

**The effect of resource stoichiometry on fish and macroinvertebrate
nutrient excretion**

By

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ABSTRACT

Consumer-driven nutrient cycling has been shown to be an important process in supplying inorganic nutrients to autotrophic and heterotrophic organisms in aquatic ecosystems. Theory indicates that consumer nutrient excretion is influenced primarily by an organism's nutrient composition; however, an organism's diet should also play an important role in nutrient excretion, especially if the consumer is nutrient limited. This study asks the question, how does diet influence nutrient excretion of consumers at different trophic levels? Macroinvertebrates and fish were collected from six streams and nitrogen (N) and phosphorus (P) excretion were quantified. Epilithon, leaf detritus, and seston (fine particulate organic matter in transport) were collected and analyzed for carbon (C), nitrogen (N), and phosphorus (P) content in an attempt to qualitatively assess the nutritional status of the diet of primary consumers. Macroinvertebrates were also analyzed for C, N, and P content to assess their nutritional composition in relation to their excretion and also to assess the nutritional composition of the diet of predatory insects and fish. Fish were also analyzed for C, N, and P.

Similar to theoretical predictions, fish and macroinvertebrate P excretion was negatively related to P content and the N:P excretion ratio was negatively related to the body N:P ratio. However, this relationship was driven primarily by two phosphorus rich species, mottled sculpin in the fish and crayfish in the macroinvertebrates. Some relationships did emerge between consumer excretion and diet. For example, hydropterygids had the highest macroinvertebrate P excretion, possibly explained by the low N:P of seston. However, shredders, eating on a very low N and P diet of leaf detritus, had very low N and P excretion.

The relationship between consumers, their food, and nutrient excretion is a matter of mass balance. If the food N:P ratio is higher than that of the consumer, then the N:P excretion should be higher than the consumer N:P and the food N:P, especially if organisms are P-limited. However, N:P excretion by macroinvertebrates and fish were very similar despite large differences in diet. The majority of macroinvertebrates and fish had a lower N:P excretion ratio than the predicted N:P of their food, possibly indicating that 1) consumers were either selectively consuming more P-rich foods than the diets that I assigned them or 2) consumers are generally not N or P limited or influenced by the N or P in their diet. Mottled sculpin and crayfish were the only organisms with a higher N:P excretion than their resources and both had a higher %P than the other fish and macroinvertebrates, respectively. High N:P excretion along with high phosphorus content is indicative of P-limitation. Macroinvertebrates and fish, excluding mottled sculpin and crayfish, had a lower N:P excretion and the N:P ratio of the water column. If consumers do play a role in nutrient dynamics, then consumers could alter the relative abundance of nitrogen and phosphorus by supplying more phosphorus. However, the

presence of a P-limited organism, such as mottled sclupin or crayfish, could alter the relative abundance of nitrogen and phosphorus by supplying less phosphorus.

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INTRODUCTION

Consumer nutrient cycling is an important process of supplying inorganic nutrients to autotrophic and heterotrophic communities in aquatic food webs (McIntyre et al. 2007; Schindler 2007; Evans-White and Lamberti 2006; Evans-White and Lamberti 2005; Vanni et al. 2002; Vanni et al. 1997; Schaus et al. 1997; Kitchell et al. 1979). Differences in nutrient excretion among taxa are the result of variations in consumer nutrient composition, especially phosphorus content (Vanni et al. 2002; Sterner and Elser 2002). Variations in the relative abundance of different elements in a consumer's body, that is its stoichiometry, is primarily related to phylogeny (Evans-White et al. 2005; Fagan et al. 2002). Recently, however, differences in body stoichiometry alone have been unable to explain variability in nutrient excretion among different taxa, possibly indicating the importance of dietary nutrients (Torres and Vanni 2007). Variability in consumer stoichiometry is also due to body size, growth rate, ingestion rate, and resource allocation (Cross et al. 2005; Gaholt and Vanni 2005; Stener and Elser 2002).

Organisms assimilate elements from their food to maintain internal homeostasis and then excrete excess nutrients. Food resources such as detritus, algae, and animal tissue can vary extensively in their C:N:P values, creating a large degree of variation between consumers and their food resource. Most studies have shown that consumers display strict homeostasis despite changes in nutrient availability (Sterner and Elser 2002; Bowman et al. 2005); however, some studies have indicated possible non-homeostasis in consumers (Cross et al. 2003; Glaholt & Vanni 2005; Frost and Elser. 2002). Assuming consumers maintain homeostasis, differences in resource nutrient ratios should alter nutrient excretion. If a nutrient becomes limiting, organisms must either eat a nutritionally superior diet or differentially excrete non-limiting nutrients. The implications of altered resource stiochiometry, whether nutrient deficient or more nutrient rich, include stress or alleviating stress associated with requirements of reproduction and growth (Bowman et al. 2005; Urabe et al. 2003; Frost and Elser 2002; Elser et al. 2000a), which also may have implications for nutrient excretion (Dodds et al. 2004; Vanni 2002; Shindler and Eby 1997; Elser and Urabe 1999). Vanni (2002) indicated that because

nutrient excretion is a function of mass balance, nutrient excretion must be influenced by the composition of a consumer's body and its food.

Organizing trophic relationships into elemental interactions creates a framework for testing predictions of how nutrient availability affects elemental relationships between consumers and their resources (Sterner and Elser 2002; Cross et al. 2003; Bowman et al. 2005; Frost et al. 2005). This framework can also be used to predict consumer nutrient excretion (Glaholt and Vanni 2005; Sterner and Elser 2002; Vanni et al. 2002; Vanni 2002; Elser and Urabe 1999). Elemental imbalance is defined as the dissimilarity in elemental composition between a consumer and its resource (Sterner and Elser 2002; Bowman et al. 2005). Because nutrient excretion is influenced by body stoichiometry and resource stoichiometry, evaluating excretion among different resource nutritional regimes may help predict the relationship between diet and nutrient limitation (Hood et al. 2005).

Stoichiometric theory indicates that N:P excretion should be positively correlated with N:P food (Vanni 2002). If consumers are limited by N or P in their diet, N and P excretion rates should vary as a function of their specific nutrient limitation. Schindler and Eby (1997) found that fish N:P excretion was not variable despite differences in diet. Thus, they concluded that because most fish were not influenced by their dietary N or P, fish were not N or P limited, but energy limited. Sterner and George (2002) found that the N:P ratio of the diet of cyprinids closely matched that of the fish's body; thus, they assumed that N:P excretion should also be similar to the N:P of the fish's body. However, Hood et al (2005) found that a species of tropical catfish was limited by phosphorus in its diet and had an extremely high N:P excretion ratio.

I predict that if the N:P elemental imbalance is large between a consumer and its food resource, then the non-limiting nutrient, in this case nitrogen, will be excreted in excess. If N:P imbalance is small, then there will be less excretion of nitrogen and phosphorus should be excreted in greater amounts. This principle should also apply to C:P and C:N ratios. For example, when C is high (high C:P or C:N imbalances), then P or N may be limiting and will result lower N or P excretion.

Studies have evaluated the influences of nutrient availability on elemental imbalances (Bowman et al. 2005; Cross et al. 2003). However, evaluating the entire

elemental process from resource to consumer to excretion is important because excretion can shed light on nutrient allocation and nutrient limitation that may otherwise be incorrectly predicted from imbalances alone. Evaluating differences in nutrient excretion should provide a framework to understand nutrient flow through food webs, starting with basal resources through consumers and then back to inorganic nutrients. Also, because nutrient excretion is a function of consumer stoichiometry and diet, comparisons of multiple consumer/resource relationships, across and within trophic groups, will aid in accurately assessing specific diets of consumers, which has been a point of debate (Benke and Wallace 1997; Benke et al. 2001; Mihuc 1997; Mihuc and Minshall 1995). For example, if a specific consumer has a lower N:P excretion than what is predicted from their available resource, the consumer may be selectively eating a resource with a lower N:P. Finally, imbalances along with excretion can give insights into nutrient retention and constraints imposed on organisms feeding on a variety of resources.

In this study, I evaluated the stoichiometry of the dominant macroinvertebrates and fish, their excretion, and their diet in six streams to determine how differences in the nutritional composition of food resources alter nutrient excretion. Specifically, I evaluated the effect of the nutrient composition of food resources on nutrient excretion by observing 1) the relationship between body stoichiometry and nutrient excretion across taxa, 2) the effect of diet on excretion among and within taxa and functional feeding groups, 3) the effect of allometry, which is the proportional changes in body chemistry and physiology with size, on excretion among and within taxa, 4) the relationship between imbalance and excretion among and within taxa, and 5) created a food web conceptual model to illustrate how all trophic levels were related in terms of N and P. Also, I used relationships between nutrient availability and excretion to assess what consumers, especially macroinvertebrates, are consuming.

METHODS

Study Sites

My field sampling was conducted at three sites, Coweeta Hydrologic Laboratory in western North Carolina, Grayson Highlands State Park in southwestern Virginia, and agricultural land in Floyd County, Virginia. At each site, two separate 100-m reach stream sections were chosen totaling 6 stream sections (2 sections, Mira Fork open and forested, were located on the same stream). Streams consisted of an array of different nutrient loading regimes as well as differences in geology, vegetation, and riparian density (Table 1). Coweeta streams are characterized by a mixed hardwood vegetation of maple, poplar, and oak, and an understory of *Rhododendron*. Catchments are underlain by diorite gneiss and metasedimentary bedrock (Swank et al. 2001) and streams averaged 700 m altitude. Shope Fork is a highly unshaded stream bordered mostly by alder; however, it also had some upstream inputs of mostly hardwoods. Ball Creek, the forested stream, consisted of a understory of *Rhododendron* with maple and tulip poplar overstory. Grayson Highlands streams are characterized by higher altitude catchments of open balds and hardwood/ conifer mixes along with rhyolite bedrock outcrops. Cabin creek (1390 m altitude) has an understory of *Rhododendron* and an overstory of birch and maple. Cabin Creek has high nitrogen concentrations possibly due to high deposition from upper reaches around 1600 m altitude. The unnamed tributary of Big Wilson (from here on: Big Wilson) is similar in altitude (1330 m) to Cabin Creek but runs through a naturally open bald with intermittent springs and sphagnum bogs with patches of *Rhododendron* and birch. In Floyd County, Mira Fork (760 m altitude) runs through agricultural land with metasedimentary and granite geology and is lightly impacted by cattle grazing upstream of the study site. The upstream section runs through an unforested field dominated by grasses and patches of alder. The forested section is lightly canopied by birch and oak species. Mira Fork has high nitrogen and phosphorus concentrations due to fertilizer runoff from agriculture.

Field Sampling Overview

In each 100-m reach, four dominant macroinvertebrate taxa and three dominant fish taxa (if present) were collected and excretion was quantified and analyzed for inorganic N and P content. Macroinvertebrates and fish were analyzed for C, N, and P. Qualitative samples of epilithon, seston (SPOM), and leaf detritus (CPOM) were collected in each stream and later analyzed for C, N, and P content. Water samples were also taken and analyzed for inorganic N and P content.

Consumer Excretion and Body Stoichiometry

Macroinvertebrates were collected with d-frame nets and sorted in the field by family. Different taxa were placed in separate containers where fresh stream water was replenished. One to four individuals (depending on size) from each taxa were placed in a bag with 30 ml of prefiltered stream water each (Schaus et al 1997; Vanni et al. 2002). Five bags were used per taxa. Bags without any macroinvertebrates were used as controls. Bags were incubated for 1 hr in stream water to keep temperature constant, and water samples were collected, filtered, and immediately put on ice at the end of the incubation period.

Fish were collected with an electroshocker at low voltage and with nets. Fish were sorted by species, and individuals were placed into bags with 500 to 1000 ml of pre-filtered water depending on size and species. Five bags were used per fish species. Bags were incubated in the stream for 30 minutes, water samples were collected and immediately put on ice at the end of the incubation period. All excretion samples were frozen and later analyzed for nitrate ($\text{NO}_3\text{-N}$), ammonium ($\text{NH}_4\text{-N}$), and soluble reactive phosphorus (SRP) on a Lachat Quickchem Flow Injection Analyzer. N and P in background water samples were subtracted from N and P in bags to correct for background. No significant changes in N and P concentration were found in the control bags. After excretion samples were taken, macroinvertebrates and fish were frozen and later dried and weighed so that a dry mass specific excretion weight could be calculated. For body stoichiometry, individual fish were analyzed; however additional

macroinvertebrates samples, besides those used in excretion quantification, were collected to quantify C, N, and P body content because of small amounts of tissue available for analysis. Three separate macroinvertebrate samples were collected for each taxa in each stream.

Macroinvertebrates and fish were ground, and a subsample was used in a Variomax CNS Macro-Elemental Analyzer to determine C and N content. P content was determined by ashing a 10-mg sub-sample followed by acid hydrolysis in 0.5 N HNO_3^- (Sturner and George 2002). Inorganic P was then found by flow injection analysis of SRP on a Lachat Quickchem Flow Injection Analyzer.

Epilithon, Seston, and Leaf Detritus Collection

Epilithon was collected by scrubbing randomly collected rocks from three sections within each stream reach. From each section, the resultant slurry was collected and put into acid-washed nalgene bottles. Seston (0.45 μm – 1 mm) was collected by placing a 10- μm mesh net in the current for approximately 24 hrs in each stream. Seston collection was repeated 2 times for each stream and samples were put into separate acid washed nalgene bottles. Leaf detritus representing the dominant vegetation was randomly collected throughout each stream and put into clean plastic bags. Leaf samples were randomly divided into 4 samples. Four water samples were collected from each stream, filtered (Gelman AE), and put in acid washed nalgene containers. All samples were immediately placed on ice and transported back to lab where they were frozen. Epilithic algae samples and seston samples were thawed, vacuum filtered, and collected on glass fiber filters. Leaf detritus, epilithon, and seston samples were dried and ground, and subsamples were analyzed for C, N, and P similar to animal tissue. However, epilithon and seston slurries were composed of a large inorganic fraction. Therefore, I hydrolyzed 10 mg of unashed sample in 0.5N HNO_3^- to assess inorganic phosphorus that was associated with mineral substrate. I calculated organic P (the P available in the consumer's diet) by subtracting the P in the inorganic fraction from the P in the initial ashed sample (total P). Because organic P was calculated from the mean of the inorganic samples and the mean of the total P, one value for organic phosphorus was reported. Subsamples of dry material were ashed to obtain ash free dry mass (AFDM). Stream

water samples were analyzed for NO₃-N, NH₄-N, and SRP by flow injection analysis with a Lachat Quickchem Flow Injection Analyzer.

Diet Nutritional Values Assigned to Consumers

Resources for scrapers, collectors, filterers, and shredders were assigned according to functional feeding group (Merritt and Cummins 1996). Gut analysis of fish was conducted by cutting from the anus to the upper foregut and removing the digestive tract. The entire tract was dissected and invertebrates were identified to order. Nutrient ratios for predatory fish diets were calculated as weighted averages (weighted by percent gut contents) of representative macroinvertebrates in each order. Nutrient ratios of macroinvertebrates were based on values found in this study and published data from Evans-White et al. (2005), Cross et al. (2003), Frost et al. (2003), and Fagan et al. (2002). I found that central stoneroller, bluehead chubs, and redbelly dace consumed a small number of insects in addition to algae. Algal consumption was estimated as the difference between the actual number of insects in the gut and potential insects in the gut based on a regression against size for predatory fish. However, herbivorous fish ate such small numbers of insects that deviations from the nutritional content of a pure algal diet were minimal.

To assess the elemental imbalance between a consumer and its food, I used Bowman et al.'s (2005) imbalance formula. One limitation in the imbalance formula of Bowman et al. (2005) is that it does not differentiate between situations where the resources are more nutrient rich than the consumer. I add a positive or negative sign to indicate the direction of the imbalance. Nutrient imbalance is calculated as:

$$\sqrt{(X : Y_{diet} - a)^2 + (X : Y_{consumer} - a)^2}$$

where X is element 1 and Y is element 2

and where a is $(X : Y_{diet} + X : Y_{consumer}) / 2$

and

if $X : Y_{diet} > X : Y_{consumer}$, then $X : Y_{imbalance}$ is positive

if $X : Y_{diet} < X : Y_{consumer}$, then $X : Y_{imbalance}$ is negative

Statistical analysis

Analysis of variance was used to analyze differences in stoichiometric variables among streams. Two-tailed t-tests were used to analyze differences between streams within the same geographical site. Linear regressions were used to analyze relationships between consumer stoichiometry and excretion across nutritional gradients. Data were log transformed and arcsin square root transformed where appropriate.

RESULTS

Resource Stoichiometry

Nitrate ($\text{NO}_3\text{-N}$) concentration in the water ranged from $79 \mu\text{g L}^{-1}$ at Shope Fork to $440 \mu\text{g L}^{-1}$ at Cabin Creek and was significantly different among sites (ANOVA: $p < 0.0001$, Table 2). Ammonia ($\text{NH}_4\text{-N}$) was less variable than nitrate, although significantly different among sites ($p = 0.03$). Mira Fork (open and forested) had the highest mean phosphorus concentrations (open; $6.9 \mu\text{g L}^{-1}$ forest; $6.7 \mu\text{g L}^{-1}$); however, phosphorus was consistently very low and not significantly different among sites (ANOVA: $p = 0.06$). N:P (molar) ratio was not significantly different among sites ($p = 0.2$), though the mean N:P ratio at Cabin Creek was approx 780, 4.7- 14 times higher than the other streams.

Table 1. Riparian canopy cover, wetted width and dominant vegetation characteristic of each stream in the study. O = open. F= forest.

Site	Stream	Riparian (% Cover)	Wetted Width (m)	Dominant Vegetation
Coweeta Hydrologic Lab, NC	Shope Fork	9	3.9	Alder, Oak, Poplar, Birch, Rhododendron
	Ball Creek	96	5.7	Rhododendron, Oak, Poplar, Birch
Floyd Co., VA	Mira Fork O.	1	3.6	Alder, Birch, Oak
	Mira Fork F.	89	4.8	Birch, Oak
Grayson Highlands State Park., VA	Big Wilson	24	1.8	Rhododendron, Birch
	Cabin Creek	99	4.7	Birch, Maple, Rhododendron

Table 2. Water chemistry for each stream. N:P (molar) ratio is total inorganic nitrogen (NO₃-N and NH₄-N) divided by soluble reactive phosphorus. Range indicated within parentheses. O = open. F= forest.

