



Article

# Trophic Trait Evolution Explains Variation in Nutrient Excretion Stoichiometry among Panamanian Armored Catfishes (Loricariidae)

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**Abstract:** Variation in nutrient excretion rates and stoichiometric ratios (e.g., nitrogen to phosphorus) by consumers can have substantial effects on aquatic ecosystem function. While phylogenetic signals within an assemblage often explain variation in nutrient recycling rates and stoichiometry, the phylogenetically conserved traits that underlie this phenomenon remain unclear. In particular, variation in nutrient excretion stoichiometry across a phylogeny might be driven by phylogenetic patterns in either diet or body stoichiometry. We examined the relative importance of these traits in explaining variation in nutrient recycling rates and stoichiometry in a diverse family of Neotropical-armored catfishes, Loricariidae, in Panamanian streams. We found significant variation in nutrient mineralization traits among species and subfamilies, but variation in nutrient excretion stoichiometry among species was best explained by trophic position rather than body stoichiometry. The variation in trophic position among Panamanian species was consistent with variation in the trophic niche of their genera across South America, suggesting that phylogenetic patterns underpin the evolution of trophic and nutrient excretion traits among these species. Such geographical variation in nutrient mineralization patterns among closely related species may be common, given that trophic variation in fish lineages occurs widely. These results suggest that information on trophic trait evolution within lineages will advance our understanding of the functional contribution of animals to biogeochemical cycling.

**Keywords:** nutrient recycling; Neotropical stream; nitrogen; phosphorus; suckermouth catfish

## 1. Introduction

Consumer-driven nutrient recycling and storage can substantially alter ecosystem function in aquatic ecosystems [1–3]. As a result, there has been considerable interest in understanding what drives patterns in the rates and ratios of key nutrients such as nitrogen (N) and phosphorus (P) recycled by consumers. The N:P ratio at which consumers recycle nutrients is thought to vary with body N:P, diet N:P, and the maximum accumulation efficiency of N or P [4]. Variance in any or all of these three parameters could explain interspecific differences in nutrient recycling, yet most studies have focused on consumer body stoichiometry (e.g., [5–8]). Indeed, the N:P ratio of consumer body tissues and consumer body mass have been identified as predictors of the rate and stoichiometric ratio of nutrient

recycling in large-scale studies of entire consumer assemblages [6,9]. On the other hand, variation in nutrient mineralization stoichiometry within species is driven largely by variation in diet and consumption rate [10–12]. The primary factors driving variation in consumer-driven nutrient recycling therefore vary with the scale of biological organization being studied, yet the relative importance of factors such as consumer body stoichiometry, diet, and assimilation efficiency at intermediate scales such as among species within a family remains unclear.

In community-scale studies, taxonomic family often predicts consumer-driven nutrient recycling due to family-scale patterns in body stoichiometry and/or dietary habits (e.g., [5,6,13]), which supports the hypotheses that body stoichiometry and trophic niche are phylogenetically conserved traits among families (e.g., [14,15]). However, phylogenetic conservatism of ecological traits can vary substantially among traits and lineages [16,17]. To predict patterns in consumer-driven nutrient dynamics among distantly-related species, it is important to understand the degree to which ecological traits are conserved within families. Thus, a weaker phylogenetic signal for body stoichiometry than for trophic niche in a particular lineage would suggest that variation in body stoichiometry should play a larger role in driving variation in nutrient mineralization stoichiometry among species of that lineage.

To examine whether variation in body stoichiometry and trophic niche can predict excretion ratios among species within a diverse family, we studied these traits in an assemblage of armored catfishes (Loricariidae). Loricariids are a primarily herbivorous group of fishes which reach the northern limit of their native range in Central America [18,19]. Many loricariids are covered with extensive bony plating, placing them among the most P-rich lineages of vertebrates [6,20–22]. These qualities have made loricariids a focus for studies of ecological stoichiometry (e.g., [21,23–25]). However, cryptic dietary [18] and stoichiometric [6] diversity among lineages suggests that this group of fishes is also ideal for testing hypotheses of phylogenetic conservatism in stoichiometric and trophic traits. Variation among genera within assemblages of loricariids may thus illuminate relationships between ecological and evolutionary stoichiometric variation.

