

James M. Hood · Michael J. Vanni
Alexander S. Flecker

Nutrient recycling by two phosphorus-rich grazing catfish: the potential for phosphorus-limitation of fish growth

Received: 25 August 2004 / Accepted: 30 June 2005 / Published online: 17 August 2005
© Springer-Verlag 2005

Abstract In ecosystems where excretion by fish is a major flux of nutrients, the nitrogen (N) to phosphorus (P) ratio released by fish can be important in shaping patterns of algal biomass, community composition, primary production, and nutrient limitation. Demand for N and P as well as energy influences N/P excretion ratios and has broad implications in ecosystems where nutrient recycling by fishes is substantial. Bioenergetics and stoichiometric models predict that natural fish populations are generally energy-limited and therefore N/P recycling by fishes is relatively invariant. Yet, the potential for P limitation of growth has not been examined in herbivorous fishes, which are common in many aquatic habitats. We examined N/P excretion ratios and P demand in two P-rich herbivorous catfishes of the family Loricariidae, *Ancistrus triradiatus* (hereafter *Ancistrus*) and *Chaetostoma milesi* (hereafter *Chaetostoma*). Both fishes are common grazers in the Andean piedmont region of Venezuela where we conducted this study. Mass balance (MB) models indicate that these fishes have a high P demand. In fact, our *Ancistrus* P MB model predicted negative P excretion rates, indicating that *Ancistrus* did not consume enough P to meet its P demand for growth. Direct measurement of excretion rates showed positive, but very low P excretion rates and high N/P excretion ratios for both taxa. To obtain measured P excretion rates of *Ancistrus* from the MB model, gross growth efficiency must be reduced by 90%. Our results suggest that growth rates of both of these

herbivorous and P-rich fish are likely P-limited. If P limitation of growth is common among herbivorous fish populations, herbivorous fishes recycle likely at high N/P ratios and act to diminish the quality of their food.

Keywords Consumer driven nutrient cycling · Ecological stoichiometry · Fish · Streams · Loricariidae

Introduction

Fish can play important roles in nutrient cycles (Vanni 2002); yet, we do not fully understand how their influence varies with species identity or even trophic guild. The importance of nutrient recycling by fish in ecosystems is dependent upon the magnitude of nutrient release (especially in relation to nutrient demand), the nitrogen to phosphorus ratio (hereafter N/P ratio) at which nutrients are released, and whether nutrients are released in soluble or particulate forms. The state in which nutrients are released is important because nutrients released in soluble form, through excretion, are immediately available for algal uptake; whereas, nutrients released in particulate forms, through egestion, require further processing before being available to algae. By excreting a portion of ingested nutrients, fish can resupply or translocate a significant proportion of algal nutrient demand in both streams (Grimm 1988; Hood 2000) and lakes (Vanni 2002). The N/P ratio released by consumers can determine which nutrient limits algal growth, algal community composition, and food quality for grazers (Tilman 1982; Elser et al. 1988; Elser and Urabe 1999). Thus, to understand aquatic nutrient cycles and food web dynamics it is important to determine the factors that control N/P release ratios.

Stoichiometric theory considers N and P use and cycling by fish as a chemical equation where nutrient use and release must balance (Sterner and Elser 2002). Once ingested, nutrients can move into one of the three pools: nutrients can be incorporated into tissue, excreted, or egested as fecal material. Theory assumes that animals

Communicated by David Post

J. M. Hood · M. J. Vanni
Department of Zoology, Miami University,
Oxford, OH 45056, USA

J. M. Hood (✉)
Department of Ecology, Evolution, and Behavior,
University of Minnesota, St. Paul, MN 55108, USA
E-mail: hoodx008@umn.edu

A. S. Flecker
Department of Ecology and Evolutionary Biology,
Cornell University, Ithaca, NY 14853, USA

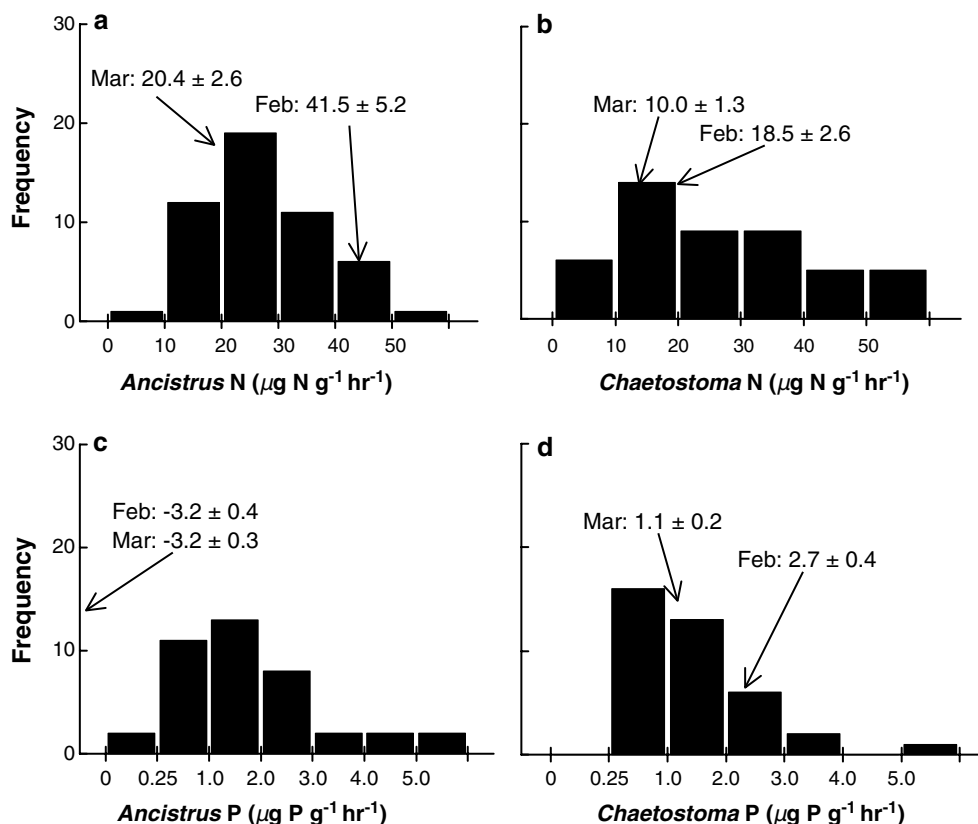
act to maintain their body C/N/P stoichiometry in the face of variation in food stoichiometry; that is, animals maintain strict nutrient homeostasis (Sterner 1990). Under this assumption, models of nutrient release predict a non-linear relationship between N/P release and food N/P (Sterner 1990; Schindler and Eby 1997). When the consumer's N/P is less than its resource, the consumer preferentially sequesters P and releases nutrients at a high N/P ratio. The N/P ratio released is fundamentally determined by the fish's nutrient demand which is, in part, determined by the consumer's body N/P ratio. Sterner's (1990) model predicts that when a group of consumers utilize the same resource with a fixed N/P, the N/P released by the consumers will be negatively related to the consumer's N/P. Interspecific patterns of N/P excretion by fishes follow stoichiometric predictions (Vanni et al. 2002). However, we do not know if N/P egestion follows a similar pattern.