	NO ₃ ⁻ -N (µg L ⁻¹)	NH ₄ ⁺ -N (µg L ⁻¹)	SRP (µg L ⁻¹)	N:P (molar)
Shope Fork	79 (64-92)	4.0 (bd – 8.2)	2.5 (bd – 5.7)	167 (36-327)
Ball Creek	95 (78-103)	3.6 (bd – 6.1)	3.4 (bd-6.1)	164 (38-411)
Mira Fork O.	290 (242-337)	7.7 (6.6 – 8.9)	6.9 (2.0 - 10)	161 (54-385)
Mira Fork F.	285 (244-344)	11 (7.1-15)	6.7 (5.1 – 7.9)	102 (74-152)
Big Wilson	130 (120-135)	13 (8.3-19)	6.4 (3.3 – 8.7)	57 (37-95)
Cabin Creek	440 (433-445)	9.4 (bd – 13)	4.3 (bd-6.3)	777 (157-2012)

Table 3. Epilithon stoichiometry for each stream. OM is percent organic matter expressed as %AFDM. Total %P is amount of phosphorus in the original rock scrubbing (original dry sample ashed and then acid hydrolyzed). Inorganic %P is the amount of phosphorus released by weak acid digestion. Organic %P is (Total %P – Inorganic %P) divided by %AFDM. %C and %N are expressed as AFDM values as well. C:P and N:P are based on organic P (%AFDM). Values in parenthesis are 1 SE. All ratios are molar. O = open. F= forest.

	OM (%AFDM)	Mean C (% AFDM)	Mean N (%AFDM)	Total P (%DW)	Inorganic P (%DW)	Organic P (%AFDM)	C:P	N:P
Shope Fork	9.24	34.7 (16.2)	6.37 (2.56)	0.080 (0.008)	0.047 (0.004)	0.3549	253	40
Ball Creek	10.3	37.1 (4.00)	5.51 (1.09)	0.055 (0.005)	0.030 (0.004)	0.2416	396	51
Mira Fork O.	16.1	34.7 (4.81)	5.27 (0.76)	0.103 (0.050)	0.036 (0.001)	0.4179	214	28
Mira Fork F.	17.8	39.1 (2.97)	6.55 (0.41)	0.088 (0.019)	0.034 (0.000)	0.3042	323	47
Big Wilson	28.4	49.7 (8.09)	7.38 (1.18)	0.085 (0.010)	0.027 (0.000)	0.2013	638	81
Cabin Creek	25.5	44.7 (5.05)	4.79 (0.46)	0.082 (0.010)	0.018 (0.001)	0.2526	457	42

Table 4. Stoichiometry of seston for each stream. OM is percent organic matter expressed as %AFDM. Total %P is amount of phosphorus in the original rock scrubbing (original dry sample ashed and then acid hydrolyzed). Inorganic %P is the amount of phosphorus released by weak acid digestion. Organic %P is (Total %P – Inorganic %P) divided by %AFDM. %C and %N are expressed as AFDM values as well. C:P and N:P are based on organic P (%AFDM). Values in paranthesis are 1 SE. All ratios are molar. O = open. F= forest.

	OM (%AFDM)	Mean C (%AFDM)	Mean N (%AFDM)	Total P (% DW)	Inorganic P (%DW)	Organic P (%AFDM)	C:P	N:P
Shope Fork	32.6	49.0 (3.9)	3.02 (0.25)	0.053 (0.003)	0.010	0.293	433	23
Ball Creek	33.3	40.5 (6.1)	2.49 (0.34)	0.043 (0.001)	0.018	0.204	513	27
Mira Fork O.	22.4	37.5 (0.6)	3.49 (0.01)	0.036 (0.002)	0.013	0.262	370	29
Mira Fork F.	12.3	40.2 -----	3.91 -----	0.022 -----	0.006	0.304	342	29
Big Wilson	-----	34.8 (4.6)	2.59 (0.11)	0.033 (0.004)	-----	-----	-----	-----
Cabin Creek	34.9	51.4 (1.4)	3.41 (0.15)	0.053 (0.001)	0.011	0.274	484	28

Epilithon percent C averaged 40% AFDM and percent N averaged 6% AFDM but there were no significant differences in epilithon %C and %N among streams (ANOVA: C, $p=0.75$; N, $p=0.75$) (Table 3). Ash free dry mass made up a small percentage of the original epilithic slurry indicative of high amounts of inorganic material. Total epilithon %P (including organic and inorganic fractions) was also not significantly different among streams ($p=0.78$). Significant differences were found in inorganic %P among streams ($p=0.001$), and 22 to 58% of P in the original slurry was inorganic. Because of differences in percent inorganic material, organic %P ($= (\text{Total P} - \text{Inorganic P}) / (\text{AFDM})$) ranged from 0.20% in Big Wilson to 0.42% in the open section of Mira Fork. Mira Fork open had the lowest epilithon C:P and N:P (C:P, 214; N:P, 28) whereas highest C:P and N:P epilithon values were found in Big Wilson (C:P, 638; N:P, 81).

Seston C (% of AFDM) averaged 42% and was not significantly different among streams (ANOVA, $p>0.5$, Table 4). Seston N (% of AFDM) was significant among streams ($p=0.05$) (Table 4). Organic material (%AFDM) was higher in seston than in epilithon, therefore, percent inorganic P made up a smaller percentage in seston (27-42%). Total %P of seston was significantly different among sites ($p=0.005$) (Table 4). Organic P (% of AFDM) ranged from 0.2% in Ball Creek to 0.3% in Mira Fork forest. Seston C:P was highest in Ball Creek (513) and lowest in Mira Fork forest (342). Shope Fork seston had the lowest N:P (23) while Mira Fork forest and Mira Fork open had the highest (29).

Table 5. Stoichiometric variables for leaf detritus in each stream. Values in paranthesis are 1 SE. All ratios are molar. O = open. F= forest.

	Mean C (%AFDM)	Mean N (%AFDM)	Mean P (%AFDM)	C:N	C:P	N:P
Shope Fork	54.9 (0.33)	1.62 (0.11)	0.070 (0.005)	40 (2.6)	2070 (137)	52 (0.5)
Ball Creek	56.0 (0.21)	1.06 (0.07)	0.044 (0.005)	63 (4.5)	3441 (371)	55 (4.8)
Mira Fork O.	53.9 (0.72)	2.44 (0.07)	0.085 (0.007)	26 (0.8)	1684 (157)	65 (4.4)
Mira Fork F.	55.9 (1.93)	2.08 (0.27)	0.075 (0.009)	32 (2.8)	1968 (187)	62 (2.6)
Big Wilson	55.1 (0.20)	1.32 (0.06)	0.048 (0.002)	49 (2.3)	3604 (223)	74 (6.5)
Cabin Creek	55.0 (0.43)	1.97 (0.05)	0.058 (0.007)	33 (1.1)	2543 (318)	77 (7.5)

Leaf detritus %C, %N, and %P were significantly different among all streams (ANOVA: C, $p=0.0002$; N, $p<0.0001$; P, $p=0.0008$) (Table 5). Leaf P was lowest in Ball Creek (0.021 % of AFDM) and Big Wilson (0.018 % of AFDM); however, only significantly different from Shope Fork, which had the highest P values (Tukey's test, $p<0.05$) (data not shown). With the exception of Cabin Creek, leaf C:P was significantly higher in Big Wilson and Ball Creek compared to the other streams, both of which were dominated by *Rhododendron* (ANOVA: $p<0.0001$, followed by Tukey's test, $p<0.05$). C:N and N:P were also significantly different among streams (C:N, $p<0.0001$; N:P, $p=0.007$). Ball Creek had significantly higher C:N than Shope Fork (Tukey's test, $p<0.05$).

Fish Stoichiometry

Body %P concentration was significantly different among fish (ANOVA: $p<0.0001$). Mottled sculpin had significantly higher body %P compared to other species, except redbelly dace (Tukey's test, $p<0.05$, Table 6). Body P concentration was not significantly different among the remaining species; however, the salmonid species had the lowest amounts of body P. Body P concentration in sculpin was 3.30 % of DW, two times higher than rainbow trout (1.66 %). N content was not as variable, although significantly different among fish species (ANOVA: $p<0.0001$). Brook trout, rainbow trout, and bluehead chub had significantly higher %N than the other fish (tukey's test: $p<0.05$). Body %C was also less variable, but also significantly different among fish (ANOVA: $p = 0.0003$). Body N:P and C:P varied among taxa (ANOVA: $p<0.0001$ and $p<0.0001$, respectively), with the lowest N:P and C:P values in mottled sculpin and redbelly dace. These two species were significantly different than the other fish species (tukey's test, $p<0.05$). The salmonid species had among the highest N:P and C:P values (Table 6). Although not significant, brook trout had a higher %P and lower C:P and N:P than rainbow trout (t-test: $p>0.05$).

Table 6. Fish C, N, P, C:P and N:P content. Numbers followed by different letters indicate significantly different fish species at the 0.05 significance level using Tukey's HSD test. Values within parentheses indicate 1SE. Ratios are molar. LND = longnose dace, RBT = rainbow trout, BT = brook trout, RBD = southern redbelly dace, BHC = bluehead chub, CS = central stoneroller, MS= mottled sculpin.

	n	Mean %C		Mean %N		Mean %P		C:P		N:P	
LND	5	47.6 (0.63)	a	9.37 (0.22)	b	2.31 (0.11)	bc	53.7 (2.87)	ab	9.06 (0.49)	b
RBT	11	47.2 (0.78)	a	11.1 (0.26)	a	1.66 (0.11)	c	77.1 (5.46)	a	15.8 (1.62)	a
BT	10	46.4 (0.36)	a	11.7 (0.07)	a	1.83 (0.06)	c	65.8 (2.25)	a	14.2 (0.48)	a
RBD	9	45.7 (0.85)	ab	9.82 (0.21)	b	2.72 (0.29)	ab	47.5 (5.35)	bc	8.68 (0.86)	c
BHC	10	45.1 (0.74)	ab	11.0 (0.16)	a	2.00 (0.13)	bc	60.4 (4.01)	ab	12.6 (0.95)	ab
CS	4	45.0 (0.68)	ab	9.17 (0.24)	b	2.21 (0.16)	bc	53.9 (4.32)	ab	9.36 (0.88)	b
MS	23	43.0 (0.69)	b	9.56 (0.10)	b	3.30 (0.15)	a	35.4 (1.86)	c	6.74 (0.33)	c

Diet and Imbalances

Mean N:P of diet was significantly different among fish species (ANOVA: $p=0.0004$). Longnose dace had significantly higher N:P of diet than the other species except mottled sculpin and central stoneroller (Tukey's test: $p<0.5$, Table 7- post hoc comparisons not shown). N:P imbalance was also differed significantly among fish species (ANOVA: $p<0.0001$) (data not shown). Longnose dace and mottled sculpin had significantly higher N:P imbalances than the other predatory fishes (salmonids) (Tukey's test: $p<0.05$). Mean C:P of diet was significantly different among species (ANOVA: $p<0.0001$), with significantly lower C:P of diet in salmonids (Tukey's test: $p<0.05$) compared to the other fish except central stoneroller. C:P imbalance was also significantly different among species (ANOVA: $p<0.0001$) with the salmonids having a significantly lower imbalance than the other species (Tukey's test: $p<0.05$). Longnose dace and mottled sculpin had significantly higher diet C:P ratios and C:P imbalances than the other predatory fish (salmonids) (Tukey's test: $p<0.05$). C:N diet and imbalance were significantly different among fish species (ANOVA: $p<0.0001$ for both) with redbelly dace and bluehead chub having the highest diet C:N ratio (7.1) as well as the highest C:N imbalance (Tukey's test: $p<0.05$). Predatory fish had a significantly lower C:N of diet (avg. 5.6) than omnivorous fish (redbelly dace, bluehead chub, and central stoneroller) (Tukey's test: $p<0.05$).

Table 7. Percent of each macroinvertebrate order found in gut analysis of each fish species from each stream and nutrient ratios of diet for each fish species in each stream. Explanations of diet nutrient ratios are described in the methods section. E=Ephemeroptera, P=Plecoptera, T=Trichoptera, O=Odonata, D=Diptera, De=Decapoda, Oth=Other, Terr=Terrestrial, Alg=Algae, Avg. Ind. = average # of individuals consumed per fish. RBT=rainbow trout, MS=mottled sculpin, CS=central stoneroller, LND=longnosed dace, BHC=bluehead chub, RBD=redbelly dace, BT=brook trout. (n refers to number of individuals of each species from each stream). Ratios are molar.

	Stream	n	E	P	T	O	D	De	Oth	Terr	Alg	Avg. Ind.	C:P	C:N	N:P
RBT	SF	6	17		30		4.8	2.4	2.8	49		12	193	5.6	36
	BC	5	9.0	7.5	16	2.5	17		0.4	48		14	184	5.6	36
MS	SF	8	22	2.3	49		14			13		5.9	253	5.8	43
	BC	5	13	6.1	31	10	24		0.6	15		11	228	5.6	42
	MFO	5	90							10		1.4	233	5.5	44
	MFF	5	27	10	53					10		3.0	239	5.7	45
CS	SF	5	1.9				2.9				95	0.8	252	6.3	40
LND	BC	5	50	17	35		5.4					9.8	266	5.7	49
BHC	MFO	5	1.5			0.9	1.3			1.5	95	0.8	213	7.6	28
	MFF	5	5.7		1.1		2.3		8.9	3.4	79	1.4	283	6.6	42
RBD	MFO	4					5.5				95	0.2	213	7.6	28
	MFF	5	16		3.9						80	1.0	309	6.6	46
BT	BW	5	3.3	2.7	13	4.9		1.3	2.2	73		14	136	5.5	34
	CC	5	15	3.3	15	1.7	7.8	1.7	3.3	49		8.4	199	5.4	40

Fish Excretion

Nitrogen and phosphorus excretion rates by fish varied significantly among species (ANOVA: N $p = 0.005$; P $p=0.0006$, Fig. 1A). Similarly, N:P molar excretion ratio was significantly different among species (ANOVA: $p<0.0001$ Fig. 1B). Central stoneroller, a herbivore, had the lowest mean N excretion rate ($3.18 \mu\text{mol NH}_4^+\text{-N g dry mass}^{-1} \text{ hr}^{-1}$, SE = 2.9), but it was only significantly different from redbelly dace, which had the highest N excretion rate (10.1) (Tukey's test, $p<0.05$) (post hoc comparisons not shown). Mottled sculpin had the lowest P excretion rate (mean = $0.148 \mu\text{mol SRP g dry mass}^{-1} \text{ hr}^{-1}$, SE=0.12); however, it was only significantly different than both dace species, which had the highest rate (tukey's test: $p<0.05$). Mottled sculpin had the highest mean N:P molar excretion ratio (mean=245, SE=73.2), significantly different than all other species except brook trout (Tukey's test, $p<0.05$). P excretion was significantly higher in rainbow trout than brook trout (t-test: $p=0.011$), and N:P excretion was significantly higher in brook trout (t-test: $p<0.0001$) (Fig. 2). P excretion was negatively related to P content (Fig. 3A); however, this relationship was weak (linear regression: $r^2=0.20$, $p<0.0001$). When only predatory fish were compared, there was a stronger negative relationship between P excretion and P content (linear regression: $r^2=0.36$, $p<0.0001$). N:P excretion was negatively related to N:P body content, although the relationship was also not strong (linear regression: $r^2=0.11$, $p<0.005$, Fig. 3B). Body %P was weakly related to dry weight (linear regression: $r^2=0.13$, $p=0.0016$, Fig. 4A), but there was no relationship between mass and P excretion (Fig. 4B).

For all fish, N:P excretion was not related to N:P imbalance due to a large amount of unexplained variation (linear regression: $r^2=0.11$, $p=0.11$, Fig. 5A). N:P excretion was high in mottled sculpin regardless of imbalance. N excretion was positively related to C:N imbalance ($r^2=0.17$, $p=0.0003$, Fig 5B) whereas P excretion was negatively related to C:P imbalance ; however, this relationship was weak (linear regression: $r^2=0.06$, $p=0.04$, Fig. 5C). Results for each individual fish species that occurred in more than one stream are discussed in the following paragraphs.

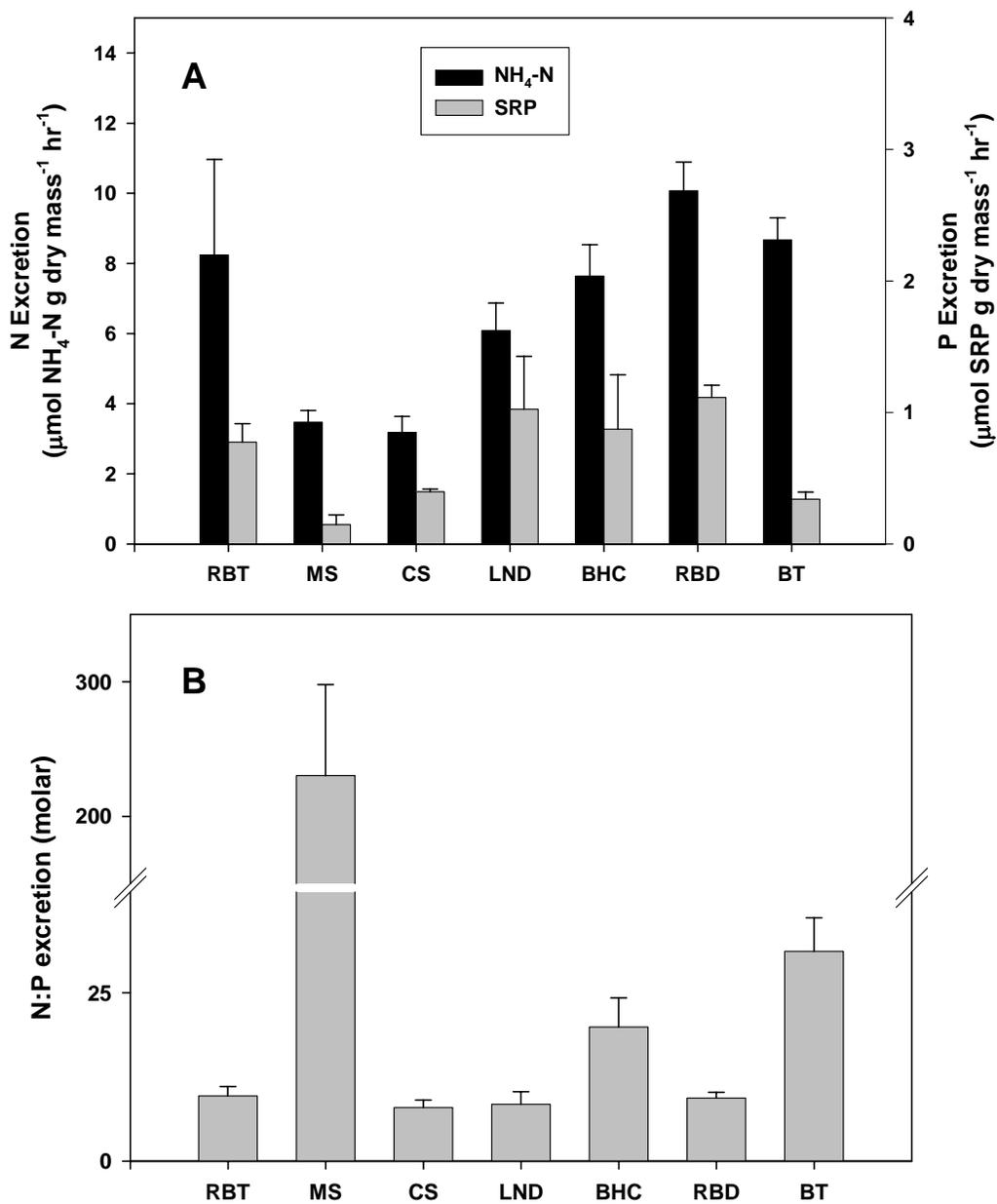


Figure 1: (A) N and P excretion rates for different fish species and (B) N:P molar excretion for different fish species. RBT= Rainbow trout, MS= mottled sculpin, CS= central stoneroller, LND= long-nosed dace, BHC= bluehead chub, RBD= southern redbelly dace, BT= brook trout. Error bars represent 1 SE.

