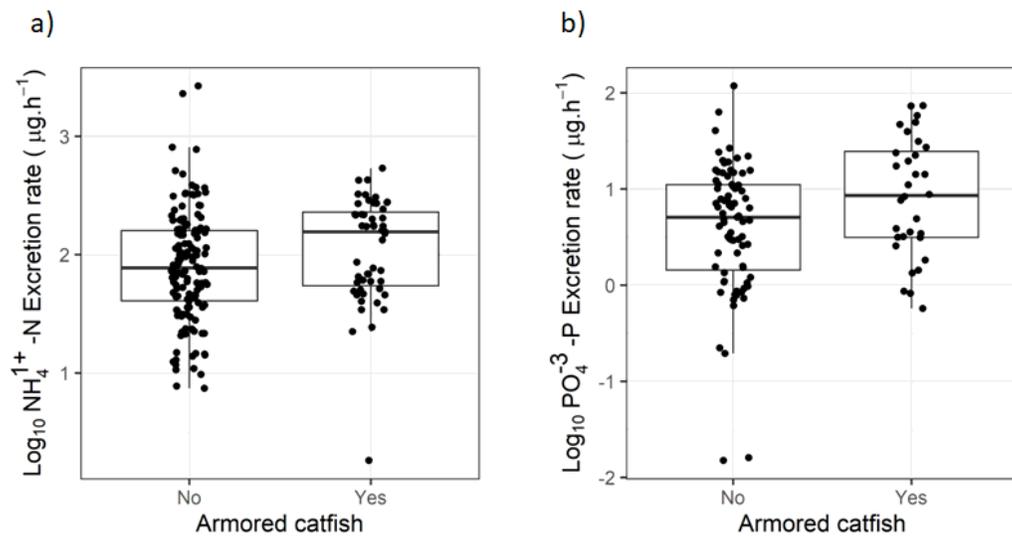


Figure 2

Body nutrient content of (a) P and (b) N:P ratio of armored versus non-armored fish species in a Brazilian stream.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryInformation.doc](#)