Patterns of C, N, and P egestion by fishes have received little attention, yet can be important since fishes contribute to particulate nutrient cycles and benthic food webs through egestion. In addition, patterns of elemental egestion reflect the role that assimilation efficiency (AE) plays in managing an organism's elemental budget. Sterner and George (2000) presented several conceptual models linking food stoichiometry, an element's AE, and the stoichiometry of fecal material. These models fall into two categories: passive and active. The passive models predict that AE remains constant

across all food nutrient concentrations or ratios (see Fig. 1a, Sterner and George 2000). In contrast, active models predict that AE is adjusted according to food elemental concentrations, in one of two ways. The AE might be adjusted to obtain elemental homeostasis. Here, AE for an element increases as the concentration of that element declines (see Fig. 1b, Sterner and George 2000). Alternatively, AE might magnify elemental imbalances if AE increases with food nutrient concentrations. Sterner and George (2000) suggest that AE does not play a role in regulating C, N, or P homeostasis.

An animal's nutrient demand affects not only egestion and excretion N/P ratios but also animal performance and food web dynamics (Schindler and Eby 1997). Therefore, it is important to understand the conditions under which nutrient limitation of fish occurs. Theory argues that while nutrient limitation of fish growth is possible, it is uncommon in natural populations. Models suggest that fish are much more likely to be energy-limited than nutrient-limited (Schindler and Eby 1997). Thus, it follows that fish excrete at moderate N/P ratios and that variation in food stoichiometry has few direct effects on fish growth (Schindler and Eby 1997). However, Schindler and Eby (1997) examined mostly planktivores and piscivores, two functional groups that consume a stoichiometrically balanced diet, that is, one in which food nutrient ratios are similar to those of the consumer. Schindler and Eby (1997) do

Fig. 1 Distributions of N excretion rates for **a** *Ancistrus* and **b** *Chaetostoma* and P excretion rates for **c** *Ancistrus* and **d** *Chaetostoma*. These excretion rates were directly measured in 1998 (Vanni et al. 2002), 1999 (this study), and 2001 (Hood et al., unpublished). The MB model estimates (± 1 SD) for February and March 1999 are shown with arrows in each figure



suggest that functional groups, which consume a stoichiometrically imbalanced diet would have a higher P demand. In particular, herbivorous fishes often consume food with very low P concentrations. The C/P ratio of algae is commonly much higher and more variable than even high C/P fishes like cyprinids (Sterner and George 2000). Thus, herbivorous fishes excrete likely at high but variable N/P ratios and may be more sensitive to P limitation of growth. Herbivorous fishes are common worldwide in streams and lakes (Gerking 1994; Matthews 1998).

Since animals rich in P will have a greater P demand, sensitivity to P limitation of growth is also likely to vary with phylogeny. Some common Neotropical fish families have a relatively high body P concentration (Vanni et al. 2002), and therefore higher P demand (Sterner and Elser 2002), than temperate fishes. Loricariids, a common family in many Neotropical aquatic habitats (Power 1990; Winemiller 1990; Flecker 1992), are covered with armor-like bony plates, thus accounting for the high-concentrations of P in their bodies (Sterner and Elser 2002). Therefore, to understand nutrient excretion by fish in an ecosystem context, it is necessary to examine N/P excretion ratios of fishes with a higher P demand than planktivores and piscivores. P-rich, herbivorous fish such as loricariids present an appropriate model organism for examining these relationships.

Here, we evaluate the generality that fishes are commonly energy-limited (i.e., not nutrient-limited) and excrete at moderate N/P ratios. We evaluate these hypotheses with two herbivorous armored catfish (Loricariidae), *Ancistrus triradiatus* and *Chaetostoma milesi*. These fishes likely have a high P demand both because they are herbivores and because they are P rich (Vanni et al. 2002). Using data collected from a stream in which *Ancistrus* and *Chaetostoma* are common, we determined the stoichiometric balance between these catfishes and their food as well as the N/P excretion ratio of these two herbivores. Furthermore, we evaluate whether these catfishes maintain nutrient homeostasis through AE. To our knowledge this is the first time the potential for P limitation of growth has been examined for herbivorous fishes. Our results demonstrate that the growth of at least one of these P-rich herbivorous catfish is likely P-limited at our study site during the dry season.

Study site and biota

This study was conducted within a 2-km reach of Rio Las Marias, Venezuela. The study site is in the Andean piedmont region of Venezuela (~225 m above sea level, 9°10' N, 69°44' W) and experiences a pronounced wet season beginning in April and ending in December. During the dry season, Rio Las Marias is a fourth-order cobble-bottom stream, averaging ~8 m in width. During this period, the stream has high-water clarity, relatively low soluble N and P concentrations, and high-algal standing crops that are N-limited (Flecker et al. 2002).

Fish, including *Ancistrus* and *Chaetostoma*, are the primary algal grazers in Rio Las Marias (Flecker 1992; Flecker et al. 2002). Armored catfish are abundant in Rio Las Marias, as is typical for many Neotropical streams. In addition to potentially affecting nutrient cycling, these and other epibenthic fishes strongly affect algal community structure and the degree of algal nutrient limitation via direct consumption of periphyton and sediment (Flecker 1992; Flecker 1996, 1997; Flecker et al. 2002; Flecker and Taylor 2004). We assessed nutrient limitation and nutrient excretion by quantifying mass balance (MB) and by directly measuring excretion rates.

Methods

Mass balance model

Mass balance models describe N or P excretion (E_i) as:

$$E_i = I_i - F_i - G_i,$$

where i is either N or P, I_i is the ingestion of i per unit time, F_i is the egestion (via feces) of i per unit time, and G_i represents the quantity of i allocated to growth per unit time (Nakashima and Leggett 1980a). Mass balance models were parameterized during the dry season for *Ancistrus* and *Chaetostoma* in February and March of 1999, as detailed below. Error was propagated through the MB model with the delta method (Agresti 1990), which uses a Taylor series analysis. To simplify the calculation of the MB model's variance, we assumed that there was no error associated with gut evacuation rate and that parameter covariance was minimal.

Ingestion

Daily ingestion was estimated as the sum of food ingested in six consecutive 4-h intervals over a 24-h period (i.e. from 0800 to 0800) following Elliott and Persson (1978). For both fish species, we estimated daily ingestion during two 24-h periods (16 February and 19 March 1999). Since neither *Ancistrus* nor *Chaetostoma* has a true stomach, we defined the first 15 cm of the fish's gut as the foregut. To determine the ingestion rate, we measured the mass of food in the foregut of ten *Ancistrus* and ten *Chaetostoma* collected from Rio Las Marias during each 4-h interval. Prior to analysis, ingestion fish were preserved in 10% formalin. In the lab, all food particles were squeezed out of the gut, placed on a pre-combusted (475°C for 4 h), preweighed glass fiber filter (Gelman AE, 25 mm diameter), which was then dried (60°C) and reweighed.

To determine fore- and hindgut nutrient contents, a second set of ten fishes were collected at midnight on both dates. These fishes were frozen and dissected as described earlier. C and N concentrations of foregut material were measured with a Perkin Elmer Series II 2400 elemental

analyzer. The P content of foregut samples was determined by ashing followed by HCl digestion and subsequent analysis of liberated SRP following Stainton et al. (1977). Before calculating daily N and P ingestion, we corrected for the effects of formalin preservation using the nutrient ration to formalin ration ratio.