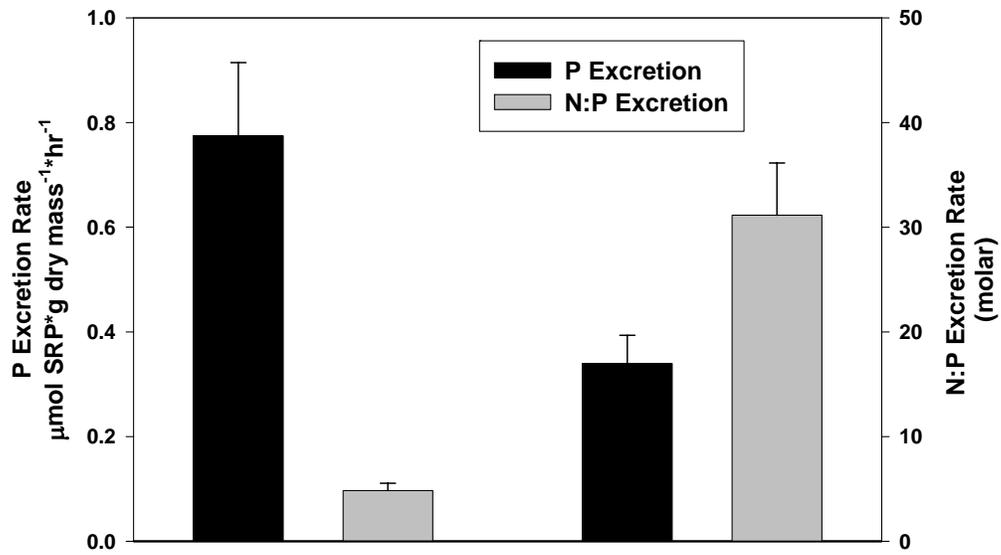


Figure 2: Comparison of two salmonids in P and N:P (molar) excretion. Rainbow trout (n=11). Brook trout (n=9). Error bars represent 1SE.

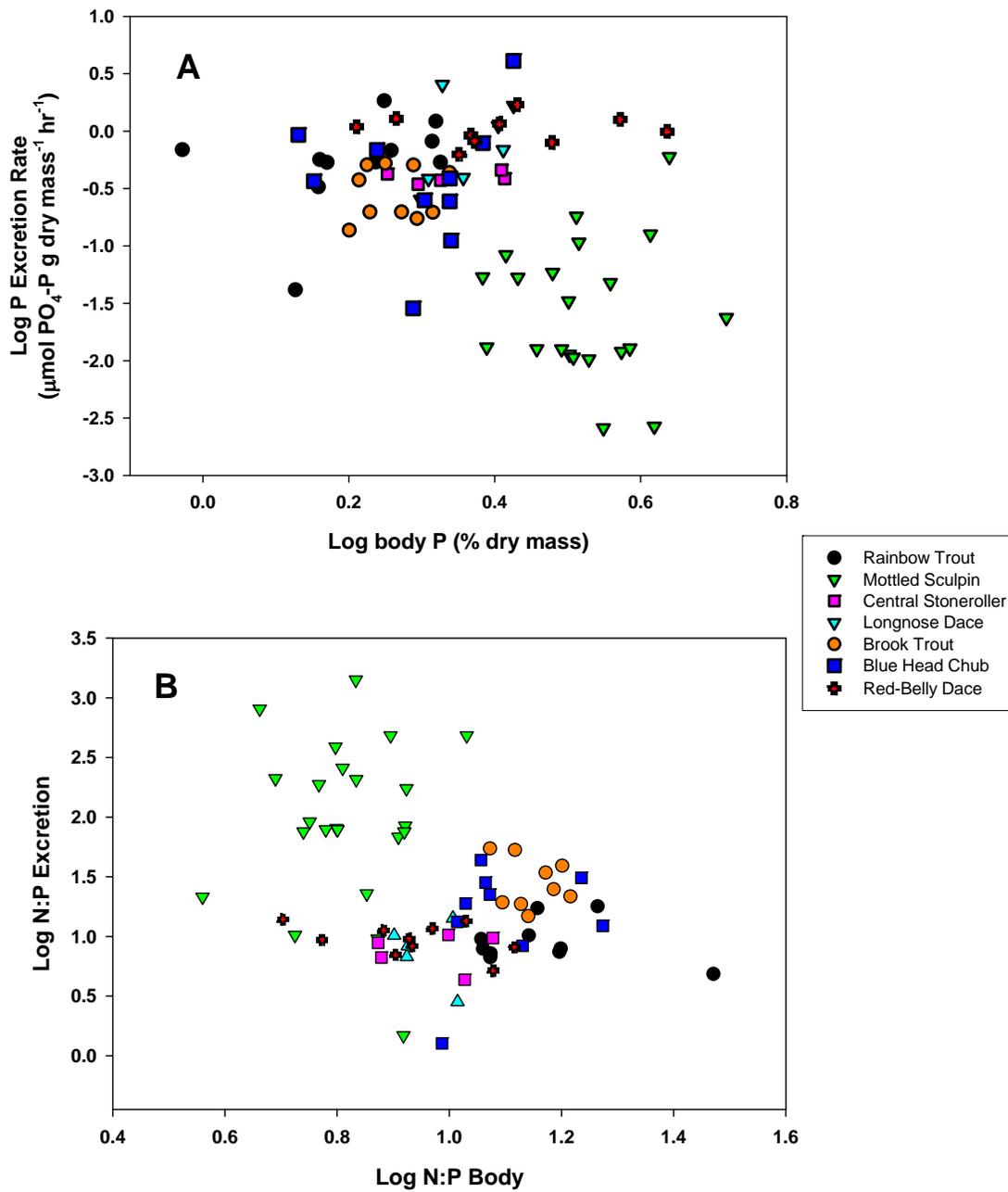


Figure 3. (A) P excretion in relation to body P and (B) N:P excretion vs body N:P ratio. Each symbol represents 1 individual. Different symbols indicate different species.

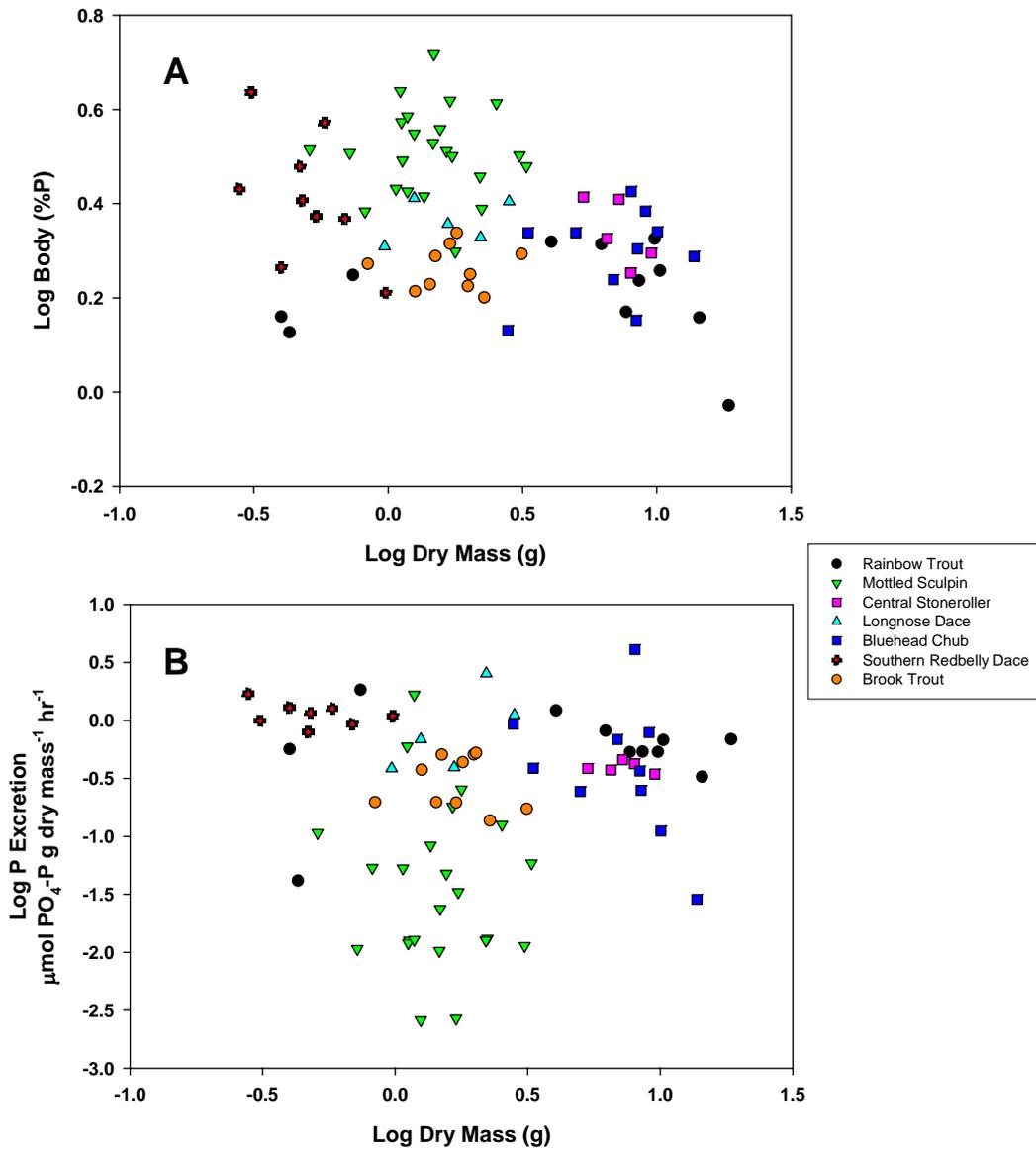


Figure 4. (A) Body P vs dry mass (g) and (B) P excretion vs dry mass (g) for all fish individuals. Each point represents an individual.

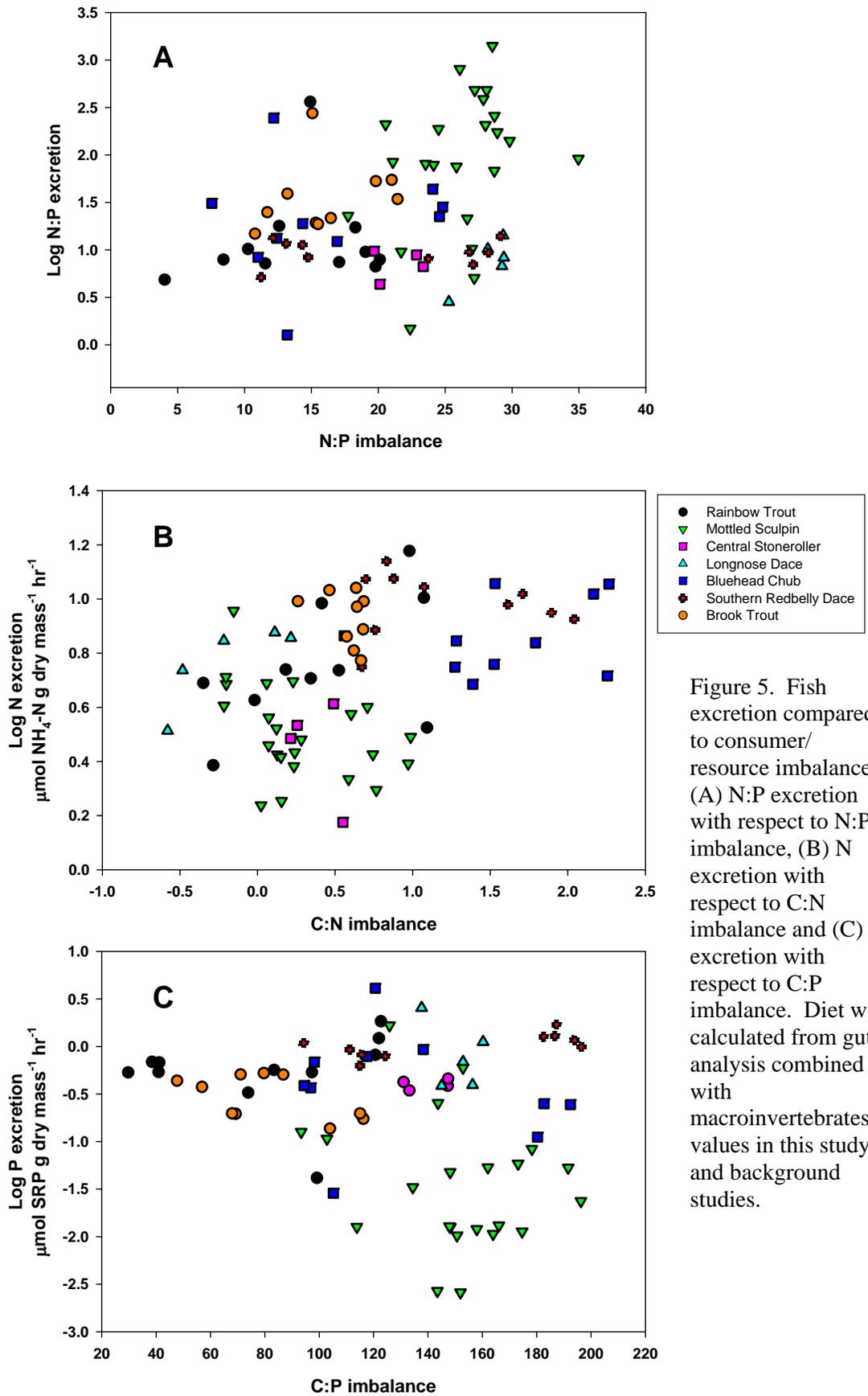


Figure 5. Fish excretion compared to consumer/resource imbalances. (A) N:P excretion with respect to N:P imbalance, (B) N excretion with respect to C:N imbalance and (C) P excretion with respect to C:P imbalance. Diet was calculated from gut analysis combined with macroinvertebrates values in this study and background studies.

Mottled sculpin—Mottled sculpin were found in four streams. There were no significant differences in N:P of diet or the N:P of body among streams. N:P of the diet was highest at the Mira Fork forest site and lowest in Ball Creek. Although not significant, sculpin with the highest body P and lowest body N:P were also in the Mira Fork open site (Table 8). Sculpin at the Mira Fork forest site had the highest N:P and C:P imbalance whereas fish in Ball Creek had the lowest (data not shown). Ball Creek sculpin had highest mean number of insect individuals found in their gut although it was not significantly different than the other streams (Table 8).

Sculpin within the Mira Fork open section had the highest mean N excretion rate ($5.13 \mu\text{mol NH}_4^+\text{-N g dry mass}^{-1} \text{ hr}^{-1}$) (ANOVA: $p=0.01$) (Fig. 6A). However, this difference may simply be due to the size difference of the sculpins sampled, especially since sculpin at Shope Fork and Ball Creek were significantly heavier than sculpin found in both sections of Mira Fork (t-test: $p=0.0008$). Linear regression showed that N excretion was negatively related to dry mass ($r^2 = 0.21$, $p=0.03$), that is, larger fish excrete less N per gram. P excretion by mottled sculpin was not significantly different among streams (ANOVA: $p=0.07$); however, it was highest at Ball Creek and lowest at the Mira Fork open site (Fig. 6B). The N:P of excretion was lowest at Shope Fork (mean, 83) and highest at the Mira Fork open site (524), but the difference among streams was not significant (ANOVA: $p=0.15$, Fig. 6C). Mottled sculpin N:P excretion was positively related to N:P diet but not significant (linear regression: $r^2=0.15$, $p=0.08$). When individuals were averaged by stream, N:P excretion was related to N:P imbalance; however, this relationship was not significant (linear regression: $r^2=0.75$, $p=0.13$, Fig 7C). When considering individuals from all streams, N:P excretion was not related to N:P imbalance (linear regression: $r^2=0.12$, $p=0.11$, Fig 7A) using Bowman's imbalance formula. However, I used my index to calculate N:P imbalance (N:P diet/ N:P consumer), N:P excretion was strongly related to the N:P imbalance index ($r^2=0.46$, $p<0.0001$, Fig. 7B) excluding two individuals whose N:P imbalance far exceeded the rest of the sample. Using Bowman's imbalance formula, P excretion was not related to C:P imbalance (linear regression: $r^2=0.07$, $p=0.20$). The predicted N:P of diet may have been

incorrect for those organisms, especially since their excretion N:P excretion was very low for the N:P of imbalance that I calculated.

Redbelly Dace –Redbelly dace occurred in the open and forested section of Mira Fork.. Body C:P, N:P, %P, and dry weight were not significantly different between streams. Body P content was negatively related to weight (linear regression: $r^2 = 0.51$; $p=0.03$). Dace in the forested section of Mira Fork had more insects in their gut, although the difference in insect diet was not significantly different. The N:P of their diet was significantly higher in Mira Fork forest (t-test: $p < 0.0001$), contributing to a significantly higher N:P imbalance (t-test: $p < 0.0001$). Differences in N:P content of diet were primarily the result of differences in nutrient content of epilithon, which composed a large percentage of the gut contents of dace. In the Mira Fork open site, epilithon had an average N:P of 28, whereas N:P of epilithon in the Mira Fork site was 47. C:P imbalance was significantly higher in the forested section (t-test: $p < 0.0001$) but C:N imbalance was not different between the two sites.

