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Does diet influence consumer nutrient cycling? Macroinvertebrate and fish excretion in streams

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Abstract. Consumer nutrient cycling supplies limiting elements to autotrophic and heterotrophic organisms in aquatic systems. However, the role of consumers in supplying nutrients may change depending on their diet and their own stoichiometry. We evaluated the stoichiometry, N and P excretion, and diets of the dominant macroinvertebrates and fish at 6 stream sites to determine if the nutritional composition of food alters nutrient excretion. We used Sterner's (1990) nutrient homeostasis model as a reference to gauge whether consumer nutrient excretion is influenced by diet. Body stoichiometry explained 61% of the variation in N:P excretion by macroinvertebrates but only 11% of the variation for fish. In both cases, the relationship was driven by 2 P-rich end-members, crayfish and mottled sculpin. Results of Akaike Information Criterion (AIC) analysis showed that family alone explained 71% of the variation in N:P excretion in macroinvertebrates and 31% of the variation in fish. Diet explained only 8% of the variation in both cases. Most consumers (9 of 11) had N:P excretion values that were well below predictions of Sterner's model. Two taxa, crayfish and sculpin, had N:P excretion that overlapped the model's predictions. Our results suggest that crayfish and sculpin may display strict homeostasis with respect to N and P and that their growth might be P-limited. Other consumers may be more flexible in their stoichiometry and not P-limited. We speculate that the extremely low excretion N:P measured for many consumers might have been the result of semiflexible homeostasis, inaccuracies in our assessment of dietary nutrients, growth-limiting nutrients other than N or P, or lack of egestion data. Our results suggest that crayfish and sculpin may alter N and P dynamics in streams by excreting low amounts of P relative to N compared to what is generally available in the water column.

Key words: stoichiometry, excretion, nutrient cycling, stream, consumers.

Consumers supply inorganic nutrients to autotrophic and heterotrophic communities in aquatic food webs (Kitchell et al. 1979, Schaus et al. 1997, Vanni et al. 1997, McIntyre et al. 2007, Schindler 2007). Variation in consumer nutrient composition influences excretion and is generally controlled by phylogeny (Fagan et al. 2002, Sterner and Elser 2002, Hendrixson et al. 2007). Thus, nutrient turnover in ecosystems may be related to the diversity and presence of particular taxa (Vanni et al. 2002). In aquatic systems with low diversity, a few taxa may strongly influence nutrient turnover (Vanni et al. 1997, Torres and Vanni 2007). Thus, the relationship between consumers and their food resources has broad implications for population dynamics, the exchange of nutrients between various components of aquatic ecosystems, and the flow of nutrients through food webs (Elser et al. 2000a, b, Sterner and Elser 2002, Frost et al. 2005).

The C:N:P of resources, such as detritus, algae, and animal tissue, varies extensively and can limit nutrients available for growth, which, consequently,

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influences nutrient excretion (Schindler and Eby 1997, Elser and Urabe 1999, Vanni 2002, Dodds et al. 2004, Frost et al. 2005). The effect on consumer nutrient excretion can be evaluated via mass–balance equations like Sterner's (1990) homeostasis model for zooplankton. If N:P of the food ($N{:}P_f$) > N:P of the consumer ($N{:}P_c$), then P is deficient in the food and

$$N{:}P_r = \frac{N{:}P_f - (GGE \times N{:}P_c)}{1 - GGE} \quad [1]$$

where $N{:}P_r$ is the nutrient ratio of released nutrients and GGE is the gross growth efficiency for the limiting nutrient (i.e., the maximum GGE achieved under scarcity of the nutrient). If N is deficient in the food, then $N{:}P_f < N{:}P_c$ and

$$N{:}P_r = \frac{N{:}P_f(1 - GGE)}{1 - \frac{GGE \times N{:}P_f}{N{:}P_c}} \quad [2]$$

Equation 1 shows that as $N{:}P_f$ becomes increasingly greater than $N{:}P_c$, $N{:}P_r$ will increase. Under these conditions, consumer growth may be limited by the P in their diet. However, an assumption of this model is that consumers maintain strict homeostasis, i.e., their internal nutrient composition remains constant relative to changes in dietary nutrients. Evidence of flexible homeostasis in consumers has been found in some studies (Sterner and Shultz 1998, Frost and Elser 2002, Cross et al. 2003, Glaholt and Vanni 2005), whereas strict homeostasis has been found in others (Bowman et al. 2005, Evans-White et al. 2005). Most metazoan consumers probably do not exhibit limitless flexibility and have a restricted range of internal composition, especially in relation to the large variability in their diet (Sterner and Elser 2002). Thus, substantial changes in dietary nutrients should influence nutrient excretion by consumers.

P-limited growth in invertebrates (mostly but not exclusively zooplankton) is well substantiated (DeMott et. al. 1998, Sterner and Shultz 1998, Frost and Elser 2002, Stelzer and Lamberti 2002, Ferrão-Filho et al. 2007). However, support for P-limited growth in fish has been mixed. For example, Schindler and Eby (1997) found that fish N:P excretion was not variable despite differences in diet and concluded that growth in most fish was not N or P limited but was energy limited. However, Hood et al. (2005) found that a tropical catfish, *Ancistrus triradiatus*, had P-limited growth and extremely low P excretion. P-limited growth is not evident across all taxa, but a general conclusion is that P-limitation is more common or pronounced in P-rich taxa than in P-poor taxa (Sterner and Shultz 1998, Hood et al. 2005, Ferrão-Filho et al. 2007), which is in accordance with stoichiometric theory.

Sterner's model shows that GGE of both elements influences excretion. GGE can vary extensively across taxa (e.g., Ferrão-Filho et al. 2007). Food quality, in terms of biochemical makeup and nutritional content, also influences GGE (DeMott et al. 1998, Anderson et al. 2004, Ferrão-Filho et al. 2007) and can lead to differences in nutrient excretion. Thus, the potential importance of various growth efficiencies to nutrient excretion should be evaluated across taxa, especially in relation to dietary nutrients.

We organized trophic relationships into elemental interactions to create a framework for testing predictions of how dietary nutrient availability affects elemental relationships between consumers and their resources and, thus, their nutrient release (Elser and Urabe 1999, Sterner and Elser 2002, Vanni 2002). One limitation of our approach is that fecal matter, which is needed to complete the mass–balance, is excluded. Sterner's model was developed for zooplankton and accurately predicted N:P excretion. However, egestion is minimal in zooplankton and may not be needed to assess nutrient turnover by these species accurately (Sterner 1990). Analyzing fecal matter would increase the predictive ability of Sterner's model for larger consumers, but trends in nutrient excretion relative to diet should be evident even without these data. Analyzing nutrient excretion also can provide estimates of the contribution of consumers to the inorganic nutrient pool in aquatic ecosystems.

We evaluated the stoichiometry, excretion, and diets of the dominant macroinvertebrates and fish at 6 sites to determine if differences in the nutritional composition of food resources altered nutrient excretion and if there was evidence of nutrient limitation. We focused on N and P dynamics. Our objectives were to: 1) determine the relationship between stream consumer nutrient excretion and diet relative to other factors, such as body composition and phylogeny, 2) use Sterner's (1990) model as a reference to gauge evidence for nutrient homeostasis and possible nutrient limitation in these consumers, and 3) determine the extent to which consumer nutrient cycling contributed to overall stream nutrient dynamics.

Methods

Study sites

We sampled fish and macroinvertebrates in June 2006 at 3 locations: Coweeta Hydrologic Laboratory (western North Carolina), Grayson Highlands State Park (southwestern Virginia), and private agricultural

land (Floyd County, Virginia). At each location, we selected 2 separate 100-m stream reaches as study sites (6 sites total). At Coweeta and Grayson Highlands, each site was on a separate stream, whereas in Floyd County, sites were on the same stream. We selected these sites because they differed in nutrient-loading regime, geology, vegetation, and riparian density, which maximized differences in resource stoichiometry among sites. Coweeta streams, Ball Creek and Shope Fork, average 700 m asl and are characterized by mixed-hardwood riparian vegetation of red maple (*Acer rubrum* L.), yellow poplar (*Liriodendron tulipifera* L.), and oaks (*Quercus* spp.) and an understory of rhododendron (*Rhododendron maximum* L.). The Shope Fork site is mostly unshaded and bordered by alder (*Alnus glutinosa* (L.) Gaertn.). Streams in Grayson Highlands State Park typically occur in balds and hardwood–conifer mixes $\geq$1600 m asl and are characterized by high N deposition because of their relatively high altitude. Cabin Creek (1390 m) has a riparian understory of rhododendron and an overstory of birch (*Betula* spp.) and red maple. Big Wilson (1330 m) runs through an open bald with patches of rhododendron and birch. In Floyd County, Mira Fork (760 m) is lightly affected by cattle grazing and fertilizer runoff. The upstream section runs through a field dominated by grasses and patches of alder. The downstream section is lightly forested by birch and oak species. Average stream temperatures ranged from 14 to 16°C at all sites during our study.

Field sampling overview

In each of the six 100-m sites, we collected 4 dominant macroinvertebrate taxa and 3 dominant fish taxa (if present) and analyzed inorganic N and P excretion. We assumed that fish and possibly macroinvertebrate excretion of organic compounds (i.e., urea) was minimal because organic compounds generally comprise $\leq$10% of total N excretion (Moyle and Cech 2004). We analyzed whole-body C, N, and P of macroinvertebrates and fish. We collected qualitative samples of epilithon, seston, and leaf detritus at each site and analyzed the C, N, and P content of these resources. We also analyzed inorganic N and P concentrations of stream-water samples.