To examine interspecific relationships between body and mineralization stoichiometry in loricariids, we focused on species in eastern Panama, where several loricariid genera have colonized the lower portion of Central America [19,26]. Panamanian loricariids represent a small fraction (~22% of genera and ~2% of species) of the whole family diversity (26). Nevertheless, this assemblage has been the subject of considerable study, so their diets and distributions are well-known relative to loricariids in other regions [20,27–29]. Further, the primarily herbivorous subfamily Hypostominae and the more trophically diverse subfamily Loricariinae are both represented by several species in Panama. Here, we test the drivers of intrafamilial variation in the rates and stoichiometric ratio of nutrient mineralization with data on the body and excretion stoichiometry of six species of Panamanian loricariids.

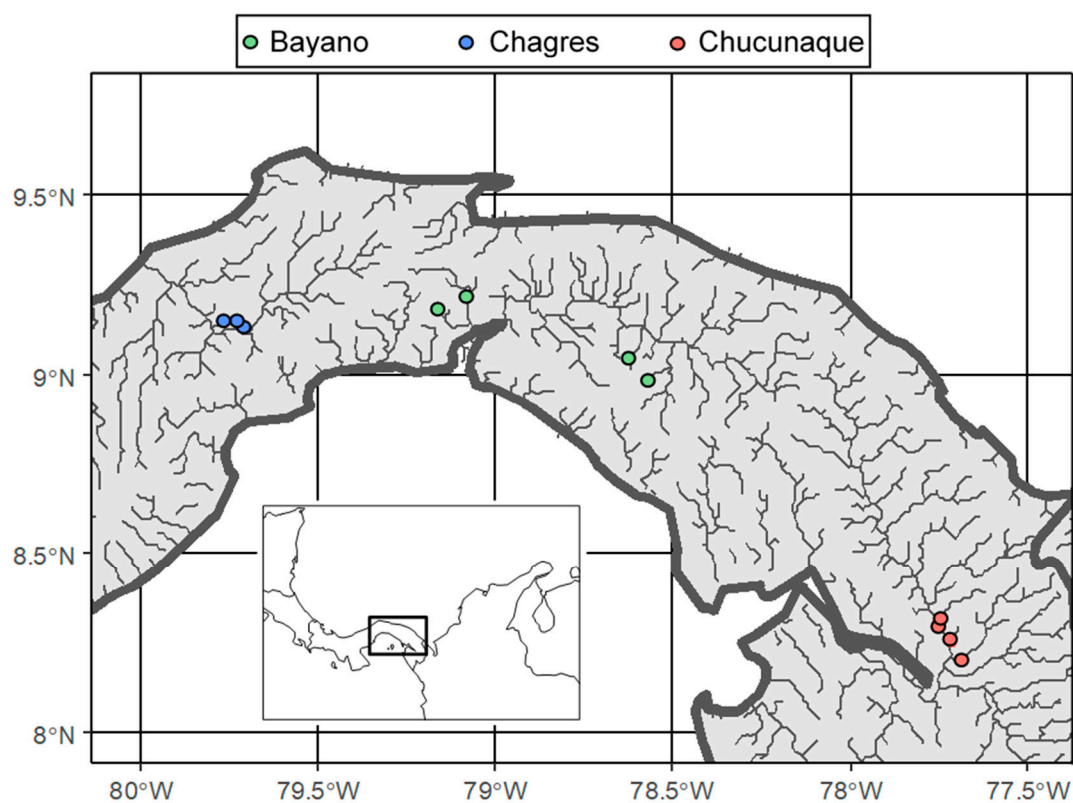
## 2. Methods

We collected fishes from 11 streams in three main river basins in eastern Panama: the Bayano, Chagres, and Chucunaque during the dry seasons of 2013 and 2014 (Table 1; Figure 1). Not all species were present or measured in each watershed (Table 2). At each sampling site, we collected water samples for chemical analyses before collecting fishes to characterize geographic variation in nutrient availability to the algae and invertebrates upon which loricariids feed. Most fishes were collected by cast net, but we also collected small numbers by electrofishing and dip nets. To test whether the collection method influenced our results, we compared excretion ratios of *Chaetostoma fischeri* collected from two rivers within the Bayano watershed, one in which we used a cast net and one in which we electrofished. We found no significant difference between rivers ( $t$ -test,  $t = 0.215$ ,  $df = 9$ ,  $p = 0.835$ ), suggesting that the collection method did not bias our results. Collected fish were held in aerated buckets of stream water until excretion measurements began. In all cases, excretion measurements were conducted within 2 hours of collection, as excretion rates of loricariids decrease over time after feeding [24]. All collections took place during daylight hours. Although some loricariids feed primarily

nocturnally [24], prior observations of several target species in one of our study rivers supports our own observations that these species feed diurnally as well (Power 1984). Further, we were most interested in standardizing methods among species and it was not feasible to collect fish at night at all study sites.