Mean N excretion as well as P excretion were significantly higher in the forested section of Mira Fork than in the open section (t-test: N: $p=0.006$ and P: $p=0.015$) even though fish in the forested section had a higher body %P. Higher N and P excretion is possibly best explained by smaller fish in the forested section. N:P excretion was not significantly different between the two reaches. Like sculpin, N excretion was negatively related to dry mass (linear regression: $r^2 = 0.74$, $p=0.003$). P excretion, on the other hand, was unrelated to body %P. P excretion and N excretion were unrelated to C:P and C:N imbalance respectively.

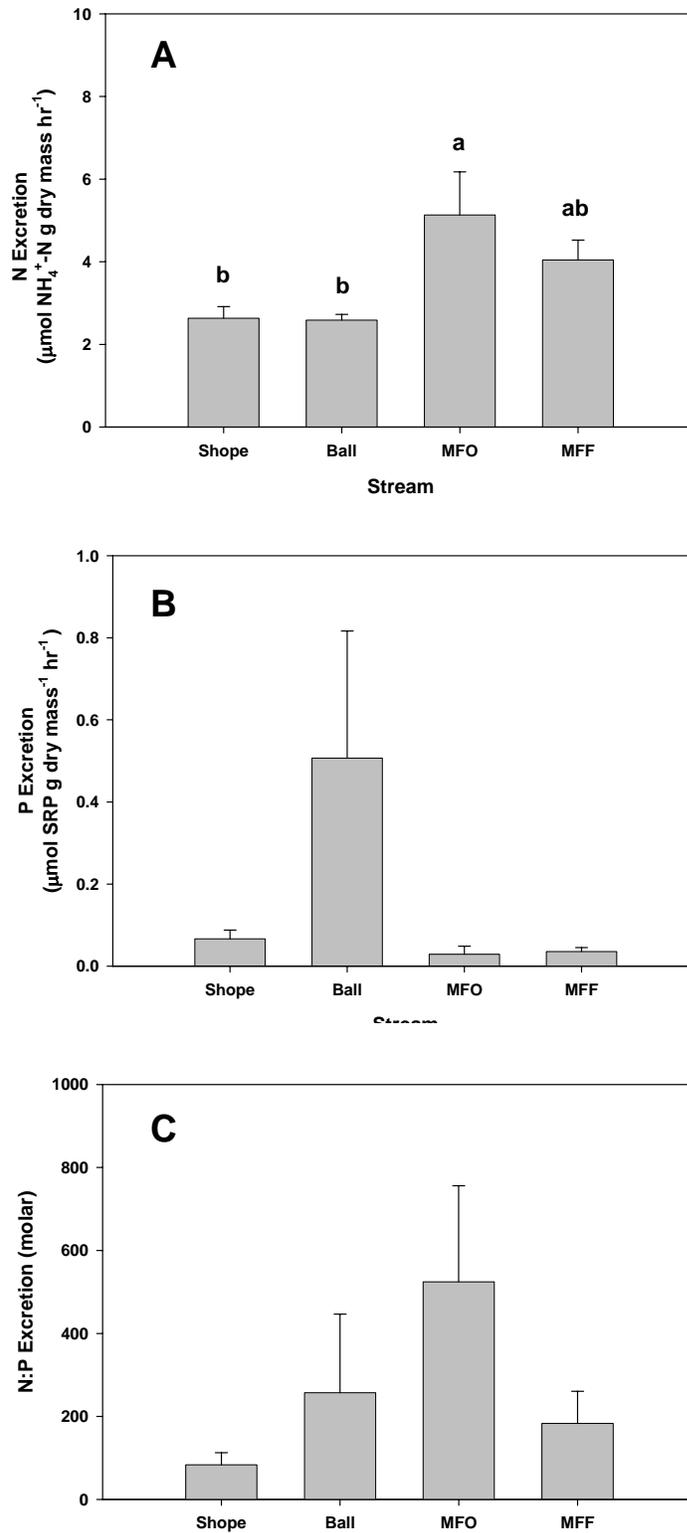


Figure 6: Mottle sculpin N excretion (A), P excretion (B), and N:P excretion (C) among all streams. Error bars represent 1 SE. MFO=Mira Fork Open. MFF= Mira Fork Forested.

Table 8: Mottled sculpin weight, %P, and N:P body. Numbers followed by different letters indicate significance among streams at the (0.05) significance level using Tukey's HSD test. Numbers in parentheses indicate 1SE.

Stream	n	Mean Dry Weight (g)		Mean %P	Mean N:P Fish
Shope	8	2.17	(0.26) a	3.37 (0.32)	6.59 (0.57)
Ball	5	1.59	(0.20) ab	3.21 (0.45)	7.45 (1.12)
MFO	5	1.10	(0.16) b	3.41 (0.11)	6.20 (0.21)
MFF	5	1.07	(0.15) b	3.16 (0.27)	6.80 (0.55)

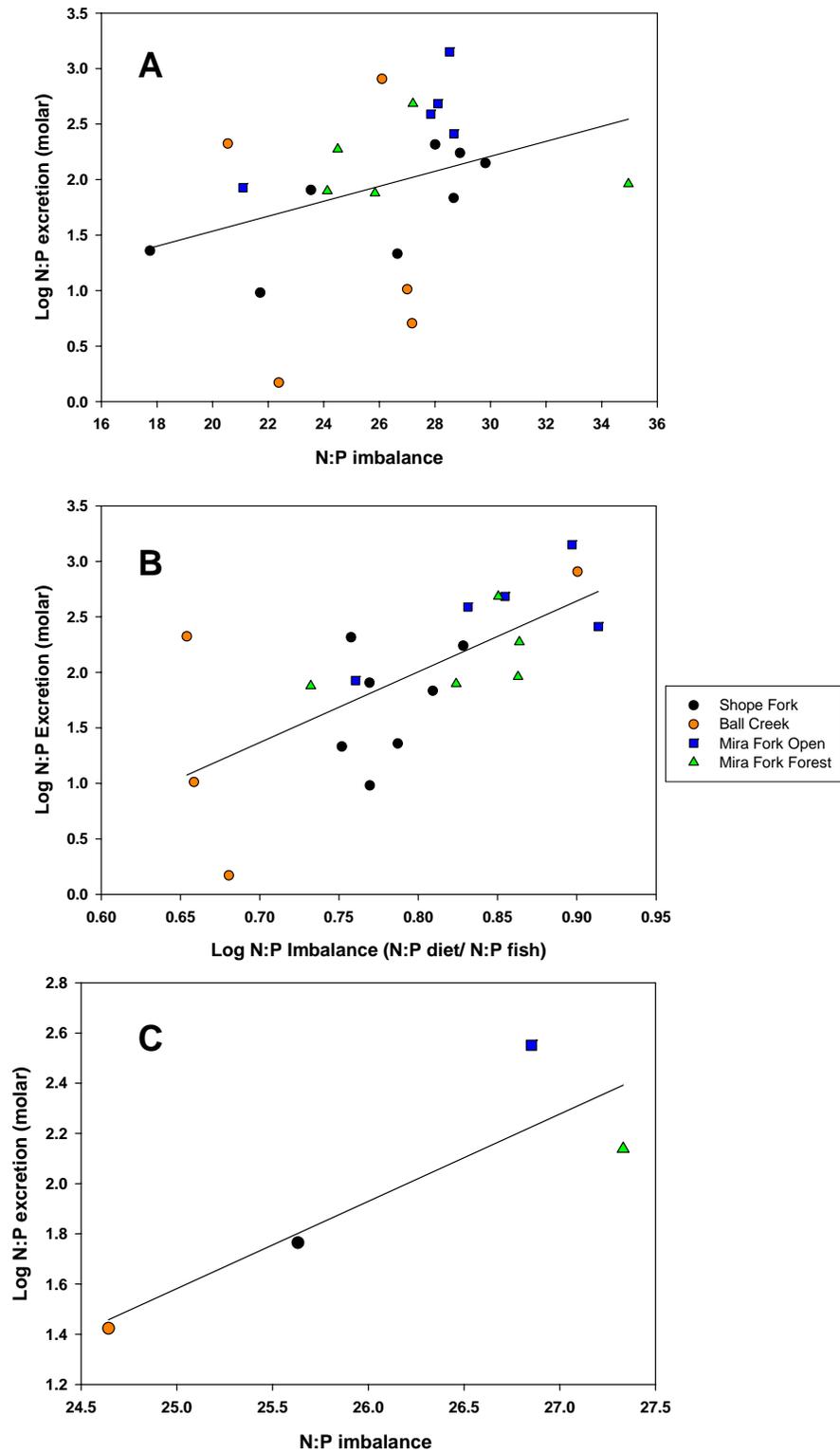


Figure 7. Mottled sculpin (A) N:P (molar) excretion with respect to Bowman et al. N:P imbalance, (B) N:P excretion with respect to N:P food/consumer index, and (C) N:P excretion vs Bowman imbalances averaged for each of the four streams. In the first two figures, each point is an individual fish. N:P values of diet were predicted from contents found in gut analysis along with background data.

Rainbow Trout— Rainbow trout were found only in Ball Creek and Shope Fork. N:P, C:P, and C:N imbalance were not different among streams. Contrary to stoichiometric theory, rainbow trout P excretion was higher in Shope Fork compared to Ball Creek (t-test: $p = 0.07$) even though fish in Shope Fork had a significantly higher body %P (t-test: $p=0.02$). Higher P excretion in Shope Fork was most likely biased by a higher proportion of smaller fish in the sample; however, average weight was not significantly different between the two streams. N:P excretion was not significantly different among streams, indicating no differential cycling of nitrogen compared to phosphorus. N excretion was negatively related to weight (linear regression: $r^2=0.73$, $p=0.0009$). P excretion and body %P was also negatively related to weight (P excretion; linear regression: $r^2=0.71$, $p=0.004$; %P; $r^2=0.50$, $p=0.034$) but only when the two smallest fish were excluded. The smallest fish were 0.42 g, over 20 times smaller than the average of the remaining fish (8.92g).

Brook Trout— Brook trout occurred in Cabin Creek and Big Wilson Creek. The body N:P ratio was not significantly different between the two streams, but the N:P of diet was significantly higher in Cabin Creek (t-test: $p=0.05$). Brook trout in Big Wilson Creek ate a mean of 73% terrestrial insects, whereas brook trout in Cabin Creek (forested) ate a mean of 49% terrestrial insects; however, percent terrestrial diet was not significantly different between the two streams (t-test: $p=0.15$). Body P content (% of DW) increased with weight (linear regression: $r^2=0.56$, $p=0.02$). C:P and N:P imbalance were higher in Cabin Creek but not significantly different (t-test: $p=0.08$ for both). One individual in Cabin Creek was excluded in the analysis because the N excretion was over 6 times higher ($53.8 \mu\text{mol NH}_4^+ \text{-N g dry mass}^{-1} \text{ hr}^{-1}$) than the average N excretion of the sample ($8.67 \mu\text{mol NH}_4^+ \text{-N g dry mass}^{-1} \text{ hr}^{-1}$). Brook trout P excretion was significantly lower and N:P excretion was significantly higher in Cabin Creek (t-test: P, $p=0.0001$; N:P, $p=0.0013$ respectively). Although not significant, brook trout N:P excretion was positively related to N:P diet ($r^2=0.42$, $p =0.08$, $n=9$) and positively related to N:P imbalance ($r^2=0.46$, $p=0.04$, $n=9$).

Bluehead Chub—Bluehead chub was found in the forested and open section of Mira Fork. N:P imbalance and the N:P of diet were significantly higher in the forested section of Mira Fork than the open section (t-test: $p=0.008$, $p=0.007$ respectively); however,

there were no differences in N:P, N, or P excretion between streams. C:P imbalance was significantly higher in the forested section of Mira Fork (t-test: $p=0.05$), and C:N imbalance was higher in the open section of Mira Fork, but not significantly higher ($p=0.08$). Epilithon nutrient content was largely responsible for the difference in diet. Body %P was unrelated to weight and P excretion.

Macroinvertebrate Stoichiometry

Body stoichiometry was significantly different among macroinvertebrate families in %C, %N, %P, C:P, N:P, and C:N (ANOVA: $p<0.0001$ for all variables, Table 9). Cambaridae, the only crustacean, had the highest body P concentration (mean, 1.06 % of DW) whereas baetid mayflies had the lowest (mean, 0.27 % of DW) (Tukey's test, $p<0.05$). Two shredders, Pteronarcyidae and Limnephilidae, had relatively high %P body concentration; however, their % P was not significantly different from the other insects (0.56 and 0.57 %, Tukey's test, $p<0.05$). P concentration seemed to be responsible for most of the variability in C:P and N:P body concentration. For example, cambarids had significantly lower C:P and N:P (mean, 89.6 and 15, respectively) compared to the other families (Tukey's test, $p<0.05$). Baetids had the highest C:P and N:P (506 and 89 respectively), two times higher than another mayfly, Heptageniidae (233, 45); however, baetid mayfly C:P and N:P content were not significantly different than other macroinvertebrates (Tukey's test, $p<0.05$). Most macroinvertebrate families had similar C and N content except for Cambaridae which had significantly lower body C (36.3 %) and N content (7.0 %) (Tukey's test, $p<0.05$). Perlids, predaceous stoneflies, had the highest mean N (12.8 %), significantly different all other macroinvertebrate families except two predatory insect families, Gomphidae and Perlodidae (Tukey's test, $p<0.05$).

Functional feeding groups differed significantly in all stoichiometric variables (%C, %N, %P, C:P, N:P, and C:N) related to their body composition (ANOVA: $p<0.0001$ for all variables) (Fig. 8). Collectors (baetids and isonychids) had the lowest P content (0.42 % of DW), but it was only significantly different from crayfish and shredders (Tukey's test, $p<0.05$) (Fig. 8, post hoc comparisons not shown). Although not significantly different than predators or filterers, collectors also had the highest N:P

and C:P body content (Tukey's test, $p < 0.05$, Fig. 8A and 8C, respectively). Crayfish had significantly higher body P content and significantly lower C and N content than all other functional feeding groups (Tukey's test, $p < 0.05$, post hoc comparisons not shown). Crayfish also had significantly higher body C:N and significantly lower C:P and N:P (Tukey's test, $p < 0.05$, Fig 8, post hoc comparisons not shown). Among insects, shredders had the highest P concentration (0.56 % of DW) followed by scrapers (0.53 % of DW), although they were not significantly different from the other insects except collectors (Tukey's test, $p < 0.05$, post hoc comparisons not shown). Also, when considering only insects, shredders and scrapers had lower C:P values (230 and 232, respectively) and lower N:P values (43; 45), but they were only significantly different than collectors. Predators had significantly higher mean body %N (11.7 %), although it was not significantly different than collectors (tukey's test, $p < 0.05$).

Table 9. Variation in macroinvertebrate body stoichiometry by family. 1 SE in parentheses. All ratios are molar. Post hoc comparison using Tukey's HSD. Different letters indicate significant differences among families at the 0.05 level.

Family	n	C (%DW)		N (%DW)		P (%DW)	
Baetidae	3	52.4 (0.120)	a	10.7 (0.083)	bc	0.27 (0.02)	c
Hydropsychidae	8	50.2 (0.674)	a	10.8 (0.291)	bc	0.43 (0.03)	bc
Perlodidae	1	50.1	ab	12.3	ab	0.39	bc
Limnephilidae	3	49.8 (0.316)	ab	9.60 (0.249)	c	0.57 (0.02)	b
Pteronarcyidae	6	49.9 (0.130)	ab	11.3 (0.072)	b	0.56 (0.02)	b
Gomphidae	3	49.3 (0.559)	ab	11.6 (0.360)	ab	0.37 (0.03)	bc
Perlidae	3	49.2 (0.259)	ab	12.8 (0.286)	a	0.47 (0.11)	bc
Corydalidae	6	49.1 (0.403)	ab	11.2 (0.210)	b	0.54 (0.04)	b
Isonychiidae	5	48.1 (0.510)	ab	10.9 (0.224)	bc	0.51 (0.05)	b
Heptageniidae	14	46.9 (0.693)	b	10.5 (0.175)	bc	0.53 (0.02)	b
Cambaridae	12	36.3 (0.533)	c	7.03 (0.107)	d	1.06 (0.03)	a

Family	n	C:N		N:P		C:P	
Baetidae	3	5.7 (0.03)	abc	89 (6.71)	a	506 (40.4)	a
Hydropsychidae	8	5.5 (0.12)	bc	59 (7.06)	bc	316 (31.1)	b
Perlodidae	1	4.7	cd	70	abc	331	abc
Limnephilidae	3	6.1 (0.14)	ab	37 (0.30)	cd	226 (5.74)	bc
Pteronarcyidae	6	5.1 (0.03)	c	45 (1.74)	bc	232 (8.19)	bc
Gomphidae	3	5.0 (0.21)	cd	70 (4.65)	abc	348 (27.9)	ab
Perlidae	3	4.5 (0.09)	d	70 (22.3)	abc	312 (95.1)	bc
Corydalidae	6	5.1 (0.15)	c	48 (2.83)	bc	244 (19.3)	bc
Isonychiidae	5	5.2 (0.08)	c	49 (4.39)	bc	254 (24.6)	bc
Heptageniidae	14	5.2 (0.08)	c	45 (1.83)	bc	233 (10.1)	c
Cambaridae	12	6.0 (0.08)	a	15 (0.48)	d	89.6 (2.55)	d

Table 10. Resource stoichiometry for each macroinvertebrate family. Resource values for scrapers, collectors, filterers, and shredders are based on resource collection and analysis. Diet values for predatory insects are based on average nutrient ratios of all insect orders. Values in parentheses are 1 SE. All ratios are molar. (n refers to number of streams as replicates).