Consumer excretion and body stoichiometry

We conducted a preliminary experiment with 3 macroinvertebrate families (Hydropsychidae, Calopterygidae, Simuliidae) and 1 fish species (blacknose dace, *Rhinichthys atratulus*) to determine appropriate incubation times for measuring NH_3 excretion. We compared time-corrected NH_3 excretion rates between incubation periods of 30 min and 1 h (4 replicates each). For the macroinvertebrates, estimates of NH_3 excretion were significantly lower after 1 h than after 30-min incubation (median = 0.7 and 1.2 $\mu g\ L^{-1}\ h^{-1}$, respectively). This result suggested that a longer incubation time might mitigate effects of handling stress and would be appropriate for assessing NH_3 excretion. However, for blacknose dace, NH_3 excretion was only slightly higher at 1 h than at 30 min (median = 30 and 25 $\mu g\ L^{-1}\ h^{-1}$, respectively). This result suggested that shorter incubations may be sufficient for assessing NH_3 excretion. Moreover, a shorter incubation would help prevent O_2 depletion, an important consideration for coldwater fish species. Whiles et al. (2009) concluded that, in general, 30-min incubations were appropriate for minimizing effects of fasting and handling stress on fish excretion estimates.

We collected macroinvertebrates with D-frame nets and sorted them by family in the field. We placed 1 to 4 individuals in the same family (depending on size) in a bag with 30 mL of prefiltered (Whatman GF/F) stream water (Schaus et al. 1997, Vanni et al. 2002). We set up 5 bags per taxon. We set up 3 bags without macroinvertebrates as controls. We incubated bags in stream water to keep temperature constant. After 1 h, we collected water samples (1 from each bag), filtered (Whatman GF/F) them, and immediately placed them on ice.

We collected fish with a backpack electroshocker at low voltage and with nets. We sorted fish by species, and placed individuals in bags with 500 to 1000 mL of prefiltered water, depending on size and species. We set up 5 bags per species. We incubated bags in the stream for 30 min, and processed water samples as described for macroinvertebrates.

We stored all excretion samples frozen until analysis for NO_3^-, NH_4^+, and soluble reactive P (SRP) on a Lachat QuickChem (Lachat Instruments, Loveland, Colorado) flow-injection analyzer using the Cd-reduction, phenate, and ascorbic acid methods, respectively. Method detection limits (MDLs; USEPA 1997) were 0.97, 1.7, and 0.95 $\mu g/L$ for NO_3-N, NH_4-N, and SRP, respectively. For values <MDL for each analyte, we assigned a value ½ MDL, a step that was necessary in only 3 cases (NH_4-N samples from 2 excretion bags for one family and a NH_4-N sample from another bag for a different family). For each family we had used 5 replicates (bags) per stream, and the use of ½ the MDL did not affect our conclusions (see Results). We corrected N and P concentrations in excretion bags by subtracting N and

P concentrations in background water samples (described below).

After excretion samples were taken, we froze macroinvertebrates and fish and later dried and weighed them so that dry-mass-specific excretion rates could be calculated. We were able to analyze body C, N, and P content of individual fish used in the excretion bags (see below). However, macroinvertebrates were too small for us to analyze individually or aggregated by bag. Therefore, we collected additional macroinvertebrates (3 separate samples per family per site) to augment measurements of body C, N, and P content.

We ground dried macroinvertebrate and fish samples and separated each sample into 2 subsamples. We used a vario MAX CNS Macro-Elemental Analyzer (Elementar Americas, Inc., Mt. Laurel, New Jersey) to determine C and N content of 1 of each pair of subsamples. We combusted (550°C for 1 h) 10 mg of each of the remaining subsamples and subjected the combusted subsamples to acid hydrolysis in 0.5 N cold HNO_3 (Sterner and George 2000) before determining inorganic P with the flow-injection analyzer. We expressed P content of macroinvertebrates and fish as % dry mass.

Water, epilithon, seston, and leaf-detritus collection and processing

We collected 2 epilithon samples from the middle of each site by scrubbing all surfaces of $\geq$10 rocks/sample. We stored the resultant slurries in separate acid-washed Nalgene® bottles. We collected 2 seston (10 μm–1 mm) samples at each site by placing a 10-μm-mesh net in the current for ~24 h and stored samples in separate acid-washed Nalgene bottles. We collected 4 samples of leaf detritus representing the dominant vegetation randomly from throughout each site and stored the samples in separate, clean plastic bags. We collected 4 water samples at each site, filtered them through precombusted filters (Whatman GF/F), and stored the samples in acid-washed Nalgene containers. We immediately placed all samples on ice and transported them to the laboratory where they were stored frozen.

We thawed and then analyzed stream-water samples for NO_3^-, NH_4^+, and SRP on the flow-injection analyzer. We thawed and filtered (precombusted Whatman GF/F) epilithic algae and seston samples. We dried leaf detritus, epilithon, and seston samples. The dried samples were ground, subsampled, and processed as described above for C, N, and P content. Because of the large inorganic P fraction in the epilithon (22–58%) and seston (9–21%) slurries, we hydrolyzed 10 mg of uncombusted sampled in 0.5 N cold HNO_3 to disassociate P from inorganic materials (Olsen and Sommers 1982). We calculated organic P (the P we assumed was available to consumers) by subtracting the P in the inorganic fraction from the P in the combusted sample (total P). We calculated organic P from the mean of the inorganic samples and the mean total P, so we report only 1 value for organic P for each subsample. We combusted subsamples of dried samples to obtain ash-free dry mass (AFDM).

Consumers may ingest some inorganic material (Forster and Gabbott 1971, Flecker 1996, Torres and Vanni 2007), but the extent to which consumers assimilate inorganic material and use inorganic-sediment-derived nutrients in growth is not clear (Conover 1966, Forster and Gabbott 1971, Lasenby and Langford 1973). We used only organic P in our analyses because little information exists on whether inorganic P subsidies can alleviate consumer P demand.

Diet nutritional values assigned to consumers

We assigned resources for invertebrate functional feeding groups (scrapers, collectors, filterers, and shredders) based on summaries in Merritt and Cummins (1996). We assigned resources for fish functional feeding group based upon gut analysis. We conducted gut analyses by cutting fish from the anus to the upper foregut and removing the digestive tract. We dissected the entire digestive tract and identified invertebrate prey to order and family where feasible. We calculated nutrient ratios for predatory fish diets as weighted averages (weighted by % gut contents) of representative macroinvertebrates in each order. Nutrient ratios of macroinvertebrates were based on values found in our study and published data from Fagan et al. (2002), Cross et al. (2003), Frost et al. (2003), and Evans-White et al. (2005). Central stonerollers (*Campostoma anomalum* Rafinesque), bluehead chubs (*Nocomis leptocephalus* Girard), and redbelly dace (*Phoxinus erythrogaster* Rafinesque) consumed a small number of insects in addition to algae. For ominivorous fish, we estimated algal consumption as the difference between the actual number of insects in the gut and potential number of insects the gut could hold (calculated from a regression of number of insects vs fish body size using predatory fish only). Omnivorous fish ate so few insects that deviations from the nutritional content of a pure algal diet were minimal.

Sterner model

We used Sterner's model (Eqs 1, 2) and our measured values for consumer body composition

Table 1. Mean P (% of ash-free dry mass [AFDM] or dry mass [DM] of macroinvertebrates), and N:P (molar) content of epilithon, seston, leaf, and macroinvertebrates collected at each of the 6 study sites. Macroinvertebrate values are means of all macroinvertebrate taxa sampled at each site. The missing % P and N:P values in Big Wilson Creek were a result of lost samples.

	Epilithon		Seston		Leaf		Macroinvertebrate	
Stream	P (% AFDM)	N:P	P (% AFDM)	N:P	P (% AFDM)	N:P	P (% DM)	N:P
Shope Fork	0.3549	40	0.293	23	0.070	52	0.5064	53
Ball Creek	0.2416	51	0.204	27	0.044	55	0.6932	37
Mira Fork Open	0.4179	28	0.262	29	0.085	65	0.6587	38
Mira Fork Forested	0.3042	47	0.304	29	0.075	62	0.6544	38
Big Wilson	0.2013	81	–	–	0.048	74	0.6610	43
Cabin Creek	0.2526	42	0.274	28	0.058	77	0.3664	73
Mean	0.2954	48	0.267	27	0.063	64	0.5951	46

and diet to predict nutrient excretion ratios. We compared our measured excretion ratios to these predictions. We replaced the accumulation efficiency (L) in Sterner's model with maximum gross growth efficiency (GGE) (Torres and Vanni 2007). GGE is the maximum gross growth efficiency (growth/ingestion) for a specific limiting nutrient, whereas L refers to the growth efficiency relative to what is removed from the resource pool. The difference between what is removed from the resource pool and what is ingested is termed *sloppy feeding*, which has been studied mostly in zooplankton (Moller 2005, He and Wang 2006). Selective consumption also occurs in fish and macroinvertebrates (Cummins 1974, Hall and Meyer 1998, Hood et al. 2005). We assumed that the nutrient composition of the measured resource and the nutrient content of what was actually ingested were the same. Torres and Vanni (2007) used GGE = 70%. However, we used values of 35% and 70% because consumer GGE may vary widely and specific values are largely unknown.