**Table 1.** Dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphate (DIP) concentrations of the sample locations across eastern Panama. Samples were collected during the dry seasons (February–April) of 2013 and 2014.

Site	Watershed	DIN ( $\mu\text{g/L}$ )	DIP ( $\mu\text{g/L}$ )	N:P (molar)
Quebrada Juan Grande	Chagres	154.6	22.8	15.0
Río Frijoles	Chagres	262.7	20.8	28.0
Río Frijolito	Chagres	319.2	46.8	15.1
Río Chechebre	Bayano	118.3	21.5	12.2
Río Curtí	Bayano	118.9	54.7	4.8
Río Mamóní	Bayano	98.3	13.1	16.6
Río Piriati	Bayano	135.1	35.8	8.4
Quebrada La Hoya	Chucunaque	139.2	8.1	38.1
Río Chucunaque	Chucunaque	144.4	14.4	22.2
Río Icuanaí	Chucunaque	172.8	29.0	13.2
Río Tupisa	Chucunaque	173.5	25.6	15.0



**Figure 1.** Map of study sites in eastern Panama. Sites are color-coded by the major basin in which they occur. Inset map shows the location of the study sites relative to Panama and surrounding countries.

**Table 2.** Number of individuals of each species collected for stoichiometric and isotopic trait sampling in each major watershed. Cells marked “X” indicate that the species was collected within that watershed but not measured in this study while cells marked with “N/A” indicate that the species was not found by us and is not known to occur in that watershed. We also measured nutrient excretion rates of three individuals of *Hemiancistrus aspidolepis* but omit them from our analyses due to a lack of body stoichiometry data for that species.

Species	Bayano	Chagres	Chucunaque
<i>Ancistrus chagresi</i>	N/A	2	N/A
<i>Chaetostoma fischeri</i>	16	X	1
<i>Lasiancistrus caucanus</i>	4	N/A	X
<i>Rineloricaria uracantha</i>	X	12	N/A
<i>Sturisoma dariense</i>	X	N/A	10
<i>Sturisomatichthys citurensis</i>	X	N/A	5

Excretion rates were measured following the protocol described by Small et al. [30]. Briefly, fish were placed into Whirl-Pak bags (Nasco, Fort Atkinson, WI, USA) filled with 1 L of filtered stream water (0.45 µm pore size filter), incubated in-stream for approximately 1 h, and then samples of the bag water were collected and compared with control bags that did not contain fish. Samples were placed on ice immediately and frozen as soon as possible. For the Chucunaque watershed, samples were placed on ice but were not frozen until 2 days after collection due to lack of electricity at the collection site. All other samples were frozen on the same day of collection. Due to this potential difference in method, we measured N excreted as both ammonium (NH<sub>4</sub>), the form in which the majority of N is excreted by fishes, and nitrate/nitrite, the forms into which it may have been converted while kept on ice. As these samples were filtered and filled without headspace, we expected any loss of inorganic N to other forms to be minimal. After collecting water samples from the bag water, we then euthanized a subset of the fish using clove oil, measured their standard length, dissected their guts, and preserved their bodies in salt for up to 3 days (following [31]). Fish were then oven-dried at 50 °C for several days to constant mass, then coarsely homogenized using a coffee grinder followed by pulverization in a ball mill. The remainder of the collected fish were preserved in formalin as vouchers and deposited into the STRI Neotropical Fish Collection and the Museo de Biología Marina of the Universidad de Panamá.