Taxa	n	Resource	C:P	C:N	N:P
Perlodidae	1	prey	260 -----	5.4 -----	49 -----
Heptageniidae	5	epilithon	377 (77.2)	7.9 (0.78)	47 (8.98)
Hydropsychidae	3	seston	477 (23.5)	19 (0.45)	25 (1.49)
Isonychiidae	2	seston	356 (13.9)	12 (0.28)	29 (0.46)
Baetidae	1	epilithon	457 -----	11 -----	42 -----
Cambaridae	4	leaf	2674 (494)	43 (8.39)	64 (3.94)
Gomphidae	1	prey	260 -----	5.4 -----	49 -----
Perlidae	1	prey	260 -----	5.4 -----	49 -----
Limnephilidae	1	leaf	3441 -----	63 -----	55 -----
Corydalidae	2	prey	260 -----	5.4 -----	49 -----
Pteronarcyidae	2	leaf	2756 (686)	52 (11.5)	54 (1.50)

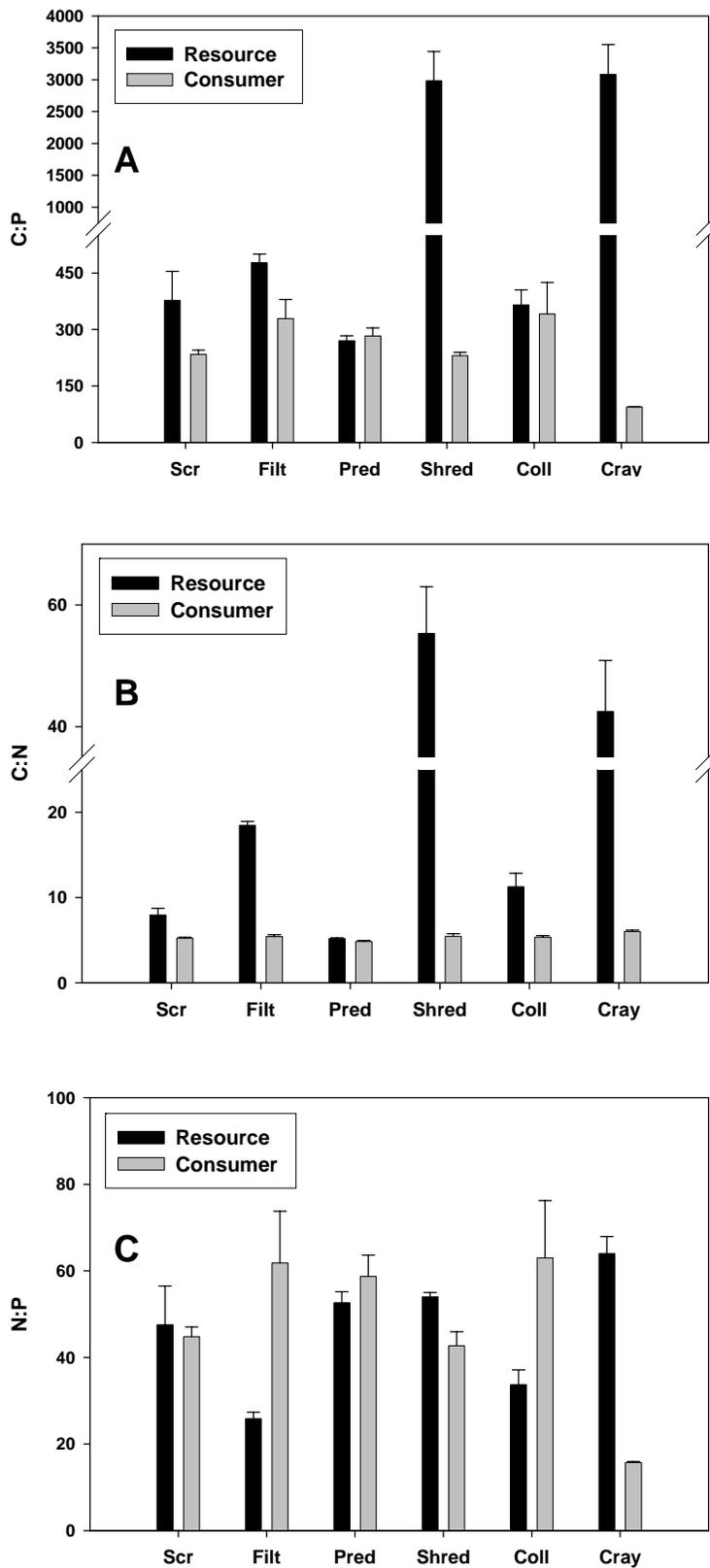


Figure 8: Functional feeding group (FFG) body stoichiometry along with corresponding resource stoichiometry. (A) FFG and resource C:P, (B) FFG and resource C:N, and (C) FFG and resource N:P. Error bars = 1SE. scr=scraper. filt=filterer. pred=predator. shred=shredder. coll=collector. cray=crayfish. Resources: scraper, epilithon; filterer, seston; predator, macroinvertebrates; shredder, leaf detritus; collector, epilithon/Seston; crayfish, leaf detritus. Ratios are molar.

Diet, Imbalances, and Excretion

Compared to macroinvertebrate stoichiometry, resource compositions were highly variable (Table 10, Fig 8). The N:P, C:P, and C:N content varied significantly among resources (ANOVA, $p < 0.0001$). Crayfish and shredder diets were significantly higher in C:P and C:N than other feeding group resources (Tukey's test, $p < 0.05$, Table 10, Fig 8A, 8B respectively, post hoc comparisons not shown). Predators had a diet C:P that was significantly lower than the diet C:P other groups except scrapers (Tukey's test, $p < 0.05$). Predators also had a significantly lower diet C:N than the other groups (Tukey's test, $p < 0.05$). Scrapers (Heptageniidae) and collectors (Baetidae and Isonychiidae) had a diet C:N significantly different than the other groups, but they were not significantly different than each other (Tukey's test, $p < 0.05$). Filterers, solely represented by Hydropsychidae, also had a C:N diet significantly different than all the other groups (Tukey's test, $p < 0.05$). The diet N:P of crayfish and shredders was higher than the other groups; however, only significantly different than filterers (Tukey's test, $p < 0.05$, Fig. 8C). Filterers had a diet N:P that was significantly lower than the other groups with the exception of collectors (Tukey's test, $p < 0.05$).

N:P, C:P, and C:N imbalances were also significantly different among groups (ANOVA: $p < 0.0001$, data not shown) with crayfish and shredders having significantly higher C:P and C:N imbalances (Tukey's test, $p < 0.05$). C:P and C:N imbalances were not significantly different among the remaining groups (Tukey's test, $p < 0.05$); however, predators had the lowest values. Crayfish had a significantly higher N:P imbalance than all other groups (Tukey's test, $p < 0.05$). Shredders had the next highest N:P imbalance, which was not significantly different than that of scrapers and predators (Tukey's test, $p < 0.05$). Filterers had the lowest N:P imbalance, but it was not significantly different than that of collectors and predators (Tukey's test, $p < 0.05$).

N, P, and N:P of excretion were also significantly different among macroinvertebrate families (ANOVA: $p < 0.0001$ for all variables) (Table 11). Perlodids exhibited the highest mean N excretion ($0.0230 \mu\text{mol NH}_4^+\text{-N mg drymass}^{-1} \text{hr}^{-1}$) (Tukey's test, $p < 0.05$), whereas pteronarcyids had the lowest ($0.0001 \mu\text{mol SRP mg drymass}^{-1} \text{hr}^{-1}$), although it was not significantly different than most of the other families (Tukey's test, $p < 0.05$). Families with lower N excretion rates were primarily composed

of shredders and predators. Hydropsychid caddisflies had the highest P excretion (mean, $0.0058 \mu\text{mol SRP mg drymass}^{-1} \text{ hr}^{-1}$), which was significantly different than that of the other families, except Perlodidae (Tukey's test, $p < 0.05$). Hydropsychids also had the lowest N:P of excretion, although it was not significantly different than that of the predator and shredder families (Tukey's test, $p < 0.05$). Cambarids had the lowest P excretion (mean, $0.00001 \mu\text{mol SRP mg drymass}^{-1} \text{ hr}^{-1}$), but not significantly different than shredders, the majority of predators, and baetids. Cambarids also had a N:P ratio of excretion significantly higher than that of the insect families (Tukey's test, $p < 0.05$).

N, P, and N:P excretion were significantly different among functional feeding groups (ANOVA: $p < 0.0001$ for all variables, Fig. 9). Shredders had the lowest N excretion rates ($0.0002 \mu\text{mol NH}_4^+\text{-N mg drymass}^{-1} \text{ hr}^{-1}$), but it was not significantly different from crayfish and predators (Tukey's test, $p < 0.05$, Fig. 9B). Scrapers had the highest N excretion rates ($0.0125 \mu\text{mol NH}_4^+\text{-N mg drymass}^{-1} \text{ hr}^{-1}$), but they were not significantly different from that of filterers or collectors (Tukey's test, $p < 0.05$, Fig. 9B). Filterers had significantly higher P excretion ($0.0058 \mu\text{mol PO}_4\text{-P mg drymass}^{-1} \text{ hr}^{-1}$) than the other functional feeding groups (Tukey's test, $p < 0.05$) whereas the remaining groups were not different (Fig. 9A). Shredders and crayfish had the lowest P excretion rates ($0.0001 \mu\text{mol SRP mg drymass}^{-1} \text{ hr}^{-1}$). N:P excretion was significantly higher in crayfish (544) than all other groups, whereas N:P excretion was lowest in filterers (1.52), which was significantly different than all groups except shredders (Tukey's test, $p < 0.05$, Fig. 9C).

Table 11. Mean N, P, and N:P excretion for different macroinvertebrate families. N and P excretion in $\mu\text{mol mg dry mass}^{-1} \text{hr}^{-1}$. Number in parentheses indicate 1 SE. N:P is a molar ratio. Post hoc comparison using Tukey's HSD. Different letters indicate significant differences among families at the 0.05 level.

Taxa	n	Mean N Excretion		Mean P Excretion		Mean N:P	
Perlodidae	5	0.0230 (0.0021)	a	0.004572 (0.00081)	ab	5.39 (0.63)	bcd
Heptageniidae	25	0.0125 (0.0015)	b	0.001500 (0.00033)	bc	21.3 (8.00)	b
Hydropsychidae	14	0.0083 (0.0012)	bc	0.005789 (0.00084)	a	1.52 (0.18)	d
Isonychiidae	10	0.0077 (0.0030)	bcd	0.002089 (0.00150)	bc	12.0 (2.88)	bc
Baetidae	5	0.0071 (0.0017)	bcde	0.000547 (0.00019)	bc	24.9 (10.8)	b
Cambaridae	18	0.0018 (0.0002)	de	0.00001 (1.87e-5)	c	544 (138)	a
Gomphidae	5	0.0009 (0.0001)	cde	0.000167 (6.74e-5)	c	12.0 (4.54)	bcd
Perlidae	5	0.0004 (0.0001)	cde	0.000211 (0.00012)	c	4.23 (1.17)	bcd
Limnephilidae	5	0.0003 (0.0001)	cde	0.000203 (0.0001)	c	2.78 (1.34)	bcd
Corydalidae	10	0.0003 (0.0001)	de	0.000040 (1.87e-5)	c	13.3 (3.65)	bc
Pteronarcyidae	10	0.0001 (0.0000)	e	0.000066 (2.27e-5)	c	6.28 (2.63)	cd

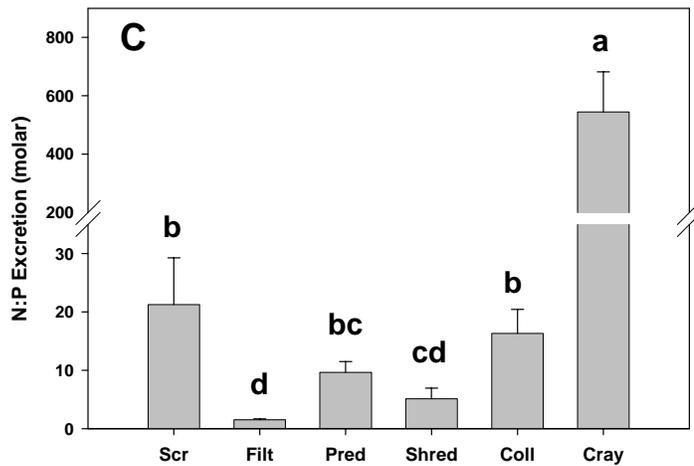
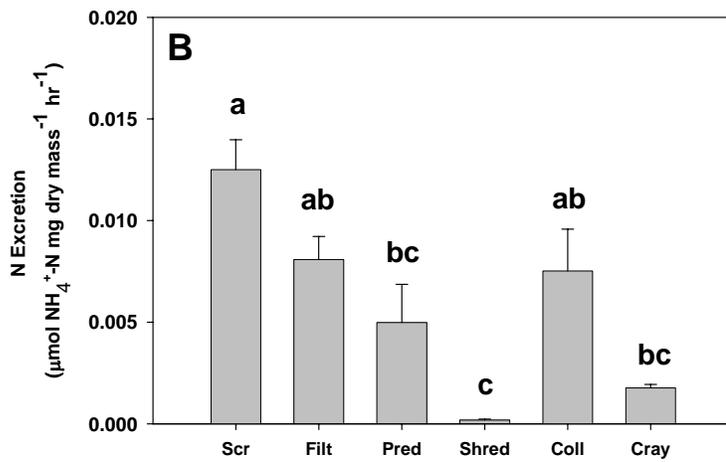
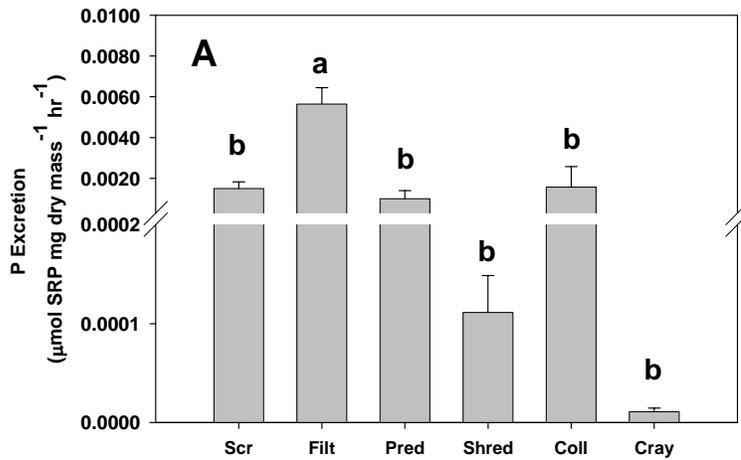


Figure 9: Functional feeding group (A) P excretion, (B) N excretion, and (C) N:P excretion. Error bars = 1SE. scr=scrapper. filt=filterer. pred=predator. shred= shredder. coll=collector. cray=crayfish. Ratios are molar. Post hoc comparison using Tukey's HSD. Different letters indicate significant differences among families at the 0.05 level.

P excretion, expressed as $\mu\text{mol SRP mg drymass}^{-1} \text{ hr}^{-1}$, was negatively related to mass (linear regression: $r^2=0.43$, $p<0.0001$); however, no relationship was found between non-mass normalized P excretion ($\mu\text{mol SRP hr}^{-1}$) and mass (linear regression: $r^2=0.05$, $p=0.02$, Fig. 10A). Hydropsychids excreted extremely high non-mass normalized P compared to all other individuals, regardless of their small size compared to other macroinvertebrates, especially crayfish (Fig. 9A). N excretion ($\mu\text{mol NH}_4\text{-N hr}^{-1}$) was also negatively related to mass (linear regression: $r^2=0.57$, $p<0.0001$) and non-mass normalized N excretion was positively related to mass when crayfish were included (linear regression: $r^2=0.45$, $p<0.0001$, Fig. 10B). When crayfish were excluded, no relationship existed between non-mass normalized N excretion and mass (Fig. 10C). Despite their large size, predators and shredders excreted extremely low amounts of N.

A negative relationship was found between macroinvertebrate P excretion and body % P (linear regression: $r^2=0.51$, $p<0.0001$, Fig. 11A) and between N:P excretion and body N:P (linear regression: $r^2=0.61$, $p<0.0001$, Fig. 11B). There was a positive relationship between N:P excretion and N:P imbalance for all macroinvertebrate families that was driven primarily by Cambaridae (linear regression: $r^2=0.47$, $p=0.0003$, Fig. 12A). Cambaridae had a high N:P imbalance along with a high N:P of excretion. Pteronarcyids and limnephilids had higher N:P imbalances compared to most of the other taxa; however, their N:P excretion ratio was low compared to the N:P excretion ratio of other families with lower imbalances. Baetid mayflies excreted very high N:P ratios for their low imbalances compared to other taxa.

Nitrogen excretion rate was negatively related to C:N imbalance (linear regression: $r^2=0.27$, $p=0.01$, Fig. 12C) and P excretion rate was negatively related to C:P imbalance (linear regression: $r^2=0.38$, $p=0.002$, Fig. 12B). Families of the same functional feeding group were acting similarly regarding their relationship between N and P excretion and C:N and C:P imbalance. For example, predators had very low N and P excretion relative to a small C:N and C:P imbalance, whereas scrapers and collectors had slightly higher imbalances and high N and P excretion. Crayfish and shredders had very large imbalances and very low N and P excretion (Fig. 12B and 12A, respectively).