Gut evacuation rate

Measurement of ingestion rate requires information on both gut contents (above) and the rate at which food passes through the gut (gut evacuation rate). The gut evacuation rate of *Ancistrus* was measured with a serial slaughter technique (Salvatore et al. 1987). Fishes were sacrificed at predetermined times and the passage of a dyed food was tracked through the gut. Methodological details are described in Hood (2000). Gut evacuation rate was calculated as an exponential function following Persson (1981) and Heroux and Magnan (1996). One serial slaughter experiment was conducted with *Ancistrus* in March 1999; this rate was used for *Chaetostoma*.

Assimilation efficiency and egestion

Daily nutrient egestion (F_i) was calculated following Fisher and Gray (1983):

$$F_i = I_i(1 - AE_i).$$

Nutrient AE_i was calculated as the ratio of hindgut nutrient content to foregut nutrient content, where both foregut nutrient and hindgut nutrient concentrations were corrected for ash content (Conover 1966). Here, we assume that minimal nutrient assimilation occurs in the foregut of these fishes and that nutrient assimilation is complete for hindgut samples. Therefore, we use hindgut samples as a surrogate for feces. Dissection methods and sample processing were the same as described earlier in the Ingestion section. Inorganic matter samples were ashed at 550°C for 5 h. Foregut–hindgut nutrient relationships were compared with reduced major axis (RMA) analysis using the software (S)MATR (Sokal and Rohlf 1995; Falster et al. 2003); although, the 95% confidence intervals for the intercept were calculated with RMA for JAVA (Bohonak and Van Der Linde 2004). Both packages produced identical results but (S)MATR does not calculate error for regression intercepts. The RMA techniques provide a superior estimate of bivariate relationships compared to typical least-squares regression because the residual variance is minimized in both x -dimension and y -dimension (Sokal and Rohlf 1995).

N and P growth

Daily nutrient growth is the product of the daily dry mass growth rate ($\text{mg dry mass day}^{-1}$) and the fish's body N or P content (mg N or P mg^{-1} dry mass). During February and March 1999, daily growth rates of *Ancistrus* and *Chaetostoma* were measured in two sepa-

rate cage experiments. *Ancistrus* or *Chaetostoma* were reared in 1×1 m Vexar cages (6 mm diameter mesh) in Rio Las Marias at commonly occurring stream densities (*Ancistrus*: 6 m^{-2} ; *Chaetostoma*: 10 m^{-2}) for approximately 1 month. Cages were placed in the habitat where each species was most abundant. Each cage bottom was tiled with stream rocks to mimic the natural substrate and provide food. To avoid potential changes in the algal community or standing crop, we replaced cage rocks with fresh stream rocks midway through each month-long experiment.

Each fish was weighed and measured at the beginning of the experiment and after approximately 1 month. Growth rates were calculated for each size class as the difference between initial and final masses divided by the experiment's duration. *Ancistrus*' growth rate increased with body size (Hood 2000), so we used the growth rates of size classes most similar to the fish used to estimate ingestion (*Ancistrus*: February: 3.3 g [wet wt], March: 3.1 g; *Chaetostoma* (March): 2.9 g). Fish body C, N, and P contents were determined for a subset ($n=4/\text{month}$) of the fish collected for nutrient ingestion in February and March following Vanni et al. (2002).

Model simulations

We conducted additional simulations with the MB model to evaluate the sensitivity of *Ancistrus* and *Chaetostoma*'s P balance. For *Ancistrus* we manipulated the MB model to first obtain zero P excretion rates and then to obtain the excretion rates similar to those directly measured in March. Here, using the March values we manipulated several combinations of parameters on a percentile basis. First, we simultaneously varied food P (holding ingestion rate constant), growth rate (holding body P constant), and P AE. We excluded body P from this analysis because fish are thought to have a homeostatic body P content, at least over short periods of time (Sterner and George 2000), and no evidence to the contrary was observed in this study. In this analysis, we capped P AE at the maximum observed, 75%. To determine the effect of growth rate and food P content on *Ancistrus*' P balance, we manipulated each parameter in isolation. For *Chaetostoma* we manipulated both growth rate and food P in isolation to obtain zero P excretion rates.

Direct excretion measurements

Excretion was measured directly in March 1999 following Vanni et al. (2002). To describe diel differences, N and P excretion rates were measured during both daytime and night-time periods. Briefly, one or two *Ancistrus* or *Chaetostoma* were incubated intraspecifically in 3.8 L bags (Ziploc) containing 1 L of filtered stream water ($n=5$ for each species). Following the incubation period, which was always less than 1 h, SRP and NH_4 samples were taken. The protocol for treatment and analysis of water

chemistry samples and the equations required to calculate excretion rates are described in Vanni et al. (2002). Day and night rates were combined, assuming a 12-h day, to estimate daily excretion.

Biofilm C, N, and P samples

Biofilm samples were collected from Rio Las Marías for comparison with fish gut contents. Samples were collected from one riffle and one pool, near fish collection sites, in February and March of 1999. Biofilm samples were taken from rocks (12 rocks per site) with a toothbrush and a circular 4.5 cm² plastic template. From each rock, one sample was filtered (Gelman AE, 25 mm) for C and N analysis and another for P analysis. Carbon and nutrient concentrations were determined as described in the Ingestion section. For each month, we compared biofilm and foregut C, N, and P concentrations with a one-way ANOVA. Post hoc comparisons were conducted with a Scheffé test in Statistica (StatSoft Inc.).

Results

N and P excretion rates

Both species excreted nutrients at high N/P ratios (Table 1). For these catfishes, high excretion N/P ratios were achieved by excreting N at moderate rates and P at very low rates (Table 1). Except for the *Ancistrus* P

excretion model, the MB models and direct measurements produced statistically similar excretion rates (Table 1). These mass specific N and P excretion rates are similar to other directly measured excretion rates of these catfishes (Fig. 1). Furthermore, our model performed well in terms of generating low excretion rates, relative to rates observed for other taxa (Vanni et al. 2002). However, the *Ancistrus* P excretion model did not balance and produced negative P excretion rates during both months (Table 1).

Evaluation of magnitude of P imbalance

We used model simulations to determine the magnitude of *Ancistrus*' P imbalance. To simply achieve zero P excretion rates it was necessary to simultaneously change the March values by decreasing growth rate, increasing food %P, and increasing P AE by 30% each. A simultaneous 48% change in all three of these parameters was required to achieve P excretion rates similar to the direct measurements. Alternatively, if only growth rate was manipulated, a 58% decrease was required to achieve a P excretion rate of zero. Here, when only growth rate was manipulated, gross growth efficiency for C (GGE_C, i.e. growth rate divided by ingestion rate) declined from the observed value of 9.6 to 4.1% before *Ancistrus*' P budget balanced; GGE_C had to be reduced to 1.0% to produce the observed P excretion rate. Finally, when only food P content was varied, a 130% increase (3.2 SD) in food P content was necessary to achieve positive excretion rates.