Statistical analysis

We used analysis of variance (ANOVA) to test for differences in % N, % P, and N:P among resources using sites as replicates and among taxa using individuals (fish) or groups of individuals (macroinvertebrates) as replicates. We used linear regressions to analyze relationships between consumer stoichiometry and excretion ratios across nutritional gradients. We used $\log(x)$- or $\arcsin\sqrt{(x)}$-transformed values where appropriate. We used Akaike's Information Criterion (AIC; Burnham and Anderson 2002) to test alternative hypotheses when determining which variables explained the most variation in excretion ratios. Alternative hypotheses or models with the lowest AIC score are considered best in terms of maximizing the overall amount of variability explained while minimizing the total number of variables (most parsimonious explanation). We used stepwise regression (SAS JMP 7.0; SAS Institute, Cary, North Carolina) to determine the variation explained by N:P of consumers; N:P of the diet; family, functional feeding group, and dry mass of the consumer; and site.

Results

Resource stoichiometry

Percent P and N:P ratios differed among epilithon, seston, leaf detritus, and macroinvertebrates (excluding crayfish) (ANOVA, $p < 0.0001$ for % P and N:P; Table 1). Percent P of leaves was significantly lower than % P of epilithon, seston, and macroinvertebrates, which were not significantly different (Tukey's test, $p < 0.05$). N:P of seston was significantly lower than N:P of epilithon, leaves, and macroinvertebrates, which were not significantly different (Tukey's test, $p < 0.05$).

Consumer stoichiometry

All body stoichiometry variables differed significantly among macroinvertebrate families (ANOVA, all $p < 0.0001$; Appendix 1, C:P and C:N results not shown). Cambaridae, the only crustacean family, had significantly higher body % P and baetid mayflies had significantly lower body % P than the other macroinvertebrate families (Tukey's test, $p < 0.05$; Appendix 1). All body stoichiometric variables also differed significantly among functional feeding groups (ANOVA, all $p < 0.0001$; Appendix 2). Crayfish were treated as a separate functional feeding group and had significantly higher body % P (1.06% dry mass [DM]) than all other groups (Tukey's test, $p < 0.05$).

Body % P differed significantly among fish species (ANOVA, $p < 0.0001$). Mottled sculpin had signifi-

cantly higher body % P than other species, except redbelly dace (Tukey's test, $p < 0.05$; Appendix 3). Body % P concentration did not differ significantly among the remaining species, but salmonid species had the lowest body % P. Body N:P and C:P varied among species (ANOVA, both $p < 0.0001$). Body N:P and C:P were significantly lower for mottled sculpin and redbelly dace than for the other fish species (Tukey's test, $p < 0.05$).

Relationships between body N:P and excretion N:P

N and P concentrations in the macroinvertebrate excretion bags were 107 to 456 μg NO_3-N/L (median = 325), 0.85 to 788 μg NH_4-N/L (median = 38.3), and 4.07 to 68.2 μg SPR/L (median = 11.7). N and P concentrations in the fish excretion bags were 102 to 452 μg NO_3-N/L (median = 246), 23.8 to 854 μg NH_4-N/L (median = 129), and 5.22 to 586.5 μg SPR/L (median = 23.0). Median % difference values between duplicates were 0%, 2.8%, and 10.7% for NO_3-N, NH_4-N, and SRP, respectively. Accuracy (% recovery) values were 109%, 107%, and 99% for NO_3-N, NH_4-N, and SRP, respectively. We measured NO_3^- in the excretion bags in case of rapid nitrification of NH_4^+, but we never observed an increase in NO_3^-. N and P concentrations in control bags did not change significantly during incubation.

N, P, and N:P excretion differed significantly among macroinvertebrate families (ANOVA, all $p < 0.0001$; Appendix 1). Macroinvertebrate P excretion and body % P were negatively related (linear regression, $r^2 = 0.51$, $p < 0.0001$; Fig. 1A) as were macroinvertebrate N:P excretion and body N:P (linear regression, $r^2 = 0.61$, $p < 0.0001$; Fig. 1B). However, these relationships were strongly influenced by the high body % P of crayfish. Hydropsychid caddisflies excreted the most P, whereas cambarid crayfish excreted the least (Appendix 1). Likewise, hydropsychid excretion had the lowest N:P, whereas cambarid crayfish excretion had the highest N:P (Appendix 1). N, P, and N:P excretion differed significantly among functional feeding groups (ANOVA, all $p < 0.0001$; Fig. 2A–C). Crayfish excretion had significantly higher N:P (544) than all other groups, whereas filterer excretion had significantly lower N:P (1.52) than all other groups except shredders (5.11) (Tukey's test, $p < 0.05$; Appendix 2).

N, P, and N:P excretion differed significantly among fish species (ANOVA, N: $p = 0.005$; P: $p = 0.0006$, N:P: $p < 0.0001$; Appendix 3). P excretion was negatively related to body % P (Fig. 3A). However, this relationship explained only a small amount of variation in P excretion (linear regression, $r^2 = 0.20$, $p < 0.0001$). Excretion N:P excretion was negatively related to body N:P, but this relationship also explained only a small amount of variation in N:P excretion (linear regression, $r^2 = 0.11$, $p < 0.005$; Fig. 3B). These relationships were strongly influenced by the high P content of mottled sculpin. Mottled sculpin had the lowest P excretion and highest excretion N:P. However, P excretion values in mottled sculpin differed only from that of dace and chub, and N:P excretion did not differ from that of brook trout (Tukey's test, $p < 0.05$; Appendix 3).

AIC model development

Tables 2 and 3 show the results of the alternative statistical models for variation in N:P excretion. We used all replicates for macroinvertebrate excretion instead of site means in the AIC analyses. Thus, values for the regression of body N:P vs N:P excretion differ from results shown in Fig. 1. For macroinvertebrates, the best (lowest AIC score) model included family, body N:P, and diet N:P, and explained 72% of the variation in N:P excretion (Table 2). Family alone explained 71% of the variation in N:P excretion, and adding diet N:P, body N:P, site, and functional group explained little more variation. Diet N:P explained little variation in N:P excretion when considered alone or in combination with other variables (Table 2). Functional feeding group, which is strongly related to family, explained 61%, whereas body N:P explained 42% of the variation in N:P excretion (Table 2). For fish, the best model included family, site, body N:P, and diet N:P, and explained 52% of the variation in N:P excretion (Table 3). Family alone explained 31%, whereas body N:P only explained 11% of the variation in N:P excretion (Table 3). Diet N:P explained little variation in N:P excretion.

Sterner's model predictions

Ranges of measured N:P excretion by most macroinvertebrate taxa (9 of 11) were lower than N:P excretion values predicted by Sterner's model (Table 4, Fig. 4A–C). Cambarid N:P excretion was higher than predicted by the model, whereas heptageniid mayflies N:P excretion overlapped model predictions. Ranges of measured N:P excretion overlapped model predictions for only 3 of 7 fish species (Table 5, Fig. 5A–C). Mottled sculpin N:P excretion was similar to or exceeded model predictions. Brook trout and bluehead chub N:P excretion overlapped model predictions. N:P excretion of the remaining 4 species was well below model predictions.

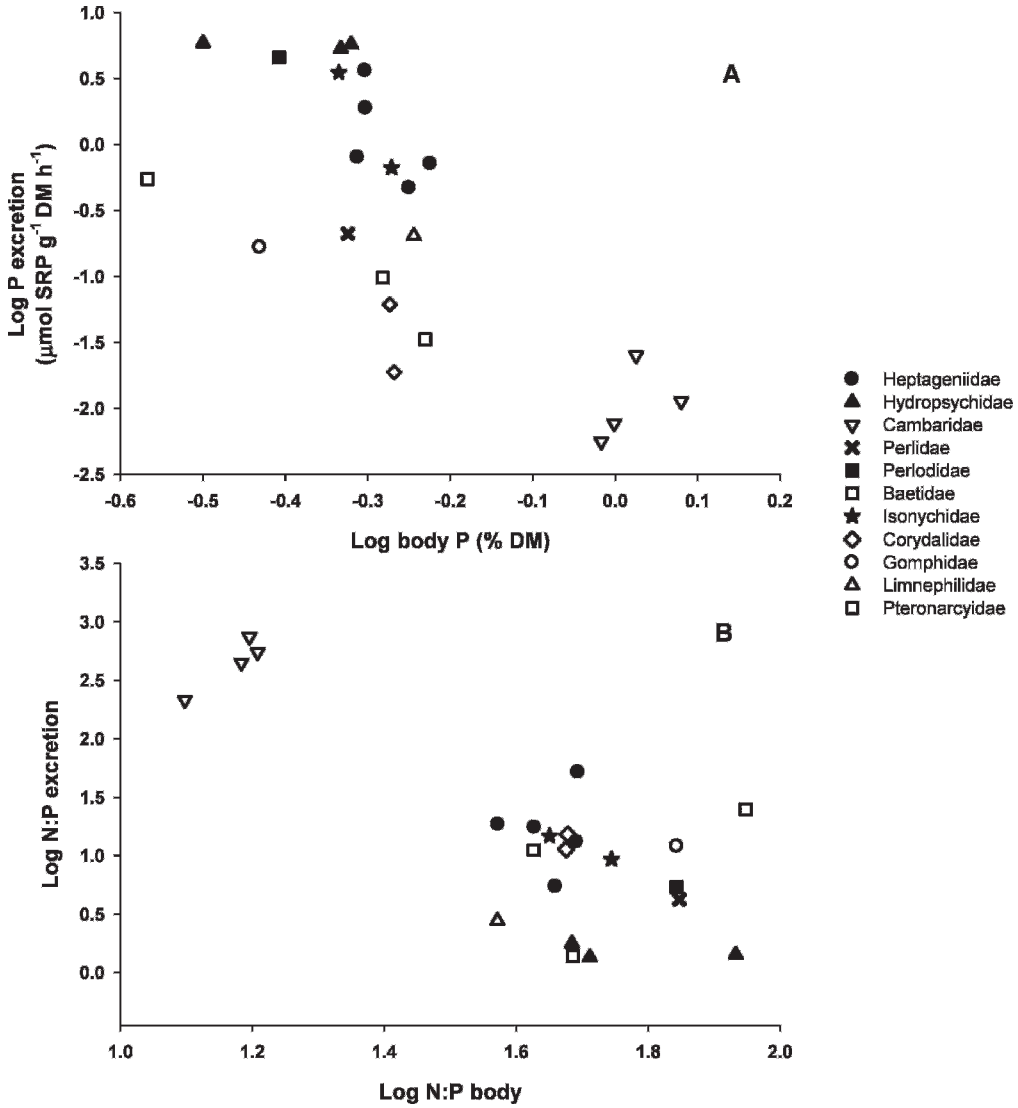

FIG. 1. Macroinvertebrate P excretion vs body % P (A) and N:P (molar) excretion vs body N:P (B). Each symbol represents the nutrient composition of the average body and nutrient excretion from each site for each representative taxon. SRP = soluble reactive P, DM = dry mass.