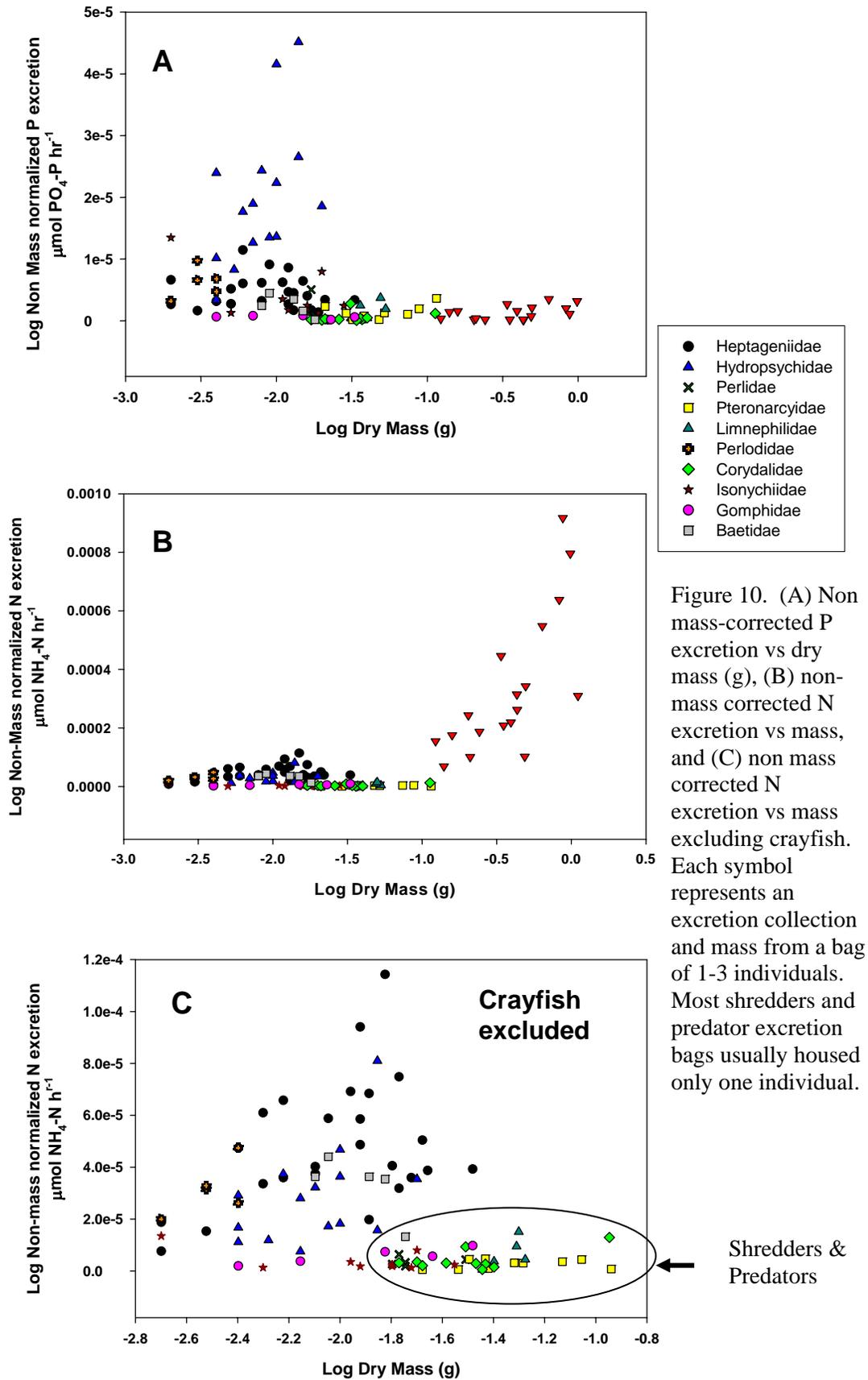


Figure 10. (A) Non mass-corrected P excretion vs dry mass (g), (B) non-mass corrected N excretion vs mass, and (C) non mass corrected N excretion vs mass excluding crayfish. Each symbol represents an excretion collection and mass from a bag of 1-3 individuals. Most shredders and predator excretion bags usually housed only one individual.

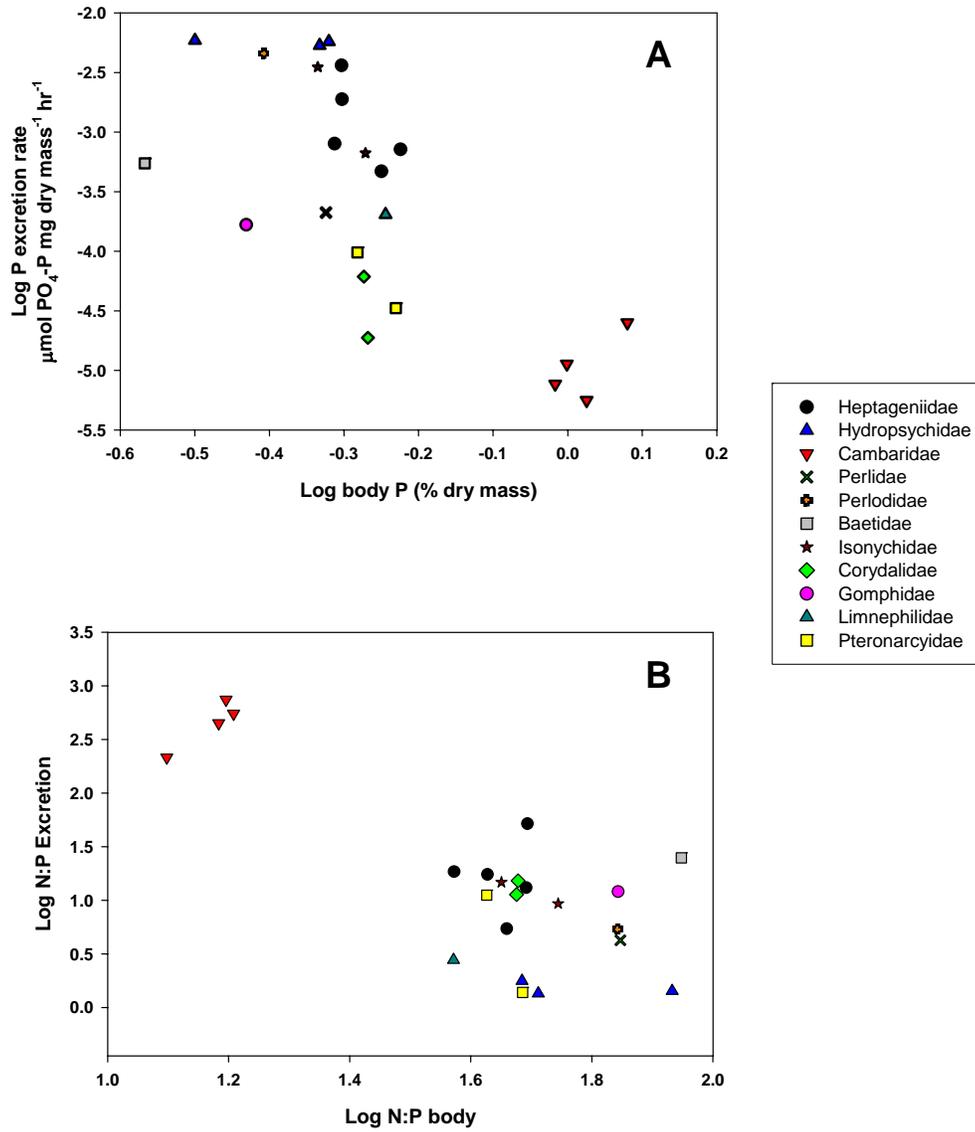


Figure 11. (A) Macroinvertebrate P excretion vs body %P and (B) N:P excretion vs body N:P. Each symbol represents the nutrient composition of the average of mass collection and the average nutrient excretion from each stream from each representative taxa.

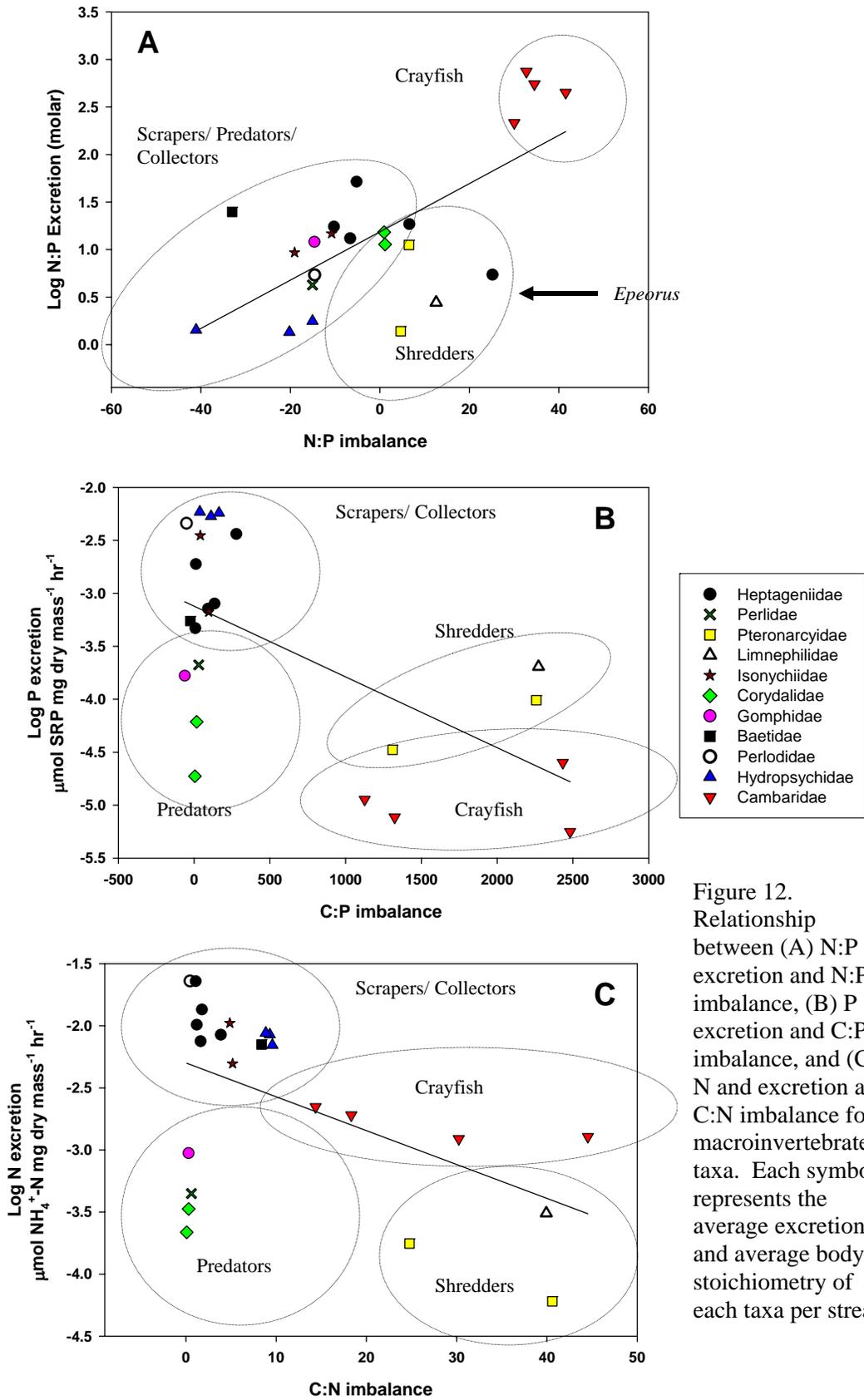


Figure 12. Relationship between (A) N:P excretion and N:P imbalance, (B) P excretion and C:P imbalance, and (C) N and excretion and C:N imbalance for macroinvertebrate taxa. Each symbol represents the average excretion and average body stoichiometry of each taxa per stream.

Excretion and stoichiometry across nutrient gradients

Pteronaryciids had lower body C:P and N:P in Shope Fork than in Ball Creek (t-test: $p=0.05$; $p=0.07$), which may have been influenced by a significantly lower leaf C:P and C:N ratio (t-test: $p=0.005$; $p=0.005$). The lower leaf C:N ratio contributed to a lower C:N imbalance in Shope Fork (25) compared to Ball Creek (41). N and N:P excretion was higher in Shope Fork although not significantly higher (t-test: N, $p=0.13$ and N:P, $p=0.11$).

Heptageniids were found in Shope Fork, Mira Fork open and forested sections, Big Wilson Creek, and Cabin Creek. Heptageniid P excretion and N excretion was significantly different among the four streams (ANOVA: $p=0.004$, $p=0.0008$ respectively). P excretion was significantly higher in Big Wilson Creek than the other streams except Shope Fork (Tukey's test, $p<0.05$, post hoc comparisons not shown). N excretion was significantly higher at Shope Fork than the other streams except Big Wilson. N:P excretion was not significantly different among streams (ANOVA: $p=0.07$) although it was lowest at Big Wilson. Heptageniid weight (g dry mass) was significantly different among streams (ANOVA: $p=0.002$). Individuals at Big Wilson Creek were significantly smaller than those at the other streams except Shope Fork (Tukey's test: $p<0.05$). This was possibly due to the dominance of *Epeorus* at Big Wilson Creek as compared to dominance by the large genus *Stenonema* at the other streams. Multiple relationships were seen between epilithon composition and heptageniid excretion. Opposite my predictions, N:P excretion was negatively related to epilithon N:P (linear regression: $r^2=0.48$, $p=0.0003$, Fig. 13A) and P excretion was positively related to epilithon C:P (linear regression: $r^2=0.23$, $p=0.01$, not shown). However, compatible with predictions, N excretion was negatively related to epilithon C:N (linear regression: $r^2=0.20$, $p=0.03$). This may have been due to a positive relationship between weight and epilithon C:N (linear regression: $r^2=0.15$, $p=0.07$), especially seeing that N excretion was negatively related to weight (linear regression: $r^2=0.39$, $p=0.001$). Non-mass normalized N excretion was positively related to weight (linear regression: $r^2=0.34$, $p<0.0001$); however, non-mass normalized P excretion was unrelated. There was a strong

relationship between body C:N and the C:N of epilithon (quadratic polynomial regression: $r^2=0.57$, $p=0.01$) indicating consumer non-strict homeostasis (Fig. 13B). Crayfish (Cambaridae) were dominant in Ball Creek, Mira Fork open and forested sections, and Big Wilson Creek. Crayfish N excretion, P excretion, and excretion N:P were not significantly different among streams (ANOVA: $p=0.18$, $p=0.35$, and $p=0.68$ respectively). N excretion was negatively related to C:N imbalance, however, not significant different due to low replicates ($r^2=0.76$, $p=0.13$, $n=4$, Fig. 14). Like heptageniids, crayfish showed indications of consumer non-homeostasis when compared to leaf detritus nutrient composition. Crayfish body C:N was weakly related to leaf C:N (quadratic polynomial regression: $r^2=0.41$, $p=0.04$, Fig. 15A). Crayfish body N:P was related to leaf N:P (quadratic polynomial regression: $r^2=0.65$, $p=0.003$, Fig. 15B) when excluding one individual which was an extreme outlier from Big Wilson Creek. There was no relationship between crayfish and epilithon nutrient content.

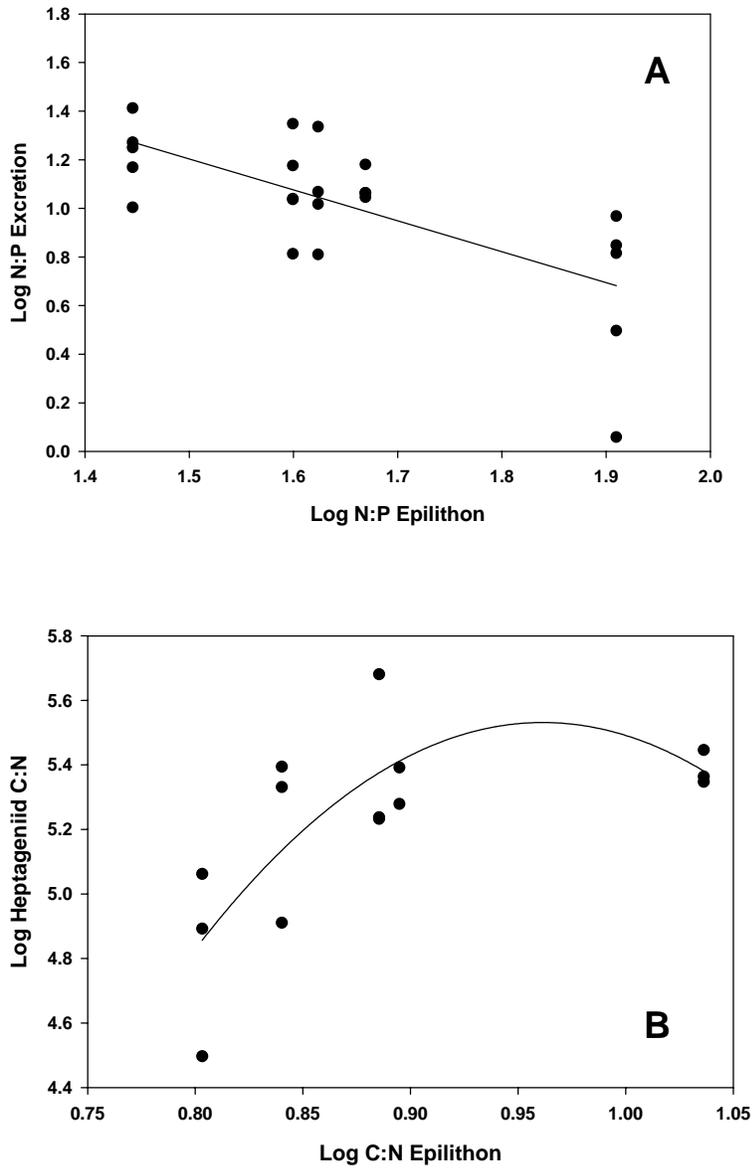


Figure 13. Linear regression showing (A) heptageniid N:P excretion vs epilithon N:P across 5 streams. (B) Polynomial linear regression showing heptageniid C:N vs epilithon C:N across 5 streams. Each point represents a separate sample of individuals from a given stream.

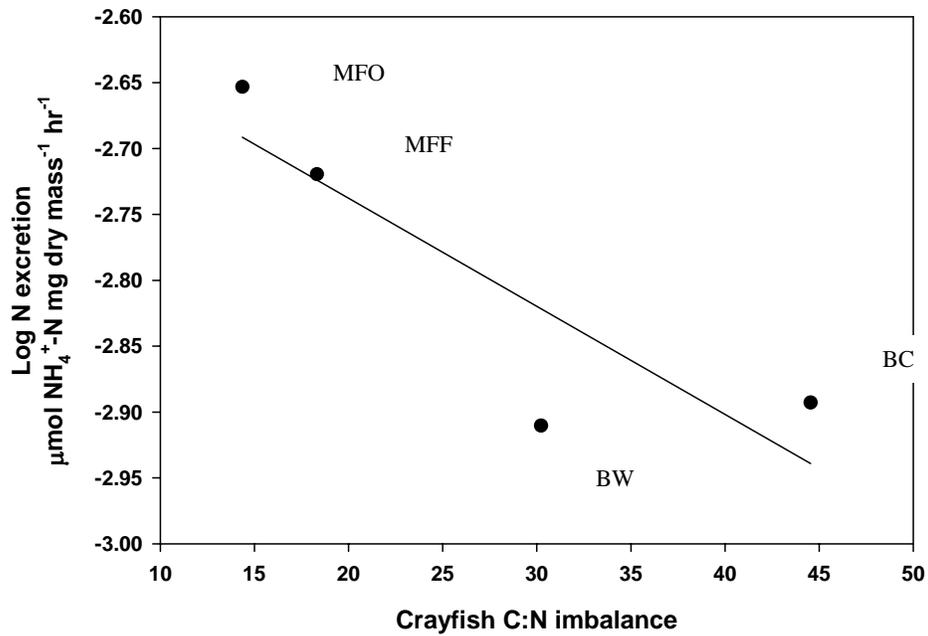


Figure 14. Crayfish N excretion vs C:N imbalance across 4 streams. Each point represents calculated imbalance of the average body stoichiometry and average leaf detritus vs the average N excretion for multiple individuals at each stream. MFO=Mira Fork open section, MFF= Mira Fork forested section, BW = Big Wilson, BC= Ball Creek.