Table 1 Parameters (X ± 1 SD) calculated for *Ancistrus* and *Chaetostoma* mass balance models for February and March 1999

Parameter	Units	Ancistrus		Chaetostoma	
		February	March	February	March
Daily ingestion	mg g ⁻¹ day ⁻¹	44.5 (15.3)	31.5 (8.6)	43.0 (7.7)	22.3 (4.6)
Gut evacuation rate	h ⁻¹	0.65 (0.015) ^a			
C AE	%	47.3 (19) ^b		49.8 (18) ^b	
N AE	%	47.9 (18) ^b		49.6 (21) ^b	
P AE	%	55.3 (20) ^b		48.7 (12) ^b	
C Egestion (model)	mg C g ⁻¹ day ⁻¹	4.6 (3.7)	4.2 (3.0)	4.4 (5.4)	2.1 (1.3)
N Egestion (model)	mg N g ⁻¹ day ⁻¹	1.4 (1.5)	0.8 (0.8)	0.5 (1.1)	0.3 (0.6)
P Egestion (model)	mg P g ⁻¹ day ⁻¹	0.07 (0.58)	0.05 (0.60)	0.09 (0.62)	0.05 (0.50)
Growth rate	mg (dry) g ⁻¹ day ⁻¹	3.8 (0.07)	3.0 (0.05)	^c	0.5 (0.01)
Body C	% Dry mass	42.4 (1.5)	40.9 (2.1)	42.4 (1.5)	40.9 (2.1)
Body N	% Dry mass	8.1 (0.3)	8.4 (0.4)	8.1 (0.4)	7.8 (0.2)
Body P	% Dry mass	4.4 (0.3)	4.5 (0.5)	4.6 (0.4)	4.4 (0.3)
N Excretion (direct)	μg N g ⁻¹ h ⁻¹		21.1 (25.9) ^d		17.8 (22.0) ^d
N Excretion (model)	μg P g ⁻¹ h ⁻¹	41.5 (33.3)	20.4 (16.8)	18.5 (17.0)	10.0 (8.6)
P Excretion (direct)	μg N g ⁻¹ h ⁻¹		1.8 (1.0) ^d		0.9 (0.2) ^d
P Excretion (model)	μg P g ⁻¹ h ⁻¹	-3.2 (2.2)	-3.2 (1.6)	2.7 (2.3)	1.1 (1.0)
N/P Excretion	Molar		57.3 (56.0) ^d		63.9 (41.1) ^d

All fish masses are wet masses

^a Only one gut evacuation was conducted. *Ancistrus*' March gut evacuation rate was used in all models

^b *Ancistrus* and *Chaetostoma* C, N, and P assimilation efficiencies were an average of each species' assimilation efficiencies in both February and March

^c Only one growth experiment was conducted for *Chaetostoma*. *Chaetostoma*'s March growth rate was used in the February *Chaetostoma* model

^d Excretion experiments were only conducted in March

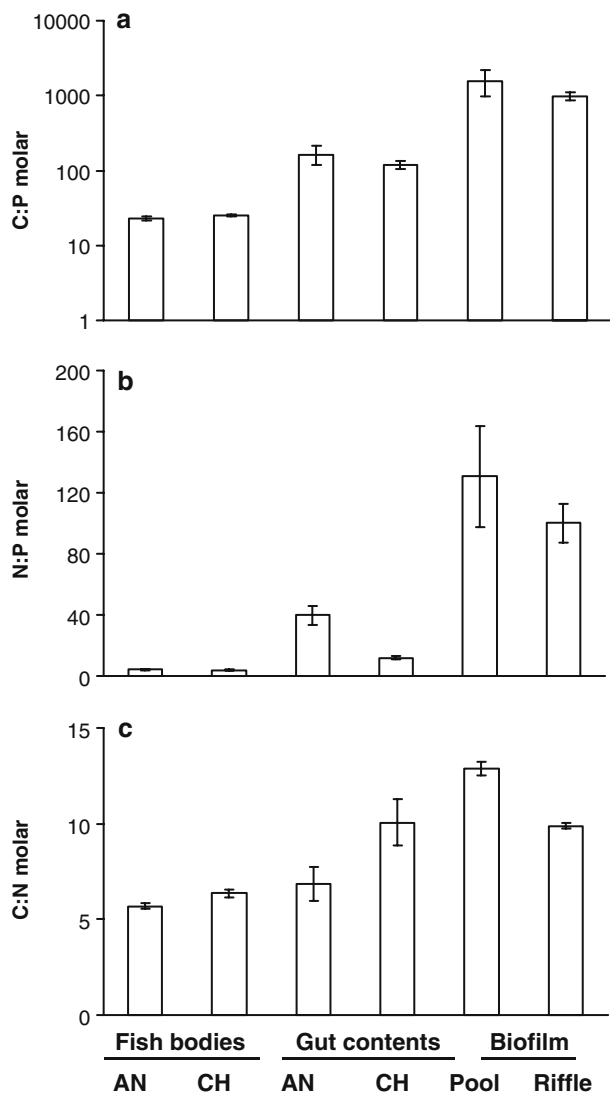


Fig. 2 Stoichiometric relationships of **a** C/P molar, **b** N/P molar, and **c** C/N molar (mean \pm SE) between *Ancistrus* (AN) and *Chaetostoma* (CH) bodies and foregut contents and riffle versus pool biofilm. The plot of **a** C/P ratios uses a log scale while the plot of **b** N/P and **c** C/N ratios uses an arithmetic scale

We also evaluated *Chaetostoma*'s sensitivity to P limitation of growth. In March, *Ancistrus*' GGE_C was more than four times higher than *Chaetostoma*'s (9.6% vs. 2.2%, respectively). Therefore, we manipulated *Chaetostoma*'s growth rate, and thus its GGE_C, until its P excretion rate was zero. *Chaetostoma*'s P excretion rate reached zero when its GGE_C was increased a little more than 100%, from 2.2 to 4.8% (33.8 SD of growth rate).

Comparison of fish, fish gut contents, and biofilm

Both *Ancistrus* and *Chaetostoma* consumed food that was stoichiometrically out of balance with their body tissues in terms of N/P and especially C/P (Fig. 2). While both catfishes had relatively high P material in their

Table 2 Elemental concentrations (% dry mass, \pm 1 SD) of *Ancistrus* and *Chaetostoma*'s gut contents and pool and riffle biofilm in February and March 1999

	February	March
Carbon		
<i>Ancistrus</i> gut contents	17.9 (6.6)	21.6 (7.4)
<i>Chaetostoma</i> gut contents	20.5 (11.8)	18.0 (2.7)
Riffle Biofilm	28.7 (10.6)	37.5 (9.4)
Pool Biofilm	23.7 (7.3)	38.2 (9.4)
Nitrogen		
<i>Ancistrus</i> gut contents	5.3 (2.2)	4.0 (1.5)
<i>Chaetostoma</i> gut contents	2.3 (1.6)	2.3 (0.9)
Riffle Biofilm	3.4 (2.0)	4.5 (1.2)
Pool Biofilm	2.1 (0.7)	4.4 (2.1)
Phosphorus		
<i>Ancistrus</i> gut contents	0.36 (0.13)	0.26 (0.12)
<i>Chaetostoma</i> gut contents	0.43 (0.22)	0.44 (0.13)
Riffle Biofilm	0.10 (0.03)	0.11 (0.03)
Pool Biofilm	0.08 (0.03)	0.09 (0.05)

foregut compared to the biofilm, these fishes also had extremely high body P concentrations (Table 1). In comparison, differences among fish C/N, the C/N ratio of foregut contents, and biofilm C/N were relatively slight (Fig. 2).