Discussion

Relationships between body N:P and excretion N:P

Stoichiometric principles predict that consumer nutrient excretion and egestion should be related to body elemental ratios (Kitchell et al. 1979, Sterner and Elser 2002, Vanni 2002, Vanni et al. 2002, Glaholt and Vanni 2005). We found negative relationships between P excretion and body % P and N:P excretion and body N:P for macroinvertebrate taxa and fish species, but the relationships were driven by P-rich end-members (crayfish, mottled sculpin; Figs 1A, B, 2A, B).

On average, crayfish had >2× more P than insects (Appendix 1), and they excreted very little P. Low P excretion by crayfish in a mesocosm experiment caused periphyton P content to be significantly lower in the presence of crayfish than in the presence of snails (Evans-White and Lamberti 2005). P is essential to carapace formation in crustaceans (hydroxyapatite–Ca-associated P), and carapace P makes up 14% of total body P in *Daphnia* (Vrede et al. 1999). Thus, molting could cause substantial P loss and P limitation in crustaceans by affecting their overall P budget (Hessen and Rukke 2000, Faerøvig and Hessen 2003). Crayfish and insects also might differ in terms of P requirements for ontogenetic shifts and allocation of energy to reproduction, RNA content, and growth rate (Sommer et al. 2003).

Among fish species, mottled sculpin had the lowest P excretion (high N:P excretion) and highest body % P

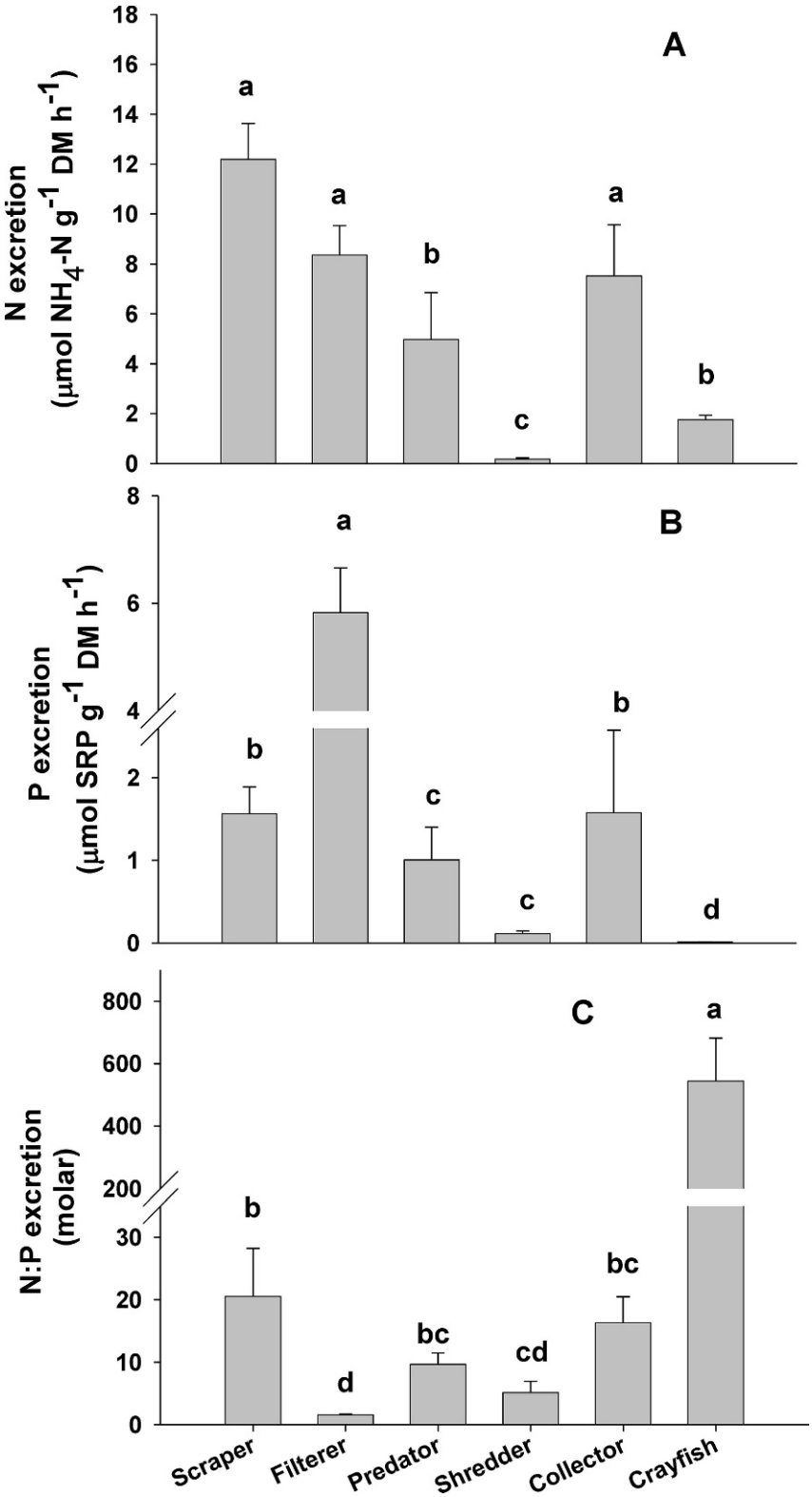

FIG. 2. Mean (±1 SE) functional feeding group N excretion (A), P excretion (B), and N:P (molar) excretion (C). Bars with the same letter are not significantly different (Tukey's test, $\alpha = 0.05$).

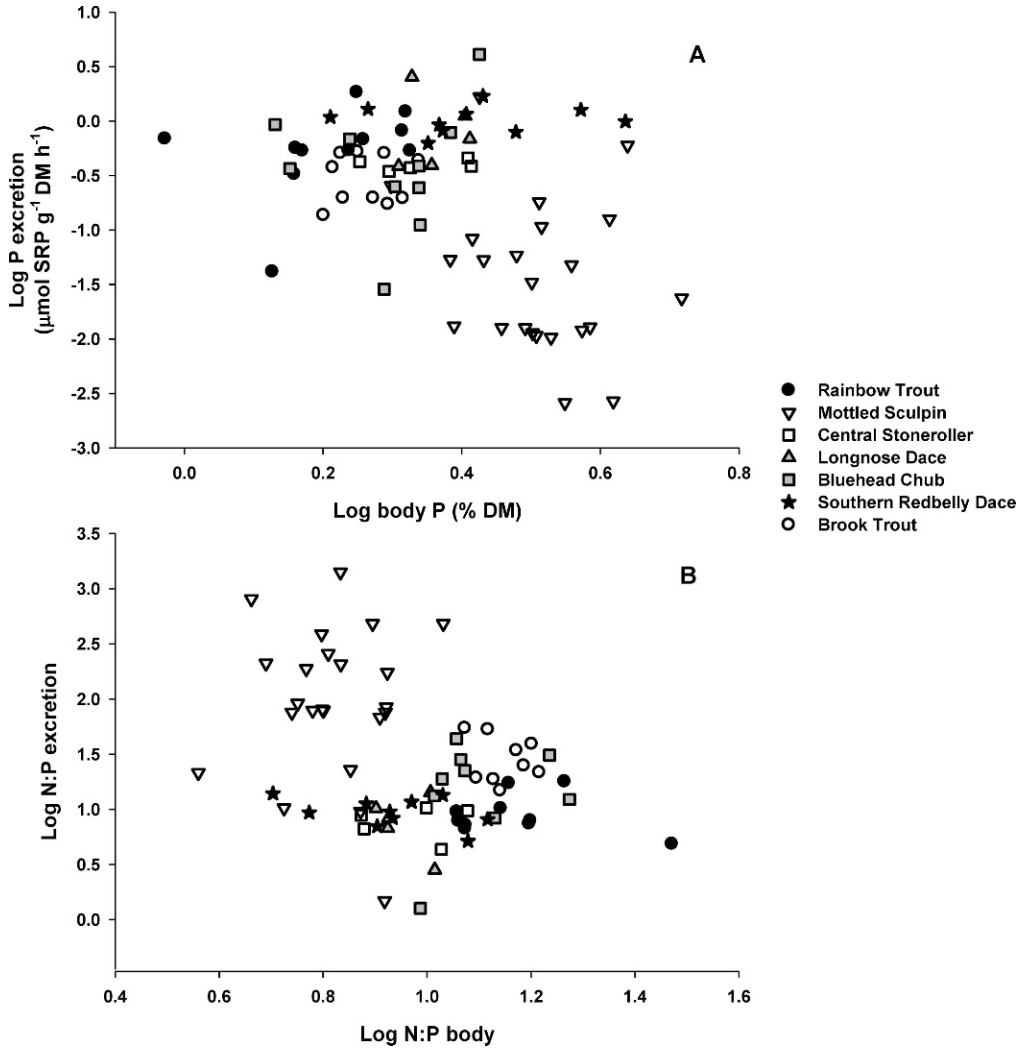

FIG. 3. Fish P excretion vs body % P (A) and N:P (molar) excretion vs body N:P (B). Each symbol represents 1 individual. Different symbols indicate different species. SRP = soluble reactive P, DM = dry mass.