We used a Lachat QuikChem Flow Injection Analysis System (Hach Ltd., Loveland, CO, USA) to measure dissolved inorganic phosphate (PO<sub>4</sub>), ammonium (NH<sub>4</sub>), and the combined concentration of nitrate (NO<sub>3</sub>) and nitrite (NO<sub>2</sub>) in excretion and water samples. Dissolved inorganic N (DIN) was then calculated as the sum of ammonium and nitrate/nitrite. This is less than the total dissolved N, which includes some dissolved organic forms of N, but encompasses the inorganic N available to primary producers. Homogenized whole fish body samples were analyzed for body C:N and stable isotope ratios on a Thermo Delta V Advantage isotope ratio mass spectrometer (Thermo Scientific, Waltham, MA, USA), and for total P by ignition (550 °C) followed by digestion in 1 M HCl, with P detection by automated molybdate colorimetry on a Lachat Quikchem 8500 (Hach Ltd., Loveland, CO, USA). We did not extract lipids or perform lipid corrections from stable isotope samples because measured fish C:N was near or below the threshold for suggested lipid correction in all species [32]. Thus, we assumed that lipid contents were sufficiently low and similar among species that a correction was unnecessary. Sample sizes of fishes collected for excretion, body stoichiometry, and stable isotope analyses are presented in Table 2.

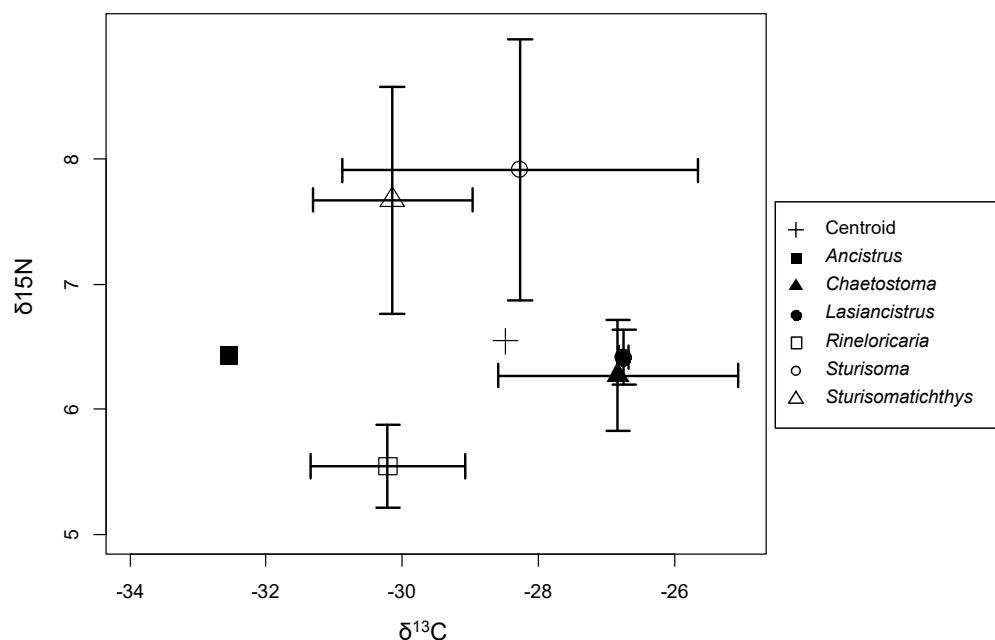
We calculated the centroid for stable isotope data across all sites following Lujan et al. [18] to visually compare variation among individual species. We then conducted single ANOVAs with Type I sum of squares for each response variable to test whether trophic traits, body stoichiometric traits, and excretion stoichiometric traits differed with body mass and among species and subfamilies as well as watersheds. These fixed effects were included in the models in the order listed based on our predictions of which factors would be most important. As we did not have all trait data from the same individual

fish, we then used correlation tests to test whether mean excretion N:P significantly differed with predictor traits among species. We assessed normality and heteroscedasticity of residuals using Q-Q plots and residuals vs. fitted value plots, respectively, and log-transformed data when assumptions were not met. All statistical analyses were performed in the R computing environment v.3.5.1 [33].

### 3. Results

Dissolved inorganic nitrogen (DIN) ranged from 98.3 to 319.2  $\mu\text{g N/L}$  while dissolved inorganic phosphate (DIP) ranged from 13.1 to 54.7  $\mu\text{g P/L}$  (Table 1). Water chemistry was similar among basins, but streams in the Bayano basin, which were downstream of larger human communities, had lower N:P than streams in the other two basins (Table 1). Stream temperatures were consistently between 26 and 30  $^{\circ}\text{C}$ , conductivity was fairly low ( $<320 \mu\text{S/cm}$ ), and pH was slightly alkaline (7.5 to 8.0). As the stoichiometric traits we measured in fishes did not vary among watersheds, watershed differences were not considered in further analyses.