Ancistrus and *Chaetostoma*'s food was higher in P content than stream biofilm (Table 2, February: $F_{3,33} = 19.9$, $P < 0.0001$; March: $F_{3,35} = 31.5$, $P < 0.001$). In February, fish gut contents and stream biofilm had similar C concentrations, while in March gut contents were lower in C than stream biofilm (Table 2; February: $F_{3,30} = 2.2$, $P = 0.084$; March: $F_{3,33} = 15.1$, $P = 0.002$). The relationship between fish gut content N and stream biofilm N varied between months. In February, fish gut contents differed in N concentrations and only *Ancistrus*' foregut contents differed from pool biofilm (Table 2; $F_{3,31} = 5.4$, $P = 0.004$, AN vs. CH: $P = 0.018$, AN vs. pool: $P = 0.012$). In March, fish gut contents were similar but only *Chaetostoma*'s foregut contents differed from riffle biofilm (Table 2; $F_{3,36} = 3.4$, $P = 0.027$, CH vs. riffle: $P = 0.045$).

Patterns of C, N, and P egestion

Following Sterner and George (2000), we evaluated patterns of C, N, and P egestion by examining the relationship between foregut and hindgut elemental ratios. Here, an elemental ratio can either be the quantity of one element relative to another (i.e. C/P) or relative to the dry mass of material (i.e. %P). Sterner and George (2000) discussed three model relationships between hindgut and foregut elemental ratios. (1) The passive model would have any slope and an intercept at the origin. (2) The first active model would have a negative slope and a non-zero intercept. This is the only model, which allows for homeostatic regulation through AE. (3) The second active model would have a positive slope and a non-zero intercept. This relationship would magnify elemental imbalances between the fish and its resource.

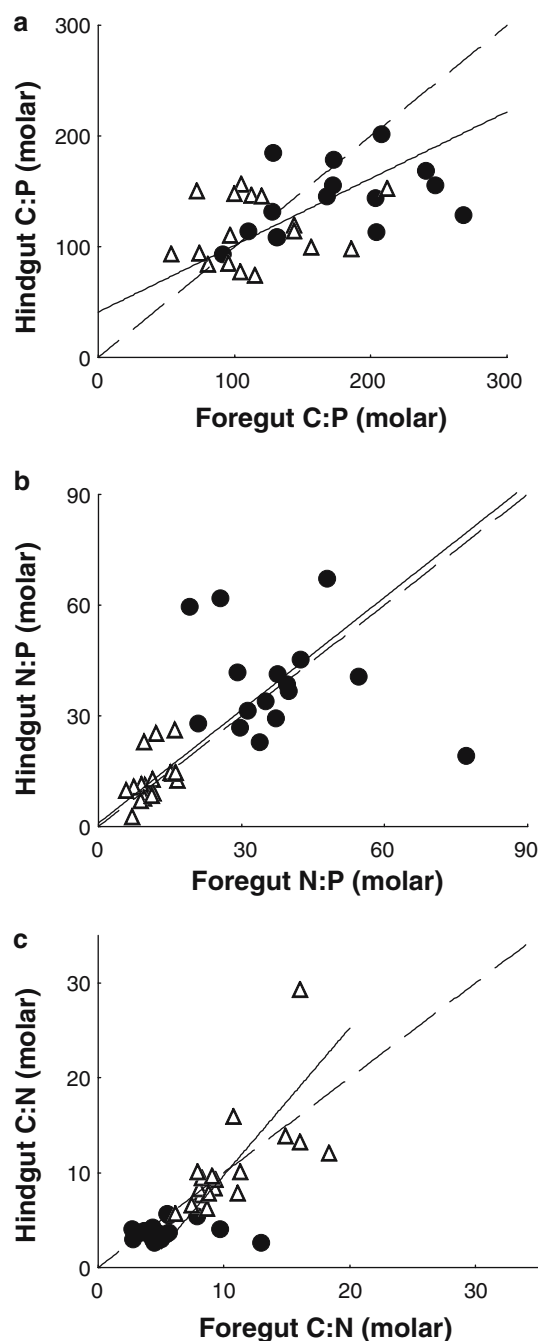


Fig. 3 Assessment of homeostatic regulation through comparison of stoichiometry of foregut and hindgut material **a** C/P ratios, **b** N/P ratios, **c** C/N ratios in *Ancistrus* (filled circle) and *Chaetostoma* (Δ). Relationships predicted by reduced major axis are shown (thick line) and, for comparison, we also show a relationship with a slope of one and an intercept at the origin (dotted line). Both catfishes exhibited similar relationships for C/P and N/P but not for C/N (Table 3). There was no correlation between *Ancistrus*' hindgut and foregut C/N ratios; so, we only show *Chaetostoma*'s relationship

Patterns of nutrient assimilation varied between elements and we observed all three-model relationships discussed earlier. First, in terms of C/P assimilation, these catfishes magnified the P imbalance they encoun-

tered in their food (model 3). That is, *Ancistrus* and *Chaetostoma* preferentially assimilated C over P when diet C/P was high; in contrast, when diet C/P was low (<100), the fish absorbed P more efficiently than C (Fig. 3a, Table 3). Second, following the passive model both catfish exhibited constant assimilation efficiencies for N relative to P across variable food N/P (Fig. 2b, Table 3). Third, *Ancistrus* and *Chaetostoma* differed in their patterns of C/N egestion. *Ancistrus* assimilated C and N from food such that hindgut C/N ratios were statistically constant over the range of food C/N encountered (Table 3). In contrast, *Chaetostoma*'s C/N assimilation acted to maintain C and N homeostasis (model 2); this species preferentially egested the element that was present in excess relative to the other (Fig. 2c, Table 3). However, this relationship is largely driven by one observation (Fig. 2c).

Finally, both catfishes used AE to maintain C and P homeostasis. Hindgut and foregut C and P concentrations were positively related with a non-zero intercept (Table 3; C and P: $p < 0.001$). Since one minus the ratio of hindgut to foregut elemental concentrations is AE, a positive intercept indicates that there was a negative relationship between C and P AE and foregut C and P concentrations, respectively. We observed no relationship between foregut and hindgut N concentrations (Table 3).

Discussion

Potential for P limitation of fish growth

We tested the generality that fish growth is energy limited with two P-rich, herbivorous armored catfishes. Our results suggest that during the dry season in Rio Las Marias the growth of both *Ancistrus* and *Chaetostoma* was at or near the boundary for P limitation of growth. Several lines of evidence support this conclusion. First, theory predicts P limitation of growth for animals consuming food with C/P ratios much higher than their body C/P (Sterner and Elser 2002). During our study both catfish taxa consumed extremely stoichiometrically imbalanced diets, which were especially deficient of P (Fig. 2). For example, Urabe and Watanabe (1992) estimated that *D. galeata*, which has a C/P of approximately 95, becomes P limited when consuming algae with C/P ratios higher than 150. Thus, *D. galeata* exhibits P limitation of growth while feeding on algae with a C/P less than two times its body C/P. In contrast, the C/P of *Ancistrus* is nine times greater than foregut material; furthermore, biofilm, the putative resource of these fishes, had a C/P ratio over four times higher than foregut contents (Fig. 2). Second, P-limited consumers are predicted to release nutrients at high N/P ratios (Sterner 1990). Our results (both MB models and direct measurements) show that both catfishes excrete at very high N/P ratios as compared to other fishes, mainly because they excrete P at very low rates, presumably by