(low body N:P; Appendix 3). C:N:P stoichiometry of fishes varies with phylogeny and generally is linked to the amount of P in scales and skeletal structures (Sterner and Elser 2002, Vanni et al. 2002, Hendrixson et al. 2007). Members of the genus *Cottus* have a broad, flattened head and large pectoral fins (Moyle and Cech 2004) that are assumed to be associated with large amounts of P-rich bone. The neotropical armored catfish, *Ancistrus triradiatus*, which has P-rich bony cranial plates, also excretes very little P (Vanni et al. 2002, Hood et al. 2005).

Crayfish and sculpin seemed to drive the relationship between body % P and N:P excretion, but body stoichiometry alone was unable to explain the large variation in nutrient excretion by the fish species. We found a stronger relationship among the 11 families of macroinvertebrates in our study. P excretion and body % P were strongly negatively related in 13 tropical fish and amphibian families (28 species) (Vanni et al. 2002). Thus, the lack of strong relationships in our study, at least for the fish, could have been a consequence of including only 3 families. However, Torres and Vanni (2007) also found that body stoichiometry failed to elucidate patterns in nutrient excretion and concluded that differences in diet or growth efficiencies might better explain excretion stoichiometry.

The role of diet and phylogeny

Body stoichiometry was unable to explain the large amount of variation in N:P excretion among taxonomic groups of consumers. Therefore, we hypothesized that diet might play a substantial role in nutrient

TABLE 2. Akaike Information Criteria (AIC) model results comparing alternative hypotheses and the variation they explain in macroinvertebrate N:P excretion. Adj. = adjusted, Δ AIC is the difference between the AIC value of the "best" model and that of each consecutive model and is an indication of relative model performance.

Model	R^2	R^2 adj.	AIC	Δ AIC
Family + body N:P + diet N:P	0.72	0.69	20.9	0
Family + body N:P + site + functional feeding group	0.74	0.69	22.6	1.7
Family	0.71	0.68	22.7	1.8
Family + body N:P + site + functional feeding group + mass + diet N:P	0.74	0.69	23.8	2.9
Family + body N:P	0.71	0.67	24.2	3.3
Functional feeding group	0.61	0.6	39.9	19
Body N:P	0.42	0.42	80.6	59.7
Body N:P + diet N:P	0.43	0.42	81.5	60.6
Site	0.22	0.19	122.28	101.38
Diet N:P	0.08	0.08	132.7	111.8

TABLE 3. Akaike Information Criteria (AIC) model results comparing alternative hypotheses and the variation they explain in fish N:P excretion. Adj. = adjusted, Δ AIC is the difference between the AIC value of the "best" model and that of each consecutive model and is an indication of relative model performance.

Model	R^2	R^2 adj.	AIC	Δ AIC
Family + site + body N:P + diet N:P	0.52	0.45	28.95	0
Family + site	0.48	0.42	30.24	1.29
Family + site + body N:P + diet N:P + functional feeding group + mass	0.54	0.44	33.3	4.35
Family	0.31	0.3	39.15	10.2
Family + body N:P + diet N:P	0.35	0.32	39.84	10.89
Functional feeding group	0.12	0.11	56.56	27.61
Body N:P + diet N:P	0.13	0.11	57.41	28.46
Body N:P	0.11	0.08	58.93	29.98
Diet N:P	0.08	0.07	59.24	30.29
Site	0.08	0.01	68.07	39.12

excretion and analyzed macroinvertebrates within functional feeding groups. Hydropsychid filterers had the highest excretion P and lowest N:P (Appendix 1, Fig. 1A, B), but their body % P did not differ from that of other insects (Appendix 1). Larger hydropsychids, such *Diplectrona*, *Hydropsyche,* and *Cheumatopsyche*, are filterers but also consume chironomids (Benke and Wallace 1997, Benke et al. 2001, Rosi-

TABLE 4. N:P ratio (molar) of diet, predicted excretion N:P based on Sterner's (1990) model with 2 different maximum gross growth efficiencies (GGE), and measured excretion N:P for 11 families of macroinvertebrates. Multiple samples were taken from each site. *n* refers to the number of sites sampled. Range is based on site means. FFG = functional feeding group.

			Diet N:P		Predicted excretion N:P Sterner's (1990) model		Measured excretion N:P	
Family	FFG	*n*	Mean	Range	GGE = 35%	GGE = 70%	Mean	Range
Heptageniidae	Scraper	5	48	28–81	23–101	15–166	21	5–52
Hydropsychidae	Filterer	3	26	23–28	17–21	9–12	1.5	1–2
Isonychiidae	Filterer	2	29	28–30	23–24	14–15	12	9–15
Baetidae	Collector gatherer	1	42	–	39	30	24	–
Pteronarcyidae	Shredder	2	53	47–69	49–83	57–130	6	1–11
Limnephilidae	Shredder	1	55	–	69	106	3	–
Cambaridae	Shredder scraper	4	64	55–74	77–106	149–213	490	215–745
Perlidae	Predator	1	48	–	46	43	4	–
Perlodidae	Predator	1	75	–	80	113	5	–
Gomphidae	Predator	1	46	–	43	38	12	–
Corydalidae	Predator	2	45	37–55	33–58	23–68	13	11–15

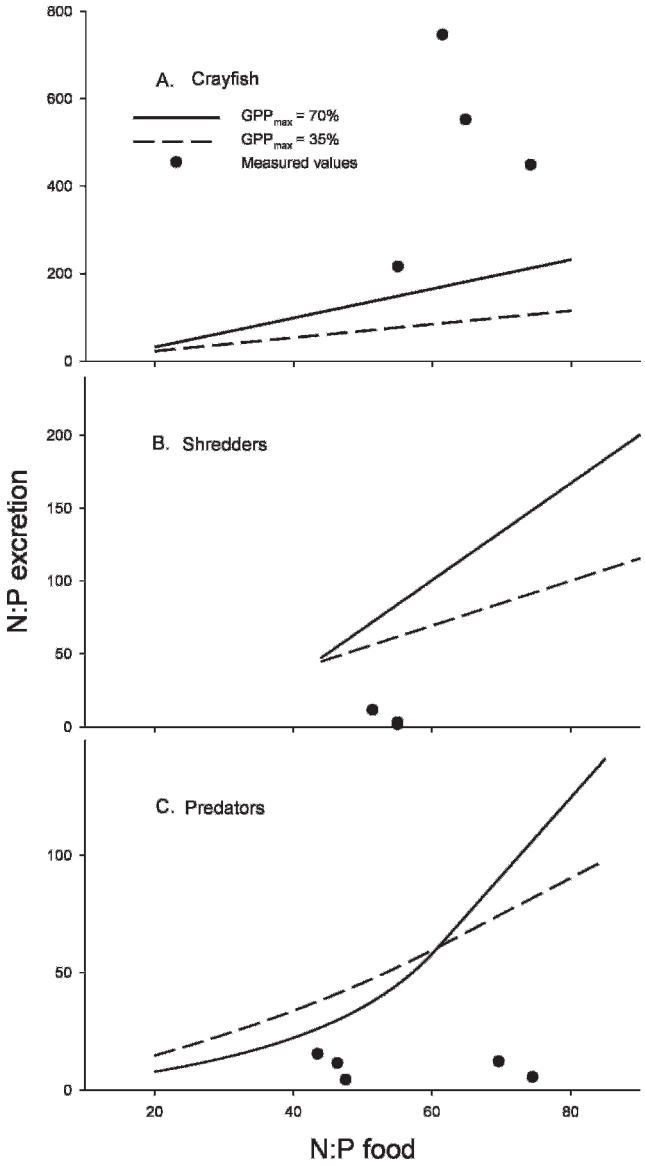

FIG. 4. Predicted N:P (molar) excretion vs N:P food calculated from Sterner's (1990) model with 2 different values of maximum gross growth efficiency (GGE_{max}) and compared to measured N:P excretion for crayfish (A), shredders (B), and predators (C). Each point represents the mean N:P excretion and mean body N:P for the group of macroinvertebrates at each site where they were found.

Marshall and Wallace 2002). Seston was the resource with the lowest N:P (Table 1), and chironomids are more P-rich than other aquatic insects (N:P $\approx$ 30) (Cross et al. 2003, Frost et al. 2003, Evans-White et al. 2005). Thus, high P excretion in hydropsychids may be the result of a low N:P diet.