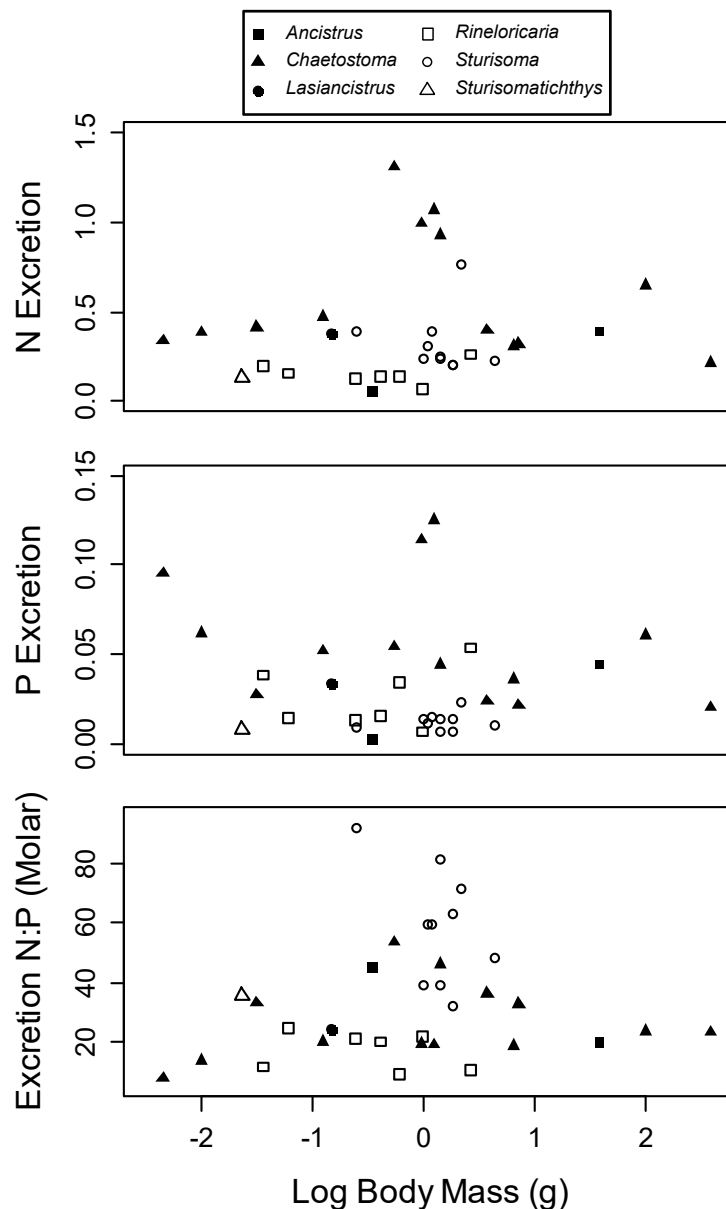
There was considerable variation in tissue-stable isotope ratios around the centroid value, with hypostomines varying more in  $\delta^{13}\text{C}$  and loricariines varying more in  $\delta^{15}\text{N}$  (Figure 2). We also found significant variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  among both species and subfamilies. We subsequently tested for variation in body stoichiometry among species and subfamilies. We found significant variation in body %C ( $F_{1,39} = 4.86, p = 0.003$ ) and %N ( $F_{1,39} = 4.13, p = 0.007$ ) among species. Further, body %C also varied among subfamilies ( $F_{1,39} = 6.09, p = 0.018$ ), while body %N did not ( $F_{1,39} = 1.84, p = 0.182$ ). In addition, body %C increased with body mass ( $F_{1,39} = 5.62, p = 0.023$ ), but neither %N nor %P varied with mass ( $p > 0.05$ ). In fact, body %P was relatively high but statistically invariant with the predictors we considered. As a result, body N:P also varied significantly among species ( $F_{2,38} = 8.60, p < 0.001$ ), but not with any other predictors. The variation among species was primarily driven by the relatively unique stoichiometry of *Sturisoma dariense*, which was lower in N:P than the other loricariine species and the hypostomine *Chaetostoma fischeri*.