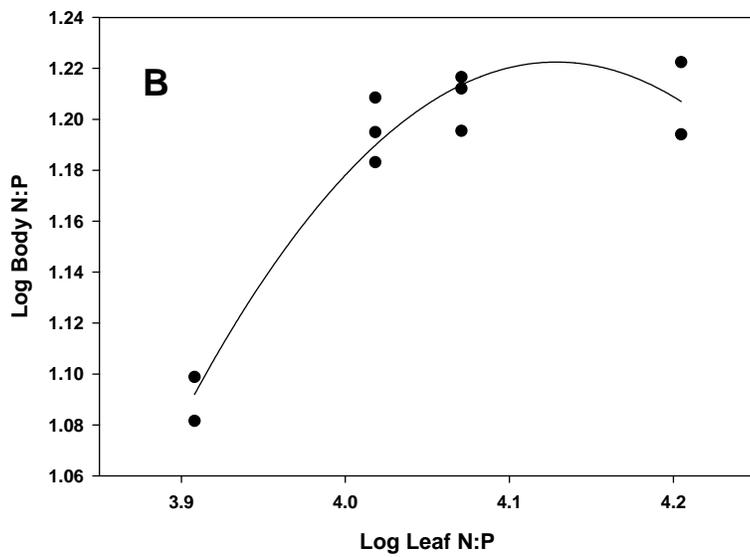
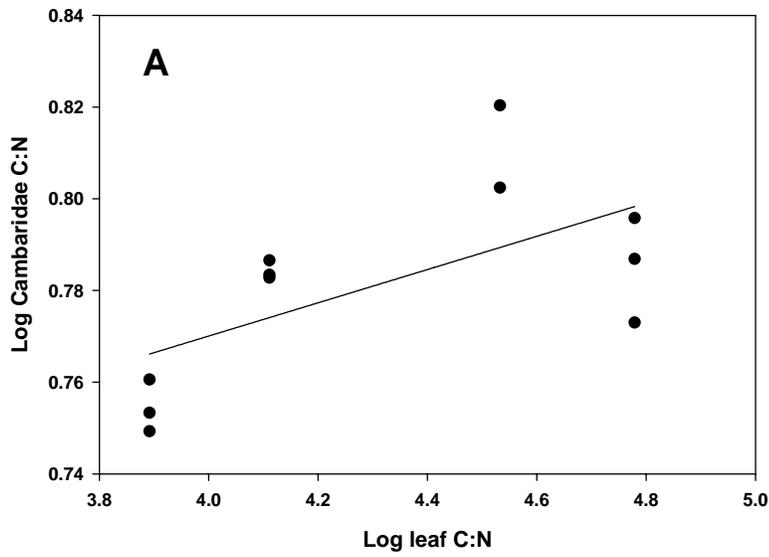


Figure 15. (A) Cambaridae C:N vs leaf C:N shown by linear regression across 4 streams and (B) Cambaridae N:P vs leaf N:P shown by polynomial linear regression across 4 streams. Each point represents a separate sample of individuals from a given stream. Each symbol represents a group of individuals from each stream lumped together and ground.

DISCUSSION

Fish Stoichiometry

Based on stoichiometric principles, consumer nutrient excretion should be related to elemental ratios (Kitchell et al. 1979; Sterner and Elser, 2002; Vanni 2002; Vanni et al. 2002; Glaholt and Vanni 2005). I was unable to explain the large variation in nutrient excretion by consumers across and within taxa based on body stoichiometry alone. In Vanni et al.'s study (2002), strong negative relationships existed between P excretion and body P content in 13 families (28 species, including fish and amphibians) found in the tropics. My lack of strong relationships may have been partially due to the small number of species (7 total) used in this study. I did, however, find support for stoichiometric theory where negative relationships existed between P excretion and P content and N:P excretion and N:P content across taxa; however, this relationship was driven by mottled sculpin, a very phosphorus rich species (Fig. 3A and 3B). Mottled sculpin had the lowest phosphorus excretion (high N:P excretion) and highest body P content (low N:P body), well above most of the other species. Large differences in C:N:P stoichiometry are associated with phylogeny, and, generally, are linked to the amount of P in skeletal structures (Vanni et al. 2002; Sterner and Elser 2002; Hendrixson et al. 2007). Members of the genus *Cottus* have a broad, flattened head, along with large pectoral fins adapted to benthic habitat in turbulent waters (Moyle and Cech 2004). The skeletal structures that compose the large head and pectoral fins are associated with large amounts of P-rich bone. One similar example is the neo-tropical armored catfish, which is characterized by P-rich cranial skeletalized plates and excretes very little P (Vanni et al. 2002; Hood et al. 2005).

The dace species and sculpin had similar phosphorus body concentrations; however, they had variable P excretion rates. For example, although southern redbelly dace had body %P only slightly lower than sculpin, they also had the highest P excretion rate. On the other hand, salmonids had higher P excretion and lower body P in comparison to mottled sculpin. Within the Salmonidae, brook trout had higher body P content than rainbow trout and a lower P excretion and higher N:P excretion (Fig. 2). Rainbow trout had the lowest body P content, similar to the findings of Hendrixson (2007) (Table 6). 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. 3). Studies have shown that detritivorous fish tend to have high P excretion (Torres and Vanni 2007; Schaus et al. 1997; Brabrand et al. 1990). Most of the cyprinids in this study were ingesting algae in addition to detritus, a possible reason for higher P excretion. However, longnose dace, which also had high P excretion, feeds exclusively on benthic macroinvertebrates (Table 7).

Across taxa, a relationship between body size and P concentration is somewhat unclear. Vanni et al. (2002) found that P excretion was negatively related to body P content and wet mass, suggesting that taxa with higher mass have higher body P content. However, for the fish in my study, larger species (salmonids, bluehead chub, central stoneroller) tended to have lower body % P (Fig. 4A), and there was no relationship between mass and P excretion (Fig. 4B). Davis and Boyd (1978) found that Centrarchid body % P increased with mass; however, Sterner and George (2002) found that there was no relationship between mass and body P content in cyprinids. In my study, relationships existed between body % P and mass, but they tended to be species-specific as found by Hendrixson et al. (2007). Rainbow trout and redbelly dace body %P was negatively related to weight; however, brook trout body % P was positively related to weight. Smaller individuals, within a taxon, generally have higher growth rates, thus higher RNA content, resulting in a higher body %P (Elser et al. 2000b; Sterner and Elser 2002; Elser et al. 2003; Frost et al. 2005). Among taxa, the lack of relationships between body P and P excretion, may be ambiguous due to differences in mass, growth rate, and growth efficiencies that are species-specific.

The deviation from stoichiometric predictions is not unprecedented. Torres and Vanni (2007) found that body stoichiometry failed to elucidate patterns in nutrient excretion, thus concluding that differences in diet or growth efficiencies may explain excretion more than stoichiometry. My results indicate that diet must be an important explanatory variable in nutrient excretion. Elemental imbalance takes into account both diet and consumer body stoichiometry and should have greater explanatory power than body stoichiometry alone (Shindler and Eby 1997; Elser and Urabe 1999; Frost et al. 2002; Evans-White et al. 2005). For example, epilithic algae had similar and at times lower N:P content than insect tissue indicating a more phosphorus-rich diet for

herbivorous fish than for predatory fish (Table 7). Therefore, despite relatively high body P, high P excretion in herbivorous or omnivorous fish might possibly be explained by high dietary P. However, growth efficiency and assimilation may be very different between herbivorous and predatory fish, leading to differences in P excretion regardless of body stoichiometry and diet. Growth efficiency and assimilation efficiency, as well as ingestion, are important variables that control the sequestering of different elements in different amounts, and thus, control differential nutrient excretion (Sterner and Elser 2002; Anderson et al. 2004; Vanni 2005; Gaholt and Vanni 2005). Herbivores and omnivores tend to have lower growth/assimilation efficiencies than predators (Sterner and Elser 2002; Elser et al. 2000a), which may result in lower allocation of P to growth and higher P excretion.

Among taxa, N:P, C:P, or C:N imbalance was not strongly related to N, P, or N:P excretion (Fig. 5). Although cyprinids had high N:P and C:P imbalances, they also had low N:P and high P excretion rates. Predatory fish had significantly lower dietary C:N than herbivorous fish, which may be important considering different nutritional constraints between different fish trophic groups (Table 7). My analysis showed that benthic predatory fishes (mottled sculpin and longnose dace) had higher dietary C:P and N:P and higher C:P and N:P imbalances than the terrestrial diet (primarily Hymenoptera) of water column predators (salmonids) (Table 7). Benthic insects are generally less P-rich than terrestrial insects (Evans-White et al. 2005; Cross et al. 2003) and less nutrient rich in general (Elser et al. 2000). Because benthic predatory fish have a lower dietary P content, they may have higher P constraints than predatory fish in the water column. Sculpin especially may be more P-limited because they had the highest body P content and among the lowest dietary P. I believe this is the reason for low P excretion rates of sculpin. Longnose dace had high C:P imbalances and the highest N:P imbalances yet high P excretion. This may be due to unique growth efficiencies of cyprinids, which may confound any difference between members of cyprinidae that eat algae vs prey. Within Salmonidae, rainbow trout had lower body P content yet very similar dietary P compared to native brook trout. Even though these species fill similar ecological niches, non-native rainbow trout may have lower P constraints because of lower body P content. Not

surprisingly, brook trout had a significantly lower P excretion and significantly higher N:P excretion than rainbow trout.

Nutrient availability must meet the assimilatory demands of the consumer, otherwise an organism can not sequester the limiting nutrient in adequate amounts, resulting in differential excretion of non-limiting nutrients. Therefore, assuming phosphorus is limiting, individuals with higher dietary N:P or higher N:P imbalances should have lower P excretion and higher N:P excretion. This trend was exhibited in mottled sculpin and brook trout; however in bluehead chub, rainbow trout, and redbelly dace, excretion was not as predicted.

For a phosphorus-rich species like sculpin, I predicted that individuals with higher dietary N:P (low P) would excrete N at higher rates. N:P imbalance had a stronger explanatory power for sculpin N:P excretion compared to dietary N:P and body N:P alone indicating the importance in the relationship between diet and body composition (Fig. 7A). Secondly, a simple ratio of dietary N:P to consumer body N:P more strongly explained N:P excretion for sculpin than Bowman et al.'s imbalance (Fig. 7B). Sculpin in the open section of Mira Fork had the lowest body N:P content along with some of the higher dietary N:P values, resulting in large imbalances. Large imbalances should be reflected in high N and N:P excretion rates. In P limited fish, small imbalances should result in less P-limitation and thus higher P excretion. Not surprisingly, sculpin in Ball Creek had the lowest imbalances and had the highest excretion of P, thus supporting my predictions (Fig. 6B). Based on allometry, smaller fish should have higher P excretion (Vanni et al. 2002; Sterner and Elser 2002); however, the smallest sculpin were found in the open section of Mira Fork and these sculpin had the lowest P excretion rates. My results suggest that mottled sculpin be P limited, especially smaller individuals.

A similar relationship between excretion and imbalance was seen in brook trout as in mottled sculpin. Brook trout had a higher dietary N:P and higher N:P imbalance in Cabin Creek resulting in higher N:P and lower P excretion. N:P excretion was positively related to dietary N:P and N:P imbalance. Fish in Big Wilson, generally ate a higher percentage of terrestrial insects, suggesting higher dietary P than a pure aquatic insect diet. Higher dietary P may have alleviated P demand and limitation resulting in higher P excretion in Big Wilson.

Because redbelly dace diet N:P, N:P imbalance, and body %P were much higher in the forested section of Mira Fork than the open section, N:P excretion should have been higher and P excretion should be lower in the forested section. N:P excretion was not different, however. Higher mass specific N and P excretion rates in the forested section of Mira Fork were primarily due to presence of smaller fish. Redbelly dace were predominantly herbivorous, although individuals in the forested section of Mira Fork ate a higher number of insects. Contrary to other studies, my data suggest that insects have a similar and at times, higher N:P ratio (less P rich) than epilithon. P excretion by redbelly dace was higher in the forested section than the open section of Mira Fork even though N:P diet, % body P, and N:P imbalance in the forested section were higher. Although redbelly dace had body %P concentrations similar to and imbalances higher than sculpin, they were responding to lower dietary P and imbalances very differently, suggesting that redbelly dace, unlike sculpin, may not be P limited. These results also suggest that phylogenetic differences may outweigh differences in diet.

Rainbow trout P and N:P excretion were also unexplained by diet and imbalances. Similar to redbelly dace, smaller rainbow trout were primarily responsible for the higher P excretion rates in Shope Fork vs. Ball Creek, even though fish in Shope Fork had higher %P and higher N:P imbalances. Body mass may outweigh stoichiometric theory in determining nutrient excretion within some species.

Our results suggest that body mass, growth efficiency, diet, and imbalances may outweigh stoichiometric variability within fish taxa and could explain large variation among taxa. However, stoichiometry, especially P content, may still be an adequate framework for predicting differences in nutrient excretion over larger phylogenetic differences. Cyprinids consistently had higher P excretion rates regardless of mass, body %P, and diet. Three explanations may be possible for the large unexplained variation in excretion:

- 1) Cyprinids, which are generally detritivores and herbivores, may not have as high growth efficiencies as predatory fish (lower acquisition of P for growth), resulting in higher P excretion. However, longnosed dace, which also had high P excretion relative to imbalance, are predators, feeding strictly on benthic insects based on my gut analyses. Phylogeny may be a stronger predictor of growth efficiencies than guild (Hendrixson et

al. 2007), especially since all of the fish with unpredicted higher P excretion were cyprinids. Therefore, growth efficiencies, determined by evolutionary history may be a large determinant of P excretion.

2) Assuming similar growth efficiencies, the diet of herbivorous cyprinids may be even more P-rich than the N:P values we assigned them based on epilithic collections. Lower dietary N:P translates to lower imbalances and should result in lower N:P excretion. Epilithon and insects had a similar %P; however, epilithon had lower %N, resulting in lower N:P values and thus sometimes lower N:P imbalances for herbivores. The actual N:P values for herbivorous or omnivorous fish may be even lower than we measured. Hood et al. (2000) found that the composition in the foregut of herbivore fish consistently had a lower N:P (higher P) than randomly collected biofilm from the stream. Sterner and George (2000) found that the nutrient composition in the foregut of cyprinid fish were very similar to the fish composition, which are dietary N:P values considerably lower than the ones I report. If I had used the algal N:P values from Sterner and George (2000), imbalances would have been very small and I might have had more power in explaining N:P excretion. This supports the contention that fish may be selectively feeding on food that is nutritionally superior in comparison to general browsing, and it also emphasizes the importance of accurately assessing diets.

3) Finally, some P excretion by consumers could be the result of non-assimilated inorganic material. Gizzard shad have been known to ingest detritus associated with sediment (Schaus et al. 1997; Torres and Vanni 2007). Brabrand et al. (1990) indicated that fish feeding on sediment tended to have higher P excretion rates than predatory fish. *Prochilodus*, a tropical detritivorous fish, ingests large amounts of sediments during yearly migrations and alters ecosystem processes via nutrient excretion (Flecker 1996, Taylor et al. 2006). However, large fluxes of phosphorus may not be entirely due to assimilatory processes. Inorganic P is often loosely attached to clays and other mineral surfaces and can be disassociated in low pH environments, such as in fish stomachs. Our inorganic P analysis method consisted of acid hydrolysis of dry matter at a pH of about 3, similar to the environment of a fish stomach (Sturm and Horn 1998). Higher P excretion rates in benthic fish could be possible due to loosely-attached mineral P in their diet that is mobilized during gut passage. Even in the case of predatory fish such as long-nose

dace, their mouth parts are adapted for gleaning benthic insects and, by association, inorganic material, from the substrate. Ingestion of inorganic P by benthic fish may alleviate P demand by converting unavailable substrate-bound P to available forms.

Fish communities have been shown to supply algal and bacterial communities with N and P, which form top down controls on ecosystem production. Using abundance data from Freeman et al. (1988), I found that the N and P flux from the fish community excretion was equal to 17 to 178% of the NH_4^+ -N and 10 to 96% of the SRP we measured leaving the lower weir on Ball Creek. This suggests that excretion, at times, may 1) make up a large percentage of the ammonia and phosphorus flux from the watershed and 2) may be influencing nutrient supply to autotrophic and heterotrophic communities within Ball Creek.

Macroinvertebrate Stoichiometry

Variation in macroinvertebrate excretion was not entirely explained by differences in body stoichiometry (Fig 11). Negative relationships existed between P excretion vs body %P and N:P excretion vs body N:P (Fig. 11); however, the relationships were influenced by one P-rich taxon, crayfish. Crayfish, on average, had 1.06 % P (as % of DW), more than twice the amount of P in insects (avg, 0.42 %P) (Table 9), and they excreted very low P in agreement with stoichiometric theory. Evans-White and Lamberti (2006) found that mesocosms containing crayfish had significantly lower periphyton P content than those with snail consumers, suggesting that crayfish had lower P excretion rates than snails. Kristiansen and Hessen (1992) found that *Astacus* crayfish had a maximum N:P excretion of 165, whereas the crayfish in my study had an average an N:P of 544. High body P in crayfish may be explained by the carapace of crustaceans. Vrede et al. (1999) indicated that P was essential to carapace formation (hydroxyapatite – calcium- associated P) in *Daphnia* and accounted for 14% of total body P. Thus, molting processes could possibly cause substantial P loss from crustaceans, causing P limitation (Hessen and Rukke 2000). The same process could cause P limitation in crayfish. Faerovig and Hessen (2003) suggested that although molting would only make up a small fraction of the total P budget of crayfish, modest losses can

have large implications on P limitation. Also, ontogenetic shifts and allocation of energy to reproduction in crustaceans can be very costly in terms of P. Other differences, such as RNA content and growth rate, may also be very different in crustaceans (Sommer et al. 2003) than insects.