Body stoichiometry of shredders was similar to that of other functional feeding groups, but their diet was among the most P deficient (Appendix 2, Table 1). The diets of *Pteronarcys* and *Pycnopsyche*, the 2 shredders in our study, consist of leaf detritus (Benke and Wallace 1997, Hutchens et al. 1997, Benke et al. 2001, Rosi-Marshall and Wallace 2002), which had higher N:P than the other resources in our study. Thus, consumption of leaves by shredders should lead to high stoichiometric imbalances. However, bulk nutrient content may be misleading (Anderson et al. 2004). Shredders ingest low-quality foods high in cellulose, lignin, and phenols, which may reduce assimilation efficiencies and slow the acquisition of nutrients necessary for growth and reproduction (Frost and Tuchman 2005). The stoichiometric imbalance experienced by shredders might have been higher than ratios suggested because resource N content can include refractory N compounds that are difficult to assimilate (Balseiro and Albarino 2006). Leaf detritus had highest C:P and C:N of all resources in our study (results not shown). We expected shredders to have a high N:P excretion ratio because of their P-deficient diet, but their N:P excretion values were low. Thus, shredder growth could have been colimited by N and P relative to C. To test this possibility, nutrients in egestion would have to be included in the mass–balance equation. For example, Balseiro and Albarino (2006) measured both shredder nutrient excretion and egestion and were able to draw strong conclusions regarding strict homeostasis and evidence for N limitation. Another possible explanation for low N:P excretion rates is that shredders may feed selectively on more nutrient-rich resources, such as microbes associated with leaf detritus (e.g., Cummins 1974), which are rich in P content.

Members of Cyprinidae (redbelly dace, longnose dace, blue head chub, and central stoneroller) tended to have high P excretion relative to their body % P (Fig. 3A). Omnivorous cyprinids may have a diet richer in P than recent literature suggests. For example, our epilithic algae N:P values were lower than values for insect tissue, a result indicating a more P-rich diet for herbivorous than for predatory fish (Table 1). Detritivorous fish tend to have high P excretion (Brabrand et al. 1990, Schaus et al. 1997, Torres and Vanni 2007). However, longnose dace, which also had high P excretion, feed exclusively on benthic macroinvertebrates (Appendix 3). Phylogeny is apparently a better predictor than feeding guild of nutrient-specific growth efficiencies (Hendrixson et al. 2007), a conclusion supported by the fact that all of the fish with high P excretion were cyprinids. However, some detritivorous fish may extract P from inorganic material and either recycle it through excretion following assimilation or convert it to soluble form while in the gut (Cosper and Reeve 1975).

TABLE 5. N:P ratio (molar) of diet, predicted N:P excretion with 2 different maximum gross growth efficiencies (GGE), and measured N:P excretion for 7 species of fish. *n* refers to number of individuals.

		Diet N:P		Predicted excretion N:P Sterner's (1990) model		Measured excretion N:P	
Species	*n*	Mean	Range	GGE = 35%	GGE = 70%	Mean	Range
Mottled sculpin	28	44	30–57	42–84	84–175	273	2–499
Longnose dace	5	49	46–52	66–74	133–151	9	3–15
Rainbow trout	10	36	28–41	34–55	56–106	21	5–23
Brook trout	9	37	27–44	34–60	60–110	36	15–65
Bluehead chub	10	38	30–45	39–62	71–121	20	1–46
Southern redbelly dace	9	37	28–47	38–67	73–136	10	5–15
Central stoneroller	4	40	39–41	55–57	108–133	8	4–11

Some patterns in our study seem to support the conclusion that diet may influence excretion, but the AIC model showed that diet explained very little variation relative to family, functional group, and body composition. Family alone explained 71% of the variation for macroinvertebrates and 31% of the variation for fish. Functional feeding group, which is closely related to family classification in macroinvertebrates, also explained a great deal of variation in N:P excretion, at least for macroinvetebrates. Thus, trophic classification, as well as diet, may explain a relatively small amount of variation relative to family (phylogeny). Body composition explained more variation in N:P excretion for macroinvertebrates (42%) than fish (11%), but this result may have been an artifact of differences in family diversity between fish and macroinvertebrates.

Comparison with Sterner's model predictions

Differences between our measured values and predictions based on Sterner's model could arise in 3 ways: 1) our organisms did not fit the assumption of the model (i.e., nutrient homeostasis), 2) our data were inadequate because we did not measure egestion, so an important component of the mass–balance inherent in Sterner's model was missing, or 3) our data were inaccurate. Inaccuracies in our data could have arisen through erroneous assignment of diets (discussed below) or inaccurate measurement of excretion rates caused by handling stress or because the animals were not at equilibrium because of their stage of growth, size, or age.

Only 2 of 11 macroinvertebrate taxa and 3 of 7 fish species had measured N:P excretion ranges that overlapped with or were higher than the model-predicted N:P excretion values (Table 5). Most measured N:P excretion values were far below model predictions, even when we set GGE at unrealistically low levels (35%). However, crayfish and mottled sculpin N:P excretion values exceeded or overlapped the model predictions. This result suggests that crayfish and mottled sculpin are more strictly homeostatic and possibly P-limited, whereas homeostasis and P-limitation in the other consumers is uncertain. Our results were similar to those of Vanni et al. (2002), who found that loricarid catfishes had higher body P content than other organisms and excreted little P. Hood et al. (2005) suggested that loricarid growth was limited by the P in their diet. In our study, a few other individuals besides crayfish and sculpin might have been influenced by P in their diet. For example, some heptageniid mayflies, brook trout, and bluehead chub also had high N:P excretion values. However, most individuals in these groups had N:P excretion values well below model predictions. Schindler and Eby (1997) suggested that N:P excretion rates should be highly variable if N or P is limiting fish growth. However, they found little variation in N:P excretion rates and concluded that P-limitation is rare in fish and that most fish are energy (C) limited. The general lack of fit between measured N:P excretion in our study and model predictions suggest several possible scenarios.

First, growth might be limited by an element other than N or P (probably C). Based on work by Urabe and Watanabe (1992), Sterner and Elser (2002) proposed a threshold elemental ratio (TER) for C:nutrient ratios. TER is calculated from the consumer's body C:nutrient ratio, assimilation, respiration, and ingestion. TERs are defined as the diet C:nutrient ratios at which nutrient limitation switches to C limitation. We compared C:P TER values ($TER_{C:P}$) determined by Frost et al. (2006) to diet C:P for several of the groups we studied (Fig. 6). Pteronarcyids (shredders) and crayfish had higher diet C:P than their $TER_{C:P}$ and low P excretion, a result suggesting P limitation. Seston C:P was higher than $TER_{C:P}$ for hydropsychids, suggesting P limitation, but hydro-

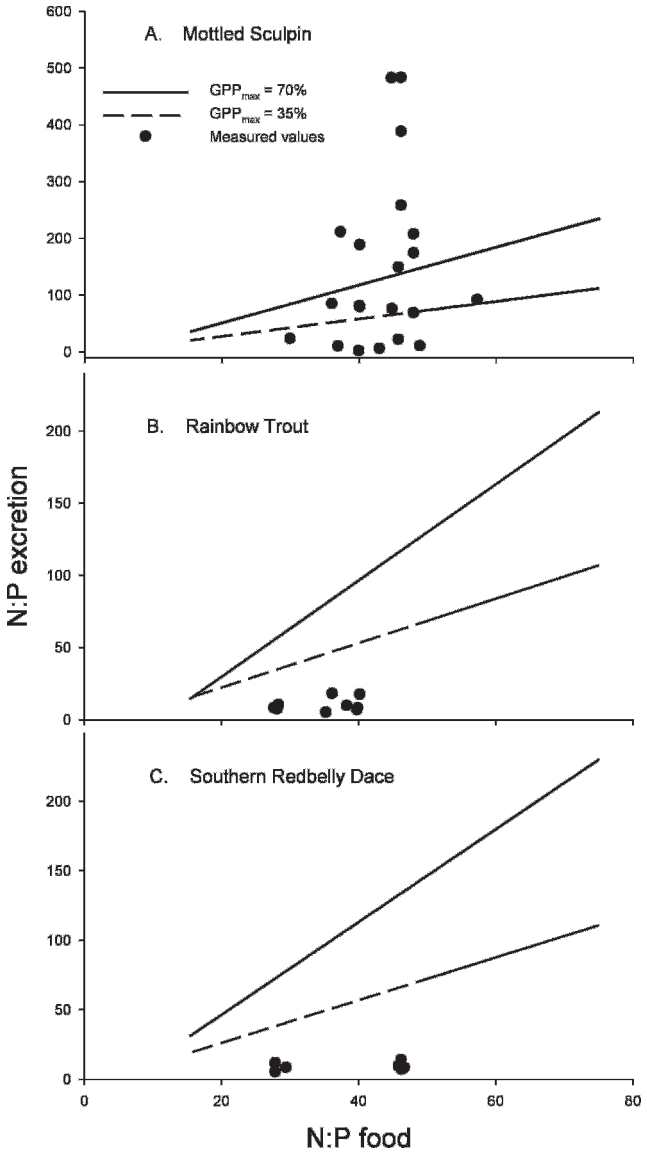

FIG. 5. Predicted N:P (molar) excretion vs N:P food calculated from Sterner's (1990) model with 2 different values of maximum gross growth efficiency (GGE_{max}) and compared to measured N:P excretion for mottled sculpin (A), rainbow trout (B), and southern redbelly dace (C). Each point represents the N:P excretion of an individual fish.

psychid P excretion was among the highest in our study. However, hydropyschids may be mostly predaceous. If we replace the seston C:P value with the lower chironomid C:P value, the data suggest C rather than P limitation and explain the high P excretion. Members of the family Perlidae, predatory stoneflies, had slightly lower P excretion than other families with $TER_{C:P}$ > diet C:P. Predators are more likely to be C than nutrient limited because their diet is biochemically similar to the biochemical makeup of their bodies (Anderson et al. 2004), and their food is more efficiently absorbed resulting in higher growth efficiencies (Anderson et al. 2005) and lower N and P waste.