Table 3 Regression statistics for the relationship: hindgut $x = \text{foregut } x \times \beta + \alpha$; where, x is an elemental ratio

	n	β	α	α 95% CI	p -Value	R^2	$H_\beta = 1$	AN = CH
N/P	33	1.02	0.884		0.001	0.323	0.875	0.084
C/P	32	0.603	41.000		0.008	0.214	0.003	0.605
C/N								0.001
<i>Ancistrus</i>	17	0.371	1.760		0.966	0.000	< 0.001	
<i>Chaetostoma</i>	18	1.550	-5.756		0.003	0.440	0.028	
$\mu\text{g C } \mu\text{g ash}^{-1}$	30	0.282	0.031	0.015–0.046	0.025	0.167	< 0.001	0.837
$\mu\text{g N } \mu\text{g ash}^{-1}$								0.001
<i>Ancistrus</i>	15	0.417	0.009		0.212	0.117	0.002	
<i>Chaetostoma</i>	15	0.857	0.039		0.857	0.003	0.327	
$\mu\text{g P } \mu\text{g ash}^{-1}$	30	0.308	0.001	0.0009–0.0021	< 0.0001	0.520	< 0.0001	0.437

Here, we evaluate two null hypotheses: (1) the slope of the relationships was one ($H_\beta = 1$), and (2) *Ancistrus* and *Chaetostoma* had similar relationships in terms of slope (AN = CH). In the foregut–hindgut comparisons of food elemental content (e.g., $\mu\text{g C } \mu\text{g ash}^{-1}$), a non-zero intercept indicates that AE is related to food elemental content

preferentially retaining P. Mass specific excretion rates of non-loricariid species of similar size were approximately 1.8 \times higher for N and 5.5 \times higher for P than excretion rates of *Ancistrus* and *Chaetostoma* (Vanni et al. 2002). Finally, according to our estimates, *Ancistrus* did not consume enough P to account for its growth. Large changes in the most sensitive parameter values (growth, food P, and AE) were required to simply obtain positive P excretion rates. In addition, simulations demonstrated that if *Chaetostoma* had the same GGE_C as *Ancistrus*, *Chaetostoma*'s P budget would also not balance. Taken together these results strongly suggest that *Ancistrus* was P limited and that *Chaetostoma* was close to P limitation of growth.

Phosphorus limitation of fish growth may be more common than formerly realized. Previously, researchers have suggested that P limitation of fish growth may be rare in natural populations (Schindler and Eby 1997). However, herbivorous and high P fishes may commonly be on the border of P limitation of growth. Herbivorous fishes face great stoichiometric imbalances with their food. The body C/P and N/P of most fishes is lower than all but the most P-rich algae (Elser et al. 2000; Tanner et al. 2000; Vanni et al. 2002). Although herbivory is not common in temperate zone freshwater pelagic fishes (where most information on stoichiometric relationships has been generated), it is common in streams, wetlands, and most freshwater ecosystem types in the tropics (Matthews et al. 1987; Wootton and Oemke 1992; Gerking 1994). Furthermore, in the Neotropics P-rich taxa such as the Loricariidae are common and diverse (Power 1990; Winemiller 1990; Flecker 1992). Phosphorus limitation of growth may be common throughout this family. Even insectivorous loricariids likely consume a stoichiometrically imbalanced diet. The average C/P of six loricariid species was 25 (Vanni et al. 2002) while the C/P of stream insects range from 93 to 877 (Cross et al. 2003).

In aquatic ecosystems with P-limited species, fishes may play a unique role in nutrient cycles. When nutrient excretion by fishes is substantial, the N/P ratio recycled can determine the status of algal nutrient limitation

(Elser et al. 1988) and algal community composition (Vanni and Layne 1997). Fishes limited by energy excrete a moderate N/P ratio, generally slightly less than the Redfield ratio of 16:1 (Schindler and Eby 1997). In contrast, animals that consume a stoichiometrically imbalanced diet will preferentially retain the limiting element. Thus, P-limited fishes will excrete at a high N/P ratio, just as we observed for *Ancistrus* and *Chaetostoma*. By changing the soluble N/P ratio, P-limited fishes may facilitate the decline of algal species that are poor P competitors, exasperate P limitation in P-limited algae, and increase algal C/P. Thus by recycling at a high N/P ratio, P-limited herbivorous fishes could indirectly decrease the quality (C/P) of their resource. Furthermore, P-limited fishes represent a potentially large P sink.

In Rio Las Marias, for example, nutrient excretion by catfish will likely raise soluble N/P ratios. During the dry season, Rio Las Marias is an N-limited stream (Flecker et al. 2002), where *Ancistrus* and *Chaetostoma* alone recycle as much as 10% of the N required for algal production (Hood 2000). Since catfish recycle at a high N/P ratio, nutrient recycling by catfish likely acts to diminish periphyton N-limitation but it also likely increases periphyton C/P, leading to further P-limitation of catfish growth. Our results suggest that the role of herbivorous and P-rich fishes in nutrient cycles and food webs requires further examination.

Patterns of C, N, and P egestion

Since herbivorous fishes consume food with far greater stoichiometric variation than their own bodies, these fishes must maintain homeostasis through either elemental assimilation in their guts or via excretory processes. Sterner and George (2000) demonstrated that cyprinid fishes do not maintain C and N homeostasis through AE. Instead, they concluded that fishes maintain C and N homeostasis through physiological processing of assimilated nutrients (i.e., via excretion). Our results are consistent with Sterner and George's (2000) only for C/P. In contrast to Sterner and George (2000),

we demonstrate that the patterns of AE vary greatly between fishes and elements. *Ancistrus* and *Chaetostoma* exhibited three different patterns of elemental assimilation (Fig. 3). Thus, patterns of fecal material stoichiometry may differ between elements and species, and be difficult to predict. This suggests that while fish stoichiometry is likely a major factor in determining the C/N/P of released elements, the physiology of an individual species may play an even greater role. When both excretion and egestion are examined, species identity appears to be a strong determinate of N/P release.

The relationship between food C, N, and P content and AE appears to be highly variable between taxa (Nakashima and Leggett 1980b; Sterner and George 2000; Galholt and Vanni 2005). Studies have shown that the relationship between AE and food nutrient concentrations may be non-existent or may be negative as we describe here. This variation may have important implications for efforts to model nutrient use or excretion by fishes. Many bioenergetics and MB studies utilize a single nutrient AE from the literature, rather than values measured in the field for the particular population being modeled (Kraft 1992, 1993; Vanni 1996; Schindler and Eby 1997). The use of generalized nutrient assimilation efficiencies may be a source of error in model prediction both because of the apparent variation between species and the variation with food nutrient content.

Modeled versus measured excretion rates

Mass balance models and direct excretion measurements are the two most common methods used to determine nutrient excretion rates by fish (Kraft 1992; Vanni 1996; Schaus et al. 1997; Schindler and Eby 1997; Vanni et al. 2002). To our knowledge, this is the first direct comparison of mass balance models and direct measurements of excretion. Previous comparisons demonstrated only that the two methods produced excretion rates within the same range (e.g., Mather et al. 1995; Vanni 1996; Schaus et al. 1997). In contrast, this study demonstrates that these two methods produce statistically similar excretion rates for the same fish population (Table 1). However, our comparison suggests that these methods produced similar results only when nutrients do not limit fish growth. When nutrients limit fish growth direct measurements are likely to be more accurate.