**Figure 2.** Stable isotope centroids for all data (cross symbol) and individual loricariid taxa averaged across all sampling sites. Dark symbols are genera in Hypostominae, and white symbols are genera in Loricariinae. Error bars represent standard deviation, and sample sizes are listed in Table 2.

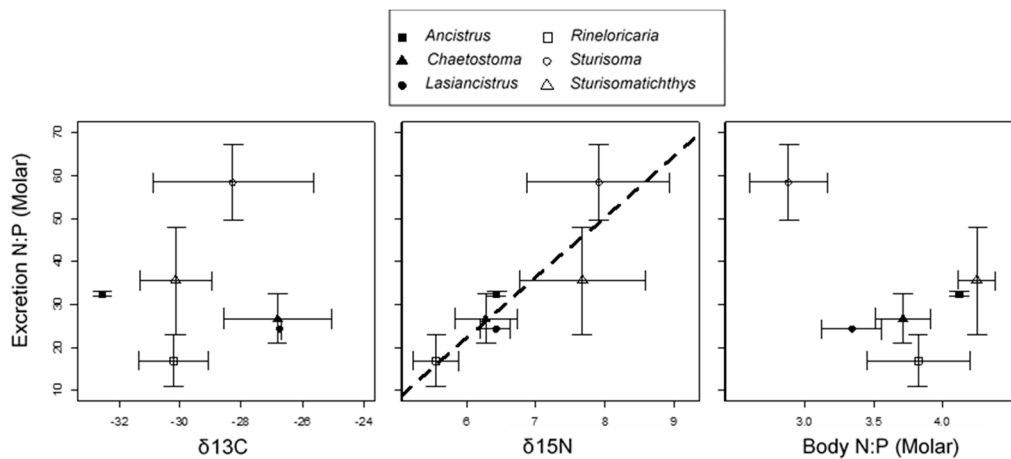
Contrary to patterns in body stoichiometry, subfamily was a significant predictor of both N ( $F_{1,27} = 13.69, p < 0.001$ ) and P ( $F_{1,27} = 18.09, p < 0.001$ ) excretion rates. Specifically, hypostomine

species excreted both N and P at higher rates than loriciariine species of a given mass (Figure 3). Notably, species differences within subfamilies were also important in explaining variation in both N ( $F_{4,27} = 4.15, p = 0.009$ ) and P ( $F_{4,27} = 2.83, p = 0.044$ ) excretion rates. As with individual elemental excretion rates, the stoichiometric ratio of excretion (N:P) also varied with both subfamily ( $F_{1,27} = 6.51, p = 0.017$ ) and species ( $F_{4,27} = 8.25, p < 0.001$ ). Loriciariine excretion had greater N:P than hypostomine excretion, which was most extremely exemplified by the loriciariine *S. dariense* (open circles in Figures 3 and 4).



**Figure 3.** Excretion rates ( $\text{mg L}^{-1} \text{hr}^{-1}$ ) and N:P ratio as a function of body mass for the two subfamilies of Loricariidae measured in this study. Dark symbols are genera in Hypostominae, and white symbols are genera in Loriciariinae. All points represent individual fish.





**Figure 4.** Relationships between excretion N:P and  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and body N:P among species of Panamanian loriciariids. Excretion N:P was positively correlated with  $\delta^{15}\text{N}$  ( $r = 0.884$ ,  $p = 0.019$ ), but not  $\delta^{13}\text{C}$  or body N:P. Dark symbols are genera in Hypostominae, and white symbols are genera in Loricariinae. Error bars represent standard deviation.

Finally, we examined species-specific relationships between excretion N:P and trophic niche or body stoichiometry. Not surprisingly, mean excretion N:P was not correlated with mean  $\delta^{13}\text{C}$  ( $r = 0.022$ ,  $p = 0.967$ ); yet, mean excretion N:P did vary with mean  $\delta^{15}\text{N}$ . Counterintuitively, this correlation was positive ( $r = 0.884$ ,  $p = 0.019$ ; Figure 4), indicating that species feeding on higher trophic levels excreted nutrients at a higher N:P ratio. Also, somewhat surprising was the lack of a significant relationship between body N:P and excretion N:P among species ( $r = -0.509$ ,  $p = 0.302$ ; Figure 4), although this could be due to the small number of species considered as the pattern of this relationship was in the expected direction.