Second, some consumers might have flexible homeostasis. Evidence has been found for both flexible and strict homeostasis in consumers (Sterner and Shultz 1998, Frost and Elser 2002, Cross et al. 2003, Bowman et al. 2005, Evans-White et al. 2005, Glaholt and Vanni 2005). We examined consumer N:P relative to respective diet N:P of consumers found at multiple sites (heptageniids and mottled sculpin). Heptageniid body N:P was not significantly related to organic or bulk N:P diet content (organic: $r^2 = 0.0005$, $p = 0.94$; bulk: $r^2 = 0.0102$, $p = 0.74$; Fig. 7A), and mottled sculpin body N:P was not significantly related to N:P diet content ($r^2 = 0.12$, $p = 0.11$; Fig. 7B). The range in body N:P of both consumers, especially heptageniids, was quite variable. However, in both cases, the range in body N:P was much lower than the range in diet N:P. We are limited in our ability to designate the extent of homeostasis, but we conclude that consumers are not "what they eat," and consumer waste (including egestion) should be influenced by diet.

Third, the actual diet of consumers may be far more P-rich than we assigned. For example, cyprinids may be P-limited ($TER_{C:P}$ < diet C:P), but their P excretion was high and most cyprinids excreted much lower N:P than Sterner's model predicted. Hood et al. (2005) found that the content in the foregut of herbivorous fish consistently had a lower N:P (higher P) than did randomly collected biofilm from the stream. The nutrient composition of the contents in the foregut of cyprinid fish (N:P ≈17) was very similar to the fish composition (N:P = 15) (Sterner and George 2000). These dietary N:P values are considerably lower than those in our study. Our N:P values for epilithon ranged from 28 to 81, much higher than the average N:P ratio of the cyprinids (~12). Thus, consumers could be selectively consuming foods that more closely meet their nutritional demands. However, we were able to assign diets accurately for fish predators, so our assessment of the N:P in their diet had little room for error. Macroinvertebrates also can exhibit selective consumption. Hall and Meyer (1998) suggested that heptageniid mayflies consumed a large portion of the bacterial fraction in biofilms, and bacteria have considerably lower N:P ratios (4–30) (Chrzanowski et al. 1996) than heptageniids (mean = 53; Appendix 1). Another possibility is that consumers could be ingesting P-rich inorganic matter (Forster and Gabbott 1971), which can become soluble in the gut (Cosper and Reeve 1975). We were unable to

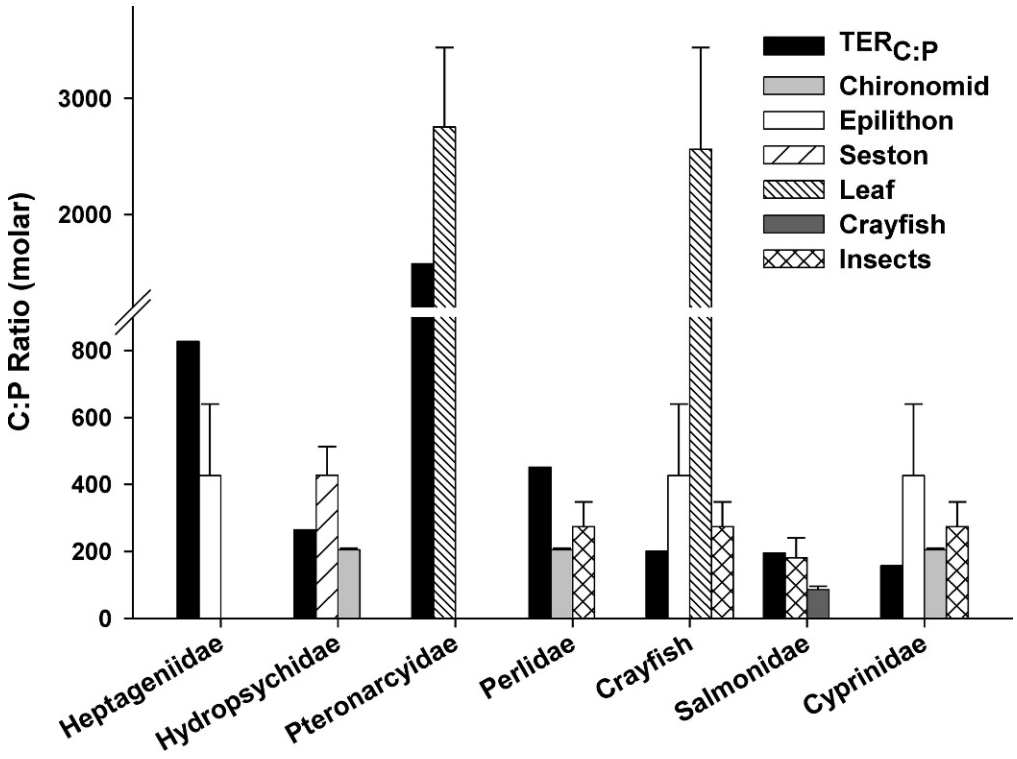

FIG. 6. C:P threshold elemental ratios ($TER_{C:P}$) compared to C:P of potential diets for several groups used in our study. TER values are from Frost et al. (2006).

differentiate among sources of P in excretion (assimilated or leached from ingested material). Thus, disassociated P from ingested inorganic matter could have led to high P excretion.

Lack of egestion data could have significantly affected our results if macroinvertebrates and fish differentially assimilated N and P. Little information is available on nutrient egestion or assimilation efficiencies in natural populations of macroinvertebrates or fish, but one example illustrates how including egestion might influence our results. Glaholt and Vanni (2005) measured N and P ingestion, egestion, excretion, and growth of bluegill sunfish (*Lepomis macrochirus*) fed earthworms in laboratory studies. N assimilation efficiency was 63%, and P assimilation efficiency was 93% (their table 1, low ration). If we apply these assimilation efficiencies to our data for southern redbelly dace with a body N:P = 8.68 (Appendix 3) feeding on a diet with N:P = 37 (Table 5), the N:P of assimilation would be 25.1. This value is greater than body N:P, so growth would be controlled by the net growth efficiency (growth/assimilation) for P. We used data from Glaholt and Vanni (2005; table 1, low ration) to calculate a P net growth efficiency of 64%. We applied this efficiency to P assimilation to calculate P growth, and then calculated the accompanying N growth based on body N:P. For both N and P, we could then calculate excretion as assimilation minus growth. The resulting N:P of excretion was 54.8. We used the same data (body N:P = 8.68, diet N:P = 37, GGE = P assimilation efficiency × P net growth efficiency = 60%) in Eq. 1 to calculate excretion N:P = 79.4. Therefore, if P assimilation efficiency > N assimilation efficiency, then the predicted excretion N:P is lower than N:P calculated using Sterner's model unless we explicitly account for the difference in N and P egestion. Nevertheless, the value remains much larger than our measured excretion N:P for redbelly dace (mean = 10; Table 5).

This example indicates that egestion may account for some of the differences between our measured and model-predicted excretion N:P. If our goal is to understand nutrient balance in stream consumers, then further measurements of nutrient assimilation and egestion are needed. However, nonassimilated (i.e., egested) nutrients may be in a relatively refractory and unavailable form. If we are trying to understand the consumer contribution to nutrients available to primary producers and heterotrophic microbes, measurements of nutrient excretion may be highly valuable (e.g., Grimm 1988, Vanni et al. 2002, McIntyre et al. 2008).