Potential sources of uncertainty

The *Ancistrus* P excretion model did not balance and predicts negative P excretion. This result is not reasonable, because even starved fishes excrete some P (Mather et al. 1995). However, as noted earlier, measured P excretion rates are quite low for loricariids, compared to other fish taxa. Therefore, obtaining negative-modeled excretion rates are more likely for these fishes than other

fishes that excrete P at higher rates, given the parameter uncertainty inherent in modeling efforts. Since negative nutrient excretion rates are not possible and it is unlikely that we missed a major aspect of *Ancistrus* P budget, *Ancistrus*' negative P excretion rates must be the result of one or more inaccurate parameters. The parameters strongly affecting *Ancistrus*' P budget are body P, P AE, food P, and growth rate. All of these parameters appear reasonable. Our estimates of *Ancistrus* body P content are similar to Vanni et al.'s (2002) and reflect *Ancistrus*' bony body. Furthermore, the MB models' assumption of nutrient homeostasis was supported by our results. Model simulations demonstrated that there is not enough variation inherent in P AE to fully account for *Ancistrus*' P imbalance.

Both food P and growth rate have a large effect on the P MB model (Hood 2000) and were not measured as explicitly as the other parameters. *Ancistrus*' daily pattern of P ingestion may not have been adequately described since we only measured food P at midnight. However, our data indicate that at midnight both catfishes consume food highly enriched in P over stream biofilm. Since algal C/P is rarely lower than 100 (Elser et al. 2000), to achieve P balance, these fishes would have to consume animal material or high P detritus during the day. Both stable isotopes and direct diet measurements demonstrate that these catfishes consume periphyton (P. McIntyre and B. Daley, personal communications). Alternatively, *Ancistrus*' negative P excretion rates may be the result of an overestimate of *Ancistrus*' growth rate. It is noteworthy that *Ancistrus* exhibited far higher growth rates than *Chaetostoma*. Yet, model simulations suggest that a 58% (37 SD) decrease in growth rate is required to merely balance *Ancistrus*' P budget. Furthermore, other studies have recorded much higher growth rates for *Ancistrus* in Rio Las Marias (Solomon et al. 2004). Regardless of the actual source of this P imbalance, our results demonstrate that P uptake and conservation have an impact on *Ancistrus* growth rates and nutrient excretion in Rio Las Marias. In addition, since P excretion rates are only sensitive to growth rate when fishes reach the border of P limitation of growth (Schindler and Eby 1997; Vanni and Headworth 2004), our observation that P excretion rates are sensitive to growth is a further evidence for P limitation of growth.

Ancistrus gut evacuation rate was used in the *Chaetostoma* MB model. Differences between *Chaetostoma* and *Ancistrus* gut evacuation rates will likely have a small effect on the MB model for several reasons. First, *Ancistrus* and *Chaetostoma* have similar gut morphology and consume similar food; two factors known to affect gut evacuation rate (Persson 1992; He and Wurtsbaugh 1993). Second, gut evacuation rate often varies more with temperature than between species (Olson and Boggs 1986; Targett and Targett 1990; Horn et al. 1995). Finally, neither the N nor P mass balance model is particularly sensitive to the gut evacuation rate (Hood 2000). Taken together, this suggests that although *An-*

cistrus and *Chaetostoma* may have different gut evacuation rates, these differences are likely to be small in comparison to variation in gut evacuation rate in response to environmental conditions.

Conclusions

We conclude that in Rio Las Marias, during the dry season *Ancistrus* and *Chaetostoma*, either experience P-limited growth or are on the border of P limited growth. Our results suggest that if herbivores and families with high body P concentrations received more attention, P limitation of fish growth would appear to be more common. Both the potential for P limitation of fish and the relationship between AE and food nutrient content indicate that N and P release by fish may be far more dynamic and complicated than previously thought. If the magnitude of nutrients recycled by herbivorous fishes is high, nutrient recycling by fish will modify the N/P ratio available to algae and potentially decrease the quality of their food. In zooplankton communities similar interactions have been predicted to affect zooplankton coexistence and the stability of food web dynamics (Andersen 1997). Finally, our results suggest that consumer-resource stoichiometry, phylogeny, and functional identity may be important in determining the role fishes play in nutrient cycling in aquatic ecosystems.

Acknowledgements We thank B. Daley, S. Harper, J. Headworth, L. Knoll, M. Munson, B. O'Shea, and B. Taylor for their help in the field or lab. M. Hughes and A. Bailer helped with error propagation. We thank E. Hall, A. Kay, P. McIntyre, D. Post, R. Sterner, K. Zimmer, and one anonymous reviewer for comments on the manuscript. Our work in Venezuela would have been far more difficult and less rewarding if not for the generosity of the Figueredo family, D. Taphorn, and B. Perez. This work was supported by NSF grant DEB 9615620 to MJV and DEB 9615349 and DEB 0321471 to ASF. Funding was also provided by Miami University's Philip and Elaina Hampton Fund for Faculty International Initiatives. All experiments and sampling discussed here comply with both USA and Venezuelan regulations.