#### 4. Discussion

Identifying the drivers of species-specific variation in consumer stoichiometry and nutrient dynamics is important to elucidate how evolutionary processes affect ecosystem processes. In this study, we found that trophic traits better explained variation in nutrient mineralization stoichiometry than body stoichiometry among species of Panamanian armored catfishes. Prior work has demonstrated that intraspecific, microevolutionary variation in these traits within species can affect nutrient recycling and ecosystem function (e.g., [8,12,34]). Our results provide additional insight, suggesting that at the macroevolutionary scale, trophic traits may also be important drivers of variation.

From a stoichiometric perspective, loriciariid catfishes have historically stood out from most other vertebrate groups for their high body P content and low P excretion rates [6,21,22]. While loriciariids are typically considered grazers of periphyton and algal detritus, considerable trophic diversity exists within the family [18,35]. We studied six species of loriciariids abundant in rivers of Eastern and Central Panama: *Rineloricaria uracantha*, *Sturisoma dariense*, and *Sturisomatichthys citurensis* in the subfamily loricariinae; and *Ancistrus chagresi*, *Chaetostoma fischeri*, and *Lasiancistrus caucanus* in the subfamily hypostominae. Among the loricariines, *Rineloricaria* is phylogenetically distant from the sister genera *Sturisoma* and *Sturisomatichthys* [19,36]. In fact, Covain et al. [36] proposed that all trans-Andean species of *Sturisoma* (including *S. dariense*) are actually paraphyletic within *Sturisomatichthys* and these two species may thus be congeneric. Among the hypostomines, *Ancistrus* and *Lasiancistrus* are sister genera, with *Chaetostoma* occupying a separate clade [19]. In spite of the close relationships among some of the genera we studied, they are all diverse genera distributed primarily throughout the Andes, and their relatives thus diverged prior to colonizing the isthmus of Panama.

Although we only sampled small assemblages of loriciariids near the northern extent of their native range, our data were remarkably consistent with phylogenetic patterns in diet observed

for the Loricariinae from 19 assemblages in the Amazon, Orinoco, and Essequibo basins in South America [18]. As in Panama, *Rineloricaria* feeds at a lower trophic position than *Sturisoma* across these assemblages [18]. We also found that within Hypostominae, *Ancistrus*, *Chaetostoma*, and *Lasiancistrus* fed at a similar trophic position but varied in  $\delta^{13}\text{C}$ , as reported for these genera from the same set of South American assemblages [18]. Further, our stoichiometric results are fairly consistent with those previously published for an Andean piedmont assemblage of loricariids in Venezuela [6]. While that assemblage lacked *Sturisoma* or *Sturisomatichthys*, they similarly found that the most P-rich species was sister to those genera, i.e., *Farlowella*. In addition, *Chaetostoma* species in both our study and in Vanni et al. [6] were richer in P than their corresponding *Ancistrus* species. Additional sampling of other loricariid assemblages will improve our understanding of phylogenetic patterns in their traits, but the consistency of our results with previous research suggests that trophic position and stoichiometric traits are relatively conserved phylogenetically within Loricariidae.

In spite of the consistency of our results with prior studies, however, the fact that *Sturisoma* and *Sturisomatichthys* exhibited the highest  $\delta^{15}\text{N}$  values of all species contrasted with the phylogenetic patterns observed by Lujan et al. [18]. This discrepancy could be explained by differences in baseline  $\delta^{15}\text{N}$  among sites. While we have no reason to believe that basal resources in the Chucunaque watershed are more  $\delta^{15}\text{N}$ -enriched than in the other basins, such a difference could explain our results [36]. Alternatively, this apparent difference from Lujan et al. [18] could also be resolved by recent phylogenetic work in this group. While Lujan et al. [18] only examined cis-Andean species of *Sturisoma*, Covain et al. [36] hypothesized that trans-Andean *Sturisoma* (including *S. dariense*) are paraphyletic with respect to *Sturisomatichthys*. Therefore, it is possible that species within the trans-Andean *Sturisoma/Sturisomatichthys* have evolved a more insectivorous diet, or otherwise feed on more protein-rich material than cis-Andean *Sturisoma sensu stricto*. Historical research on loricariid diets used evidence from variation in gut morphology to suggest most Panamanian loricariine species were more omnivorous than hypostomines and the loricariine *Rineloricaria* [27]. Collectively, this work suggests that variation in the trophic traits of loricariids is well supported, and coupled with our findings, suggests that trophic traits may be an important driver of interspecific variation in the rates and ratios of nutrient mineralization.