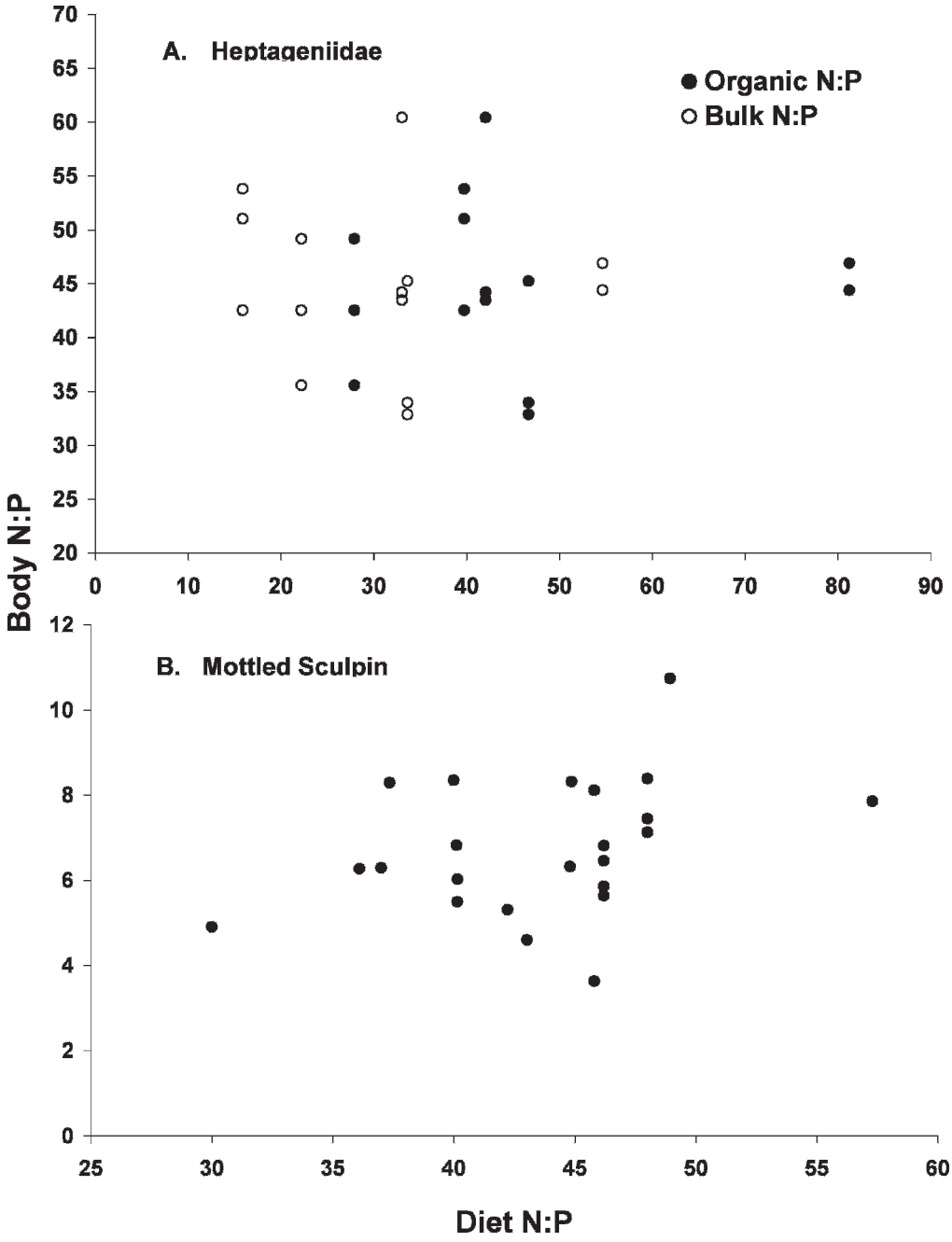

FIG. 7. Body N:P (molar) relative to diet N:P for heptageniid mayflies (A) and mottled sculpin (B) across multiple sites (5 sites for Heptageniidae, 4 sites for mottled sculpin). Diet for heptageniid mayflies was evaluated as either organic N:P (inorganic N and P removed) or bulk N:P (inorganic and organic N:P).

Consumer excretion and stream nutrient dynamics

Fish and macroinvertebrate assemblages exhibit top-down control on algal and bacterial production through N and P excretion (Schaus et al. 1997, Vanni et al. 2002). Using fish-abundance data from Freeman et al. (1988) and macroinvertebrate standing-stock data from Huryn and Wallace (1987), we compared the flux of N and P excretion from the entire consumer assemblage to the flux leaving the lower weir on Ball Creek. Excretion from the consumers was 1.5 to 2% of the total inorganic N and 12 to 119% of SRP leaving the lower weir on Ball Creek, depending on time of year. These calculations ignore reuptake, but they suggest that excretion, at times, may make up a large percentage of the P flux from the watershed

and may influence nutrient supply to autotrophic and heterotrophic microbes within Ball Creek, especially at times when stream nutrient levels are low.

The excretion N:P for most consumers, whether fish or macroinvertebrate, was lower than water-column N:P. If consumers are important contributors to nutrient dynamics in streams, then excretion values suggest that consumers alter the N:P ratio of inorganic nutrients available to microbial assemblages by providing nutrients at relatively lower N:P than is available in the water column. However, excretion N:P of organisms that showed evidence of possible P-limited growth, mottled sculpin and crayfish, was similar to or higher than water-column N:P. P-limited consumers might influence the return of different nutrients to other organisms by providing less P. For example, hydropsychid standing stock is only ⅓ that of crayfish in upper Ball Creek (Huryn and Wallace 1987), but hydropsychids excrete 160× more P than crayfish. This statement should not be interpreted to mean that some organisms are P sources and others are P sinks, an assertion that would entail a complete estimate of an ecosystem nutrient budget. All consumers excrete and are inherently temporary sources of nutrients to microbial assemblages.

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Appendix 1. Mean (±1 SE) macroinvertebrate body % P and N:P content and N, P, and N:P excretion. N and P excretion are expressed in μmol g^{-1} dry mass [DM] h^{-1}. Numbers followed by different letters indicate significantly different groups within columns at $\alpha = 0.05$ (Tukey's Honestly Significant Difference). Each of the *n* body-content samples consisted of 1 to 30 individuals depending on size. The number of excretion measurements ranged from 5 (in only 1 site) to 25 (in 5 sites).

Family	*n*	Body P (% DM)		Body N:P (molar)		N excretion		P excretion		N:P excretion (molar)	
Baetidae	3	0.27 (0.02)	c	89 (6.71)	a	7.07 (3.93)	bcde	0.55 (0.19)	bc	24.9 (10.8)	b
Hydropsychidae	8	0.43 (0.03)	bc	59 (7.06)	bc	8.07 (1.14)	bc	5.65 (0.80)	a	1.52 (0.18)	d
Perlodidae	1	0.39	bc	70	abc	23.0 (2.10)	a	4.57 (0.81)	ab	5.39 (0.63)	bcd
Limnephilidae	3	0.57 (0.02)	b	37 (0.30)	cd	0.31 (0.12)	cde	0.20 (0.10)	c	2.78 (1.34)	bcd
Pteronarcyidae	6	0.56 (0.02)	b	45 (1.74)	bc	0.12 (0.04)	e	0.07 (0.02)	c	6.28 (2.63)	cd
Gomphidae	3	0.37 (0.03)	bc	70 (4.65)	abc	0.94 (0.13)	cde	0.17 (0.07)	c	12.0 (4.54)	bcd
Perlidae	3	0.47 (0.11)	bc	70 (22.3)	abc	0.45 (0.11)	cde	0.21 (0.12)	c	4.23 (1.17)	bcd
Corydalidae	6	0.54 (0.04)	b	48 (2.83)	bc	0.28 (0.06)	de	0.04 (0.02)	c	13.3 (3.65)	bc
Isonychiidae	5	0.51 (0.05)	b	49 (4.39)	bc	7.74 (3.03)	bcd	2.09 (1.50)	bc	12.0 (2.88)	bc
Heptageniidae	14	0.53 (0.02)	b	45 (1.83)	bc	12.5 (1.47)	b	1.50 (0.33)	bc	21.3 (8.00)	b
Cambaridae	12	1.06 (0.03)	a	15 (0.48)	d	1.76 (0.17)	de	0.01 (0.004)	c	544 (138)	a

Appendix 2. Mean (±1 SE) macroinvertebrate functional feeding group (FFG) body % P and N:P content and N, P, and N:P excretion. N and P excretion are expressed in μmol g^{-1} dry mass [DM] h^{-1}. Numbers followed by different letters indicate significantly different groups within columns at $\alpha = 0.05$ (Tukey's Honestly Significant Difference). Each of the *n* body-content samples consisted of 1 to 30 individuals depending on size. The number of excretion measurements ranged from 15 (in 3 sites) to 25 (in 5 sites).

FFG	*n*	Body P (% DM)		Body N:P		N excretion		P excretion		N:P excretion (molar)	
Scraper	14	0.53 (0.02)	bc	45 (2.02)	b	12.5 (1.47)	a	1.50 (0.33)	b	21.3 (8.00)	b
Filterer	8	0.43 (0.09)	bc	59 (7.06)	ab	8.07 (1.14)	a	5.65 (0.80)	a	1.52 (0.18)	d
Predator	13	0.47 (0.04)	bc	60 (5.65)	ab	4.98 (1.88)	b	1.01 (0.39)	c	9.64 (1.85)	bc
Shredder	9	0.56 (0.01)	b	43 (1.76)	b	0.18 (0.05)	c	0.11 (0.04)	c	5.11 (1.83)	cd
Collector	8	0.42 (0.05)	c	64 (8.02)	a	7.52 (2.06)	a	1.57 (1.00)	b	16.3 (4.15)	bc
Crayfish	12	1.06 (0.03)	a	15 (0.48)	c	1.76 (0.17)	b	0.01 (0.00)	d	544 (138)	a

Appendix 3. Mean (±1 SE) fish body % P and N:P content and N, P, and N:P excretion. N and P excretion are expressed in μmol g^{-1} dry mass [DM] h^{-1}. Numbers followed by different letters indicate significantly different groups within columns at $\alpha = 0.05$ (Tukey's Honestly Significant Difference). Sample size (*n*) refers to number of individuals used in the analysis.

Species	*n*	Body P (% DM)		Body N:P (molar)		N excretion		P excretion		N:P excretion (molar)	
Mottled sculpin (*Cottus bairdi* Girard)	28	3.30 (0.15)	a	6.74 (0.33)	c	3.47 (0.33)	c	0.15 (0.07)	b	273 (67.6)	a
Southern redbelly dace (*Phoxinus erythrogaster* Rafinesque)	9	2.72 (0.29)	ab	8.68 (0.86)	c	10.1 (0.82)	a	1.11 (0.09)	a	9.90 (0.88)	b
Longnose dace (*Rhinichthys cataractae* Valenciennes)	5	2.31 (0.11)	bc	9.06 (0.49)	b	6.08 (0.79)	abc	1.02 (0.40)	a	8.92 (1.88)	b
Central stoneroller (*Campostoma anomalum* Rafinesque)	4	2.21 (0.16)	bc	9.36 (0.88)	b	3.18 (0.46)	bc	0.40 (0.02)	ab	8.28 (1.10)	b
Bluehead chub (*Nocomis leptocephalus* Girard)	10	2.00 (0.13)	bc	12.6 (0.95)	ab	7.63 (0.90)	abc	0.87 (0.41)	a	20.0 (4.32)	b
Brook trout (*Salvelinus fontinalis* Mitchill)	10	1.83 (0.06)	c	14.2 (0.48)	a	8.67 (0.62)	ab	0.34 (0.05)	ab	35.9 (4.99)	ab
Rainbow trout (*Oncorhynchus mykiss* Walbaum)	10	1.66 (0.11)	c	15.8 (1.62)	a	8.24 (2.72)	ab	0.78 (0.14)	ab	21.4 (1.39)	b