References

- Agresti A (1990) Categorical data analysis. Wiley, New York
- Andersen T (1997) Pelagic nutrient cycles: herbivores as sources and sinks. Springer-Verlag, New York
- Bohonak AJ, Van Der Linde K (2004) RMA: software for reduced major axis regression, JAVA version 1.19. In: <http://www.kimvdlinde.com/professional/rma.html>
- Conover R (1966) Assimilation of organic matter by zooplankton. *Limnol Oceanogr* 11:338–345
- Elliott J, Persson L (1978) The estimation of daily rates of food consumption for fish. *J Anim Ecol* 47:977–991
- Elser J, Urabe J (1999) The stoichiometry of consumer-driven nutrient recycling: theory, observations, and consequences. *Ecology* 80:735–751
- Elser JJ, Elser MM, MacKay NA, Carpenter SR (1988) Zooplankton-mediated transitions between N- and P-limited algal growth. *Limnol Oceanogr* 33:1–14
- Elser JJ, Fagan WF, Denno RF, Dobberfugl DR, Folarin A, Huberty A et al (2000) Nutritional constraints in terrestrial and freshwater food webs. *Nature* 408:578–580
- Falster D, Warton D, Wright I (2003) (S)MATR: standardised major axis tests and routines. Version 1.0. <http://www.bio.mq.edu.au/ecology/SMATR>
- Fisher S, Gray L (1983) Secondary production and organic matter processing by collector macroinvertebrates in a desert stream. *Ecology* 64:1217–1224
- Flecker AS (1992) Fish trophic guilds and the structure of a tropical stream: weak direct vs. strong indirect effects. *Ecology* 73:927–940
- Flecker A (1996) Ecosystem engineering by a dominant detritivore in a diverse tropical stream. *Ecology* 77:1845–1854
- Flecker A (1997) Habitat modification by tropical fishes: environmental heterogeneity and the variability of interaction strength. *J North Am Benthol Soc* 16:286–295
- Flecker AS, Taylor BW (2004) Tropical fishes as biological bulldozers: density effects on spatial heterogeneity and species diversity. *Ecology* 85:2267–2278
- Flecker AS, Taylor BW, Bernhardt ES, Hood JM, Cornwell WK, Cassatt SR, Vanni MJ (2002) Interactions between herbivorous fishes and limiting nutrients in a tropical stream ecosystem. *Ecology* 83:1831–1844
- Gallholt SP, Vanni MJ (2005) Ecosystem response to benthic-derived nutrient subsidies from omnivorous fish. *Freshwater Biol* (in press)
- Gerking SD (1994) Feeding ecology of fish. Academic Press, San Diego, CA
- Grimm NB (1988) Feeding dynamics, nitrogen budgets, and ecosystem role of a desert stream omnivore, *Agosia chrysogaster* (Pisces: Cyprinidae). *Environ Biol Fishes* 21:143–152
- He E, Wurtsbaugh W (1993) An empirical model of gastric evacuation rates for fish and an analysis of digestion in piscivorous brown trout. *Trans Am Fisheries Soc* 122:717–730
- Heroux D, Magnan P (1996) In situ determination of daily ration in fish: review and field evaluation. *Environ Biol Fishes* 46:61–74
- Hood JM (2000) The potential importance of nutrient regeneration by fish in a Neotropical stream. Thesis, Miami University, Oxford
- Horn MH, Mailhot KF, Fris MB, McClanahan LL (1995) Growth, consumption, assimilation, and excretion in the marine herbivorous fish *Cebidichthys violaceus* (Girard) fed natural and high protein diets. *J Exp Mar Biol Ecol* 190:97–108
- Kraft C (1992) Estimates of phosphorus and nitrogen cycling by fish using a bioenergetics approach. *Can J Fisheries Aquat Sci* 49:2596–2604
- Kraft C (1993) Phosphorus regeneration by Lake Michigan alewives in the mid-1970s. *Trans Am Fisheries Soc* 122:749–755
- Mather M, Vanni M, Wissing T, Davis S, Schaus M (1995) Regeneration of nitrogen and phosphorus by bluegill and gizzard shad: effect of feeding history. *Can J Fisheries Aquat Sci* 52:2327–2338
- Matthews W (1998) Patterns in freshwater fish ecology. Chapman & Hall, New York
- Matthews WJ, Stewart AJ, Power ME (1987) Grazing fishes as components of North American ecosystems: effects of *Camptostoma anomalum*. In: Matthews WJ, Heins DC (eds) Community and evolutionary ecology of North American stream fishes. University of Oklahoma Press, Norman, OK
- Nakashima B, Leggett W (1980a) The role of fishes in the regulation of phosphorus availability in lakes. *Can J Fisheries Aquat Sci* 37:1540–1549
- Nakashima BS, Leggett WC (1980b) Natural sources and requirements of phosphorus for fishes. *Can J Fisheries Aquat Sci* 37:679–688
- Olson RJ, Boggs CH (1986) Apex predation by yellowfin tuna (*Thunnus albacares*): independent estimates from gastric evacuation, stomach contents, bioenergetics, and cesium concentrations. *Can J Fisheries Aquat Sci* 43:1760–1775

- Persson L (1981) The effects of temperature and meal size on the rate of gastric evacuation in perch (*Perca fluviatilis*) fed on fish larvae. *Freshwater Biol* 11:131–138
- Persson L (1992) Rate of food evacuation in roach (*Rutilus rutilus*) in relation to temperature and the application of evacuation rate estimate for studies on the rate of food consumption. *Freshwater Biol* 12:203–210
- Power M (1990) Resource enhancement by indirect effects of grazers: armored catfish, algae, and sediment. *Ecology* 71:897–904
- Salvatore S, Mundahl N, Wissing T (1987) Effect of water temperature on food evacuation rate and feeding activity of age-0 gizzard shad. *Trans Am Fisheries Soc* 116:67–70
- Schaus M, Vanni M, Wissing T, Bremigan M, Garvey J, Stein R (1997) Nitrogen and phosphorus excretion by detritivorous gizzard shad in a reservoir ecosystem. *Limnol Oceanogr* 42:1386–1397
- Schindler DE, Eby LA (1997) Stoichiometry of fishes and their prey: implications for nutrient recycling. *Ecology* 78:1816–1831
- Sokal RR, Rohlf FJ (1995) *Biometry: the principles and practice of statistics in biological research*, 3rd edn. W.H. Freeman, San Francisco
- Solomon CT, Flecker AS, Taylor BW (2004) Testing the role of sediment-mediated interactions between tadpoles and armored catfish in a neotropical stream. *Copeia* 2004:610–616
- Stainton M, Capel M, Armstrong F (1977) The chemical analysis of freshwater. In: *Miscellaneous special report publication 25*. Freshwater Institute, Winnipeg
- Sturner RW (1990) The ratio of nitrogen to phosphorus resupplied by herbivores: zooplankton and the algal competitive arena. *Am Nat* 136:209–229
- Sturner RW, Elser JJ (2002) *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton University Press, Princeton
- Sturner RW, George NB (2000) Carbon, nitrogen, and phosphorus stoichiometry of cyprinid fishes. *Ecology* 81:127–140
- Tanner DK, Brazner JC, Brady VJ (2000) Factors influencing carbon, nitrogen, and phosphorus content of fish from a Lake Superior coastal wetland. *Can J Fisheries Aquat Sci* 57:1243–1251
- Targett TE, Targett NM (1990) Energetics of food selection by the herbivorous parrotfish *Sparisoma radians*: roles of assimilation efficiency, gut evacuation rate, and algal secondary metabolites. *Mar Ecol Prog Ser* 66:13–21
- Tilman D (1982) *Resource competition and community structure*. Princeton University Press, Princeton, NJ
- Urabe J, Watanabe Y (1992) Possibility of N or P limitation for planktonic cladocerans: an experimental test. *Limnol Oceanogr* 37:244–251
- Vanni MJ (1996) Nutrient transport and recycling by consumers in lake food webs: implications for algal communities. In: Polis GA, Winemiller KO (eds) *Food webs: integration of patterns and dynamics*. Chapman and Hall, London
- Vanni MJ (2002) Nutrient cycling by animals in freshwater ecosystems. *Ann Rev Ecol Systematics* 33:341–370
- Vanni MJ, Headworth JL (2004) Cross-habitat transport of nutrients by omnivorous fish along a productivity gradient: integrating watersheds and reservoir food webs. In: Polis GA, Power ME, Huxel GR (eds) *Food webs at the landscape level*. The University of Chicago Press, Chicago
- Vanni MJ, Layne CD (1997) Nutrient recycling and herbivory as mechanisms in the “top-down” effect of fish on algae in lakes. *Ecology* 78:21–40
- Vanni MJ, Flecker AS, Hood JM, Headworth JL (2002) Stoichiometry of nutrient recycling by vertebrates in a tropical stream: linking biodiversity and ecosystem function. *Ecol Lett* 5:285–293
- Winemiller KO (1990) Spatial and temporal variation in tropical fish trophic networks. *Ecol Monogr* 60:331–367
- Wootton JT, Oemke MP (1992) Latitudinal differences in fish community trophic structure, and the role of fish herbivory in a Costa Rican stream. *Environ Biol Fishes* 35:311–319