That trophic traits best predicted variation in excretion stoichiometry was not particularly surprising, but the nature of these relationships was unexpected. Specifically, the positive correlation between  $\delta^{15}\text{N}$  and N:P of excretion among species was surprising, given that consumers feeding at higher trophic levels typically consume more P-rich animal prey. According to the tenets of ecological stoichiometry, organisms feeding on a more P-rich diet should then excrete P at higher rates if all else is equal [4], which in turn leads to a predicted negative relationship between  $\delta^{15}\text{N}$  and excretion N:P. This surprising relationship could be explained by the variability in traits within species and low sample sizes of some species measured. If the average values included in this analysis were biased by small sample sizes or large outliers, we could expect to see a weak or counterintuitive pattern such as the one we observed. Alternatively, our results would be possible if the maximum accumulation efficiency for P increases with trophic position. Such a scenario could occur if fishes feeding at a higher trophic position consume less food, as reduced consumption rate leads to increased P retention efficiency [12]. Indeed, feeding rate typically decreases when consumers feed on high quality resources (e.g., [37,38]). High feeding rates in algivorous loricariids, relative to those of their insectivorous sister family Astroblepidae, have been proposed as a mechanism explaining how loricariids persist on a diet that is typically depauperate in P despite their high P demand [25]. Further work on this problem is needed to assess the generality of these constraints and whether they explain dietary diversification in and among this diverse family.

The results from this work expand our understanding of how consumer-driven nutrient dynamics vary within assemblages of Panamanian loricariids. However, there are three caveats in our data that are worthy of discussion. First, the use of stable isotope data to approximate trophic traits is well-supported in loricariids [18], but any analysis of trophic traits based on stable isotopes without



isotopic baseline data must be interpreted with caution [39]. In our case, the alignment of our trophic trait data with isotopic niche data from other species within these genera [18] and with gut morphology of these same species [27] supports our conclusions. However, it is likely that variation in isotopic signatures among watersheds explains some of the variation in trophic traits we ascribe to these species. Second, the small sample sizes limit the interpretation of these data. While we aimed to measure excretion rates of at least 10 individuals of each species, we were only able to do so for three of these species due to time and resource constraints (Table 2). The patterns we observed remain unchanged when the excretion data for the species for which we collected fewer individuals was excluded; however, more data to fully characterize interspecific variation in excretion rates would greatly enhance this dataset. Finally, we did not incorporate variation between sexes or among life stages into our analysis. While little is known of the reproductive biology of many of these species, at least one of our study species, *Rineloricaria uracantha*, is known to breed year-round [40]. Thus, reproductive adults may invest nutrients differently into eggs or sperm relative to each other or non-reproducing juveniles. Recent work in poeciliid fishes suggests that body and excretion stoichiometry do not vary systematically between sexes [41,42], but whether the same is true of loriciariids is not known.

Together, our results lend mixed support to the hypothesis that phylogenetic trait conservatism explains taxonomic patterns in nutrient excretion rates and ratios. Although excretion rates of N and P, as well as excretion stoichiometry, varied between subfamilies, body stoichiometry did not. Instead, conservatism of trophic traits among genera was the best predictor of excretion rates and stoichiometry. These results underscore the need to examine the degree of phylogenetic conservatism in the traits of interest when considering how the functional role of consumers in ecosystem-scale processes such as nutrient cycling varies among lineages. Shifts in trophic traits are common within many families of fishes (e.g., [18,43,44]), but the ecosystem-scale impacts of these macroevolutionary shifts are poorly studied. Our work suggests that explicitly considering the degree and causes of reduced phylogenetic conservatism of trophic traits among functionally important consumer assemblages will yield insights into how evolutionary processes shape geographic patterns in ecosystem functioning across the landscape.

**Author Contributions:** Conceptualization, E.K.M., F.A., K.A.C., O.P. and B.L.T.; Data curation, B.L.T.; Formal analysis, E.K.M. and B.L.T.; Investigation, E.K.M. and F.A.; Methodology, E.K.M., F.A. and B.L.T.; Resources, O.P.; Supervision, K.A.C.; Writing—original draft, E.K.M.; Writing—review & editing, E.K.M., F.A., K.A.C., O.P. and B.L.T.

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**Conflicts of Interest:** The authors declare no conflict of interest

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