Excluding crayfish, differences in excretion were more a function of diet and growth/assimilation efficiencies than body stoichiometry. Insect families had similar body P content, yet P excretion varied over two orders of magnitude. Hydropsychid filterers had the highest mass normalized and highest non-mass normalized P excretion and lowest N:P excretion ratio, regardless of mass or body stoichiometry (Table 11, Fig. 10). Hydropsychids had low body P, yet a very P-rich diet. Seston, hydropsychid's primary diet, had the lowest N:P value (P-rich) giving filterers the lowest N:P imbalance. Hydropsychids may have very low imbalances and possibly low nutritional constraints, resulting in their high production in streams (Huryn and Wallace 1987; Benke and Wallace 1997). Larger hydropsychids, such *Diplectrona*, *Hydropsyche*, and *Cheumatopsyche*, are filterers, but they also are predators (Benke and Wallace 1997; Benke et al. 2001; Rosi-Marshall and Wallace 2002). Most of the prey in caddisfly diets are chironomids (Benke et al. 2001), which are generally more P-rich than other aquatic insects (low N:P ~ 30) (Frost et al. 2003; Cross et al. 2003; Evans-White et al. 2005). However, my results indicate that seston had a similar or lower N:P than most stream macroinvertebrates (avg N:P=27) (Table 4). Apparently, hydropsychids are not P-limited, allowing them to excrete very high rates of P. Also, because hydropsychids capture food in their net, they have the ability to be selective in eating only nutritionally superior foods. Isonychiids also consume seston/FPOM, and although they had one of the highest P excretion rates, it was not as high as hydropsychids. One explanation is that isonychiids have a lower body N:P (49) as compared to that of hydropsychids (N:P =59). Hydropsychids therefore have a lower imbalance than isonychiids and may be able to release P at higher rates.

Shredders had extremely low N and P excretion rates (including non-mass normalized rates), regardless of mass or stoichiometry (Table 11, Fig. 9, Fig. 10). Shredders had higher P and lower N:P and C:P body values compared to other insects, yet their diet was among the most P deficient (Table 10). Elser and Urabe (1999)

indicated that at very high algal C:P values, grazer P excretion was no longer a function of body P. The same principle should be applicable to most consumer/resource relationships. Very low nutrient release rates result from an extremely N and P deficient food source along with low assimilation efficiencies that are characteristic of detritivores (Sterner and Elser 2002). Previous studies indicate that the diets of *Pteronarcys* and *Pycnopsyche*, the two shredders in this study, are primarily composed of leaf detritus (Benke and Wallace 1997; Hutchens et al. 1997; Benke et al. 2001; Rosi-Marshall and Wallace 2002). Leaf detritus had the highest N:P, C:P, and C:N values compared to other resources, resulting in the highest imbalances for shredders. Shredders ingest low quality foods (cellulose, lignin, phenols etc), which may lower assimilation efficiencies and slow the process of acquisition of nutrients necessary for growth and reproduction (Frost and Tuchman 2005). The imbalance of shredders may be even higher than ratios suggest since %N can be composed of refractory N compounds that are difficult to assimilate (Balseiro and Albarino 2006).

Predators also had low N and P excretion, although for very different reasons compared to shredders. Predators generally have high assimilation and growth efficiencies (Sterner and Elser 2002) along with diets that are very similar to their own nutritional composition (Fig. 8). As a result, predators have low C:N and C:P imbalances (Fig. 12). However, imbalances, in terms of bulk elemental ratios, may be inaccurate compared to biochemical imbalance (e.g. amino acids) (Anderson et al. 2004), which affect food quality and assimilation efficiencies. Prey have similar biochemical composition to predators, which allows efficient absorption through gut walls (high assimilation efficiency) and higher growth efficiencies (Anderson et al. 2005) resulting in low N and P waste.

Scrapers, or herbivores, generally have moderate assimilation and growth efficiencies, higher than detritivores yet lower than predators (Sterner and Elser 2002). Scrapper N:P imbalance was almost as high as shredders, however C:N imbalance was almost as low as that of predators (Fig. 12). Dodds et al. (2004) indicated that primary producers (epilithic algae) had intermediate C:N, higher than stream prey, yet lower than detritus. However, our N:P values for epilithon are lower than other studies (Table 3) (Frost and Elser 2002; Sterner and Elser 2002; Cross et al. 2003). Assuming that the

rates of N and P excretion is limited by the amount of N and P available in their diet, my N:P values of epilithon may be a more accurate assessment of nutrient availability to herbivores since their N and P excretion rates were high. Studies indicate that heptageniids, especially *Stenonema*, which were dominant in most of the streams in this study, utilize epilithic biofilms as a food source (Hall and Meyer 1998, Tank et al. 2000; Rezanka and Hershey 2003); however selectivity may occur between algal, detrital, and bacterial fractions that differ in nutrient availability (Tank et al. 2000; Rosi-Marshall and Wallace 2002). Low C:N diet and imbalances in combination with moderate assimilation and growth efficiencies were most likely responsible for the highest N excretion rates for scrapers. Grimm (1988) found that 9 to 31% of ingested N by scrapers was recycled back as ammonia (NH_4^+), and 70% of total nitrogen retained by the ecosystem was recycled as nitrogen excretion, suggesting high N turnover in scrapers. Scraper body stoichiometry does not deviate extensively from other insect taxa, especially hydropsychids, thus a higher N:P of epilithon compared to seston might have resulted in higher N turnover. High N excretion indicates that scrapers must have high N assimilation, suggesting that nitrogen content in epilithic biofilms is composed of labile organic material as compared to other resources.

My results indicate that constraints imposed by P limitation may outweigh allometry in determining excretion. Mass normalized P excretion was negatively related to dry mass as predicted. Allometry predicts that there should be a positive relationship between non-mass normalized P excretion and mass (larger organisms excrete nutrients at higher rates than smaller organisms); however, there was no significant relationship (Fig. 10A). Crayfish mass, on average, was 30 times higher than individuals from other macroinvertebrate taxa. I assumed crayfish should have extremely high non-mass normalized phosphorus excretion. However, crayfish, along with shredders and predators, had low non-mass normalized P excretion rates. Hydropsychids had the highest non-mass normalized P excretion rate, regardless of their small mass suggesting that hydropsychids may be large sources of P in streams. The difference in P excretion may be due to dietary P constraints in crayfish diet as compared to the P-rich diet of seston for hydropsychids. As predicted by allometry, mass normalized N excretion was also negatively related to dry mass, and non-mass normalized N excretion was positively

related to dry mass including crayfish (Fig. 10B). These results indicate that in ecosystems where crayfish make up a substantial biomass they could be a large source of N but a substantial phosphorus sink. When crayfish were excluded, there was no consistent pattern between non-mass normalized N excretion and mass; however, two groups emerged (Fig. 10C). Predators and shredders have low mass normalized and non-mass normalized N excretion regardless of their larger size, whereas the remaining FFG's, with the exception of one taxa (Baetidae), seemed to form a positive relationship (Fig. 10C). Excretion, under a finer scale of resolution, may be dictated more by diet and growth efficiency than allometry or body stoichiometry.

Distinct patterns in resource stoichiometry and excretion among streams suggested that changing nutrient availability in different ecosystems can alter consumer excretion. Pteronarcyds, heptageniids, and crayfish occurred in more than one stream and showed deviations in nutrient excretion that could be explained by diet. Differences in nutrient content of allochthonous inputs may alter nutrient availability to shredders and affect growth (Frost et al. 2002; Swan and Palmer 2006), thus influencing nutrient cycling. For example, pteronarcyds in Shope Fork had higher N and N:P excretion than in Ball Creek. My results show that leaf detritus had higher %N and lower C:N in Shope Creek, which was lined with N-fixing alder (Table 5). Lower leaf C:N values should correspond to higher N excretion by shredders as found by Balseiro and Albarino (2006). Leaf detritus at Shope Fork was also more P-rich (lower N:P and C:P), which may have been the result of increased microbial colonization of alder, a more labile food source than rhododendron. Interestingly, pteronarcyds at Shope Fork had lower body N:P and C:P, an indication of deviation from strict homeostasis.

The trend in heptageniid mayfly excretion was unexpected. Extremely high P excretion and low N:P excretion at Big Wilson may have been primarily due to the dominance of *Epeorus* as compared to *Stenonema*. Possibly, large differences in excretion may occur at not just the family level, but genus level also. We expect that if organisms are P-limited, increases in dietary N:P would result in differential cycling of N, thus a higher N:P excretion (Vanni 2002). Heptageniid N:P excretion, however, was negatively related to epilithon N:P suggesting N limitation instead of P limitation (Fig. 13A). Elser and Urabe (1999) predicted that excretion N:P is identical to ingested N:P,

unless the grazer accumulates a limiting nutrient that would result in N:P excretion ratios different than that of the resource. Epilithon N:P was very similar or slightly higher than heptageniid body N:P (avg. 45); however, the N:P excretion ratio was lower suggesting N retention. I also predicted that higher C:N epilithon values should lead to more N retention, thus lower N nutrient cycling (Dodds et al. 2004). N excretion was negatively related to epilithon C:N, but this may have been an artifact of larger heptageniids at higher epilithic C:N values leading to lower mass-specific N excretion. Higher C:N may cause N-limitation but also may allow for more C for growth. I also found evidence for deviation from strict homeostasis where heptageniid body C:N paralleled epilithon C:N (Fig. 13B). The increase eventually leveled off apparently to the limits of plasticity (Sturner and Elser 2002).

Crayfish also displayed non-strict homeostasis. Body N:P and body C:N increased with increasing leaf N:P and C:N (Fig. 15A and 15B), although the relationship with leaf N:P was stronger. Crayfish are known to be generalists, acting as detritivores and shredders (Creed and Reed 2004), grazers (Creed 1994; Evans-White and Lamberti 2005; Evans-White and Lamberti 2006), or predators. My results indicate that in these streams, leaf detritus or CPOM influenced crayfish stoichiometry whereas epilithon did not. Thus, leaf detritus may comprise a larger proportion of diet than algae in the streams in this study. An extremely low %P diet of leaf detritus agrees with the extremely low P excretion in cambarids. Secondly, body N:P deviation may be indicative of P-limitation in crayfish (Fig. 15B). However, crayfish N excretion was negatively related to C:N imbalance across four streams suggesting higher N retention at higher C:N values (Dodds et al. 2004, Fig. 14). Even though crayfish seem to be P-limited in general, they also are influenced by nitrogen availability.

Imbalance along with excretion may be an excellent way to accurately assess resource nutrient availability or to pinpoint actual diet. N:P excretion was positively related to N:P imbalance (Fig 12A). I predicted that N excretion should be higher at higher N:P imbalances; however, shredders did not follow this relationship. They had low N:P excretion despite high N:P imbalances. Due to extremely high diet C:N and C:P values, shredders may be co-limited by N and P resulting in low excretion of both nutrients. Baetid mayfly N:P imbalance was calculated using an epilithon value for diet;

however, baetids still seemed to have a very high N:P excretion despite having the highest body N:P (Fig. 12A). Assuming that baetids have similar assimilation efficiencies as heptageniids, their food source must have a higher N:P than epilithon. Studies have indicated that Baetids may consume mostly algal cells and fine detritus (Mulholland et al. 2000; Lancaster and Waldron 2001), which may be less P-rich than bacteria dominated biofilms in heptageniids' diet.

P excretion was negatively related to C:P imbalance and N excretion was negatively related to C:N imbalance as predicted (Fig. 12B and 12C). However, there was a large amount of unexplained variation. Predators did not follow the trend, which may be explained largely by differences in biochemical content. C:P and C:N may be a useful way to assess the nutritional status of algae, particulates, and detritus since they have similar chemical makeup (cellulose, hemicellulose, etc). Assessing the nutritional status of a totally different food source, in terms of biochemical content, may be difficult, especially in the amino acid rich diet of predators. Because predators' diet is biochemically different, using C:nutrient ratios to compare nutritional quality to herbivore/detritivores may be misleading.

Consumers have been shown to be a supply of inorganic N and P to microbial aquatic communities. Using standing stock data from Huryn and Wallace (1987), I found that although hydropsychid standing stock was over 3 times lower than that of crayfish in Ball Creek, hydropsychids were excreting 160 times the amount of P of crayfish. Even limnephilids, a shredder having 18 times lower standing stock than crayfish, were excreting more P than crayfish. This indicates that although crayfish are abundant in Ball Creek, they are a phosphorus sink. Thus, the consumer community composition can be very different in terms of nutrient turnover, possibly affecting autotrophic and heterotrophic communities differently.

Food web model

Most consumer excretion models suggest that if food N:P and consumer N:P are equal, then the N:P of excretion will also be equal (Sterner 1990; Elser and Urabe 1999). However, if food N:P is higher than the consumer N:P, then by mass balance, excretion N:P should be higher than both the consumer and food N:P. For P-limited organisms, if the food N:P is much greater than the consumer N:P, N:P excretion should exponentially increase because a greater proportion of assimilated P has to be sequestered for growth. This trend was observed with two organisms, mottled sculpin and crayfish. N:P excretion was extremely high in these organisms, possibly due to P-limitation. However, for the majority of the other consumers, my results show that herbivorous, detritivorous, and predatory macroinvertebrates and fish consistently had a lower N:P excretion ratio than their available food (Figure 16). Generally, macroinvertebrates had a lower N:P excretion than their body N:P and the fish had an N:P excretion ratio similar to or, at times, slightly higher than their body N:P. The results can indicate either of two mechanisms: 1) consumers are selectively eating foods with a lower N:P (more P-rich) to meet their N:P demands or 2) consumers are not limited by the N or P of their diet.

Schindler and Eby (1997) suggested that if N or P is limiting fish growth, then N:P excretion rates should be highly variable. However, after finding that N:P excretion rates were not variable, they concluded that P-limitation in fish is rare and that most fish are energy limited. They indicated that the majority of N:P excretion ratios of fish were around 15. Sterner and George (2002) also postulated that excretion rates by cyprinids should be also around 15, similar to their body N:P ratio. They found that the N:P of food in the foregut of the fish were very similar to the N:P in the fish body indicating a food source that exactly meets the elemental demands of the fish. However, this emphasizes another point. The N:P ratio in the foregut of the cyprinids in Sterner and George's (2002) study were very low (~17). My N:P values for epilithon ranged from 28-81, much higher than the average N:P ratio of the cyprinids in my study (~12). This suggests that consumers were selectively consuming foods that exactly met their nutritional demands. Hood et al. (2005) showed that the N:P ratio of the foregut of herbivores was much lower than the N:P ratio of randomly collected epilithon. This

relationship could be similar for macroinvertebrates as well. Hall and Meyer 1998 suggested that heptageniid mayflies consume a large portion of the bacterial fraction in biofilms, which have considerably lower N:P ratios (4-30) (Chrzanowski and Kyle 1996) than Heptageniid N:P (avg. 53). Caddisfly filterers have been shown to selectively consume nutrient rich particles from the stream column as well.

Consumers probably selectively consume foods that are rich in N and P. This highlights the need to correctly assess the diet of consumers. However, I also found that the majority of predatory fish (other than sculpin) and predatory macroinvertebrate N:P excretion was well below the average N:P value of their resource (macroinvertebrate prey). Because I conducted gut analysis on the fish predators, the assessment of their diet should be fairly accurate leaving little room for confounding error from selective consumption. Even if some fish ate terrestrial insects, which have a lower N:P (~30) than aquatic insects (avg. 55), the N:P of the fish (~14) were still considerably lower than their resource. Selective consumption may still occur; however, the low N:P excretion values indicate that most consumers are not limited by N or P.

The majority of consumers, whether fish or macroinvertebrate, had a lower N:P excretion ratio than that of the water column. If consumers are playing an important role in nutrient dynamics in streams, then excretion values suggest that consumers alter the N:P ratio of inorganic nutrients available to microbial assemblages by providing relatively lower N:P ratios (more P). However, the P-limited organisms, mottled sculpin and crayfish, were excreting extremely high N:P ratios, similar to or higher than that of the water column. P-limited organisms may alter the N:P ratio of available inorganic nutrients by providing relatively lower amounts of phosphorus. This should not be misinterpreted to mean that some organisms are sources of phosphorus and others are sinks, which would entail a complete estimate of an ecosystem nutrient budget. Because of diet differences, some consumers excrete larger amounts of phosphorus than others, such as hydropsychid caddisflies vs shredders. However, all consumers excrete and are inherently sources of nutrients, whether great or small, to microbial assemblages. My data simply suggest that the role of consumers in the dynamics of N and P in streams may be altered by the presence of P-limited organisms in food webs.

Conclusions

I found consumer stoichiometry did not account for the high variability in nutrient cycling, especially across stream ecosystems. Among taxa, body stoichiometry may still be an adequate explanatory variable in nutrient excretion, as in the case of very P-rich organisms like mottled sculpin and crayfish. Extremely low P excretion rates for sculpin and crayfish indicate that these organisms are P sinks in ecosystems where they exist primarily as a result of allocating large amounts of P to bone or exoskeleton material. On the other hand, dace and hydropsychids seem to be large sources of P possibly because of their P-rich diet. High N:P cycling in light of high N:P diets along with low P cycling due to high C:P diets suggests possible P limitation, however nutrient limitation (N or P) as compared to energy limitation (C) has been an issue of debate (Shindler and Eby 1997). Many studies, have evidence for P-limitation (Hessen and Rukke 2000; Stelzer and Lamberti 2002; Urabe et al. 2003; Hood et al. 2005; McCarthy et al. 2006; Yang et al. 2006). Also, herbivores as well as some collectors may have a more P-rich diet than even my data suggest indicating that values for epilithon and seston are misleading. Large variation in nutrient cycling within and across taxa demonstrates that under different scales of resolution, different processes dictate the turnover of nutrients such as growth efficiency and nutritional constraints.

Assessing the role of consumer driven nutrient cycling across ecosystems is important. Consumers have been shown to influence nutrient dynamics in ecosystems (McIntyre et al. 2007; Schindler 2007; Evans-White and Lamberti 2006; Evans-White and Lamberti 2005; Vanni et al. 2002; Vanni et al. 1997; Schaus et al. 1997). There is evidence that consumers may have a role in nutrient dynamics, at least in Ball Creek. Future research should specifically evaluate the role of consumers in nutrient dynamics in temperate heterotrophic streams. This is especially important considering that nutrient excretion may be influenced by diet in N and P limited systems.

Nutrient excretion provides a framework for accessing resource nutrient availability or pinpointing actual diets of different consumers. Imbalances and nutrient excretion used in conjunction with production data could yield results that shed light on to how dietary constraints affect population dynamics across ecosystems under various nutritional regimes. Using differences in nutrient cycling across ecosystems will aid in

determining differences in growth, allocation of nutrients, and resource nutrient availability along with the path of nutrients through a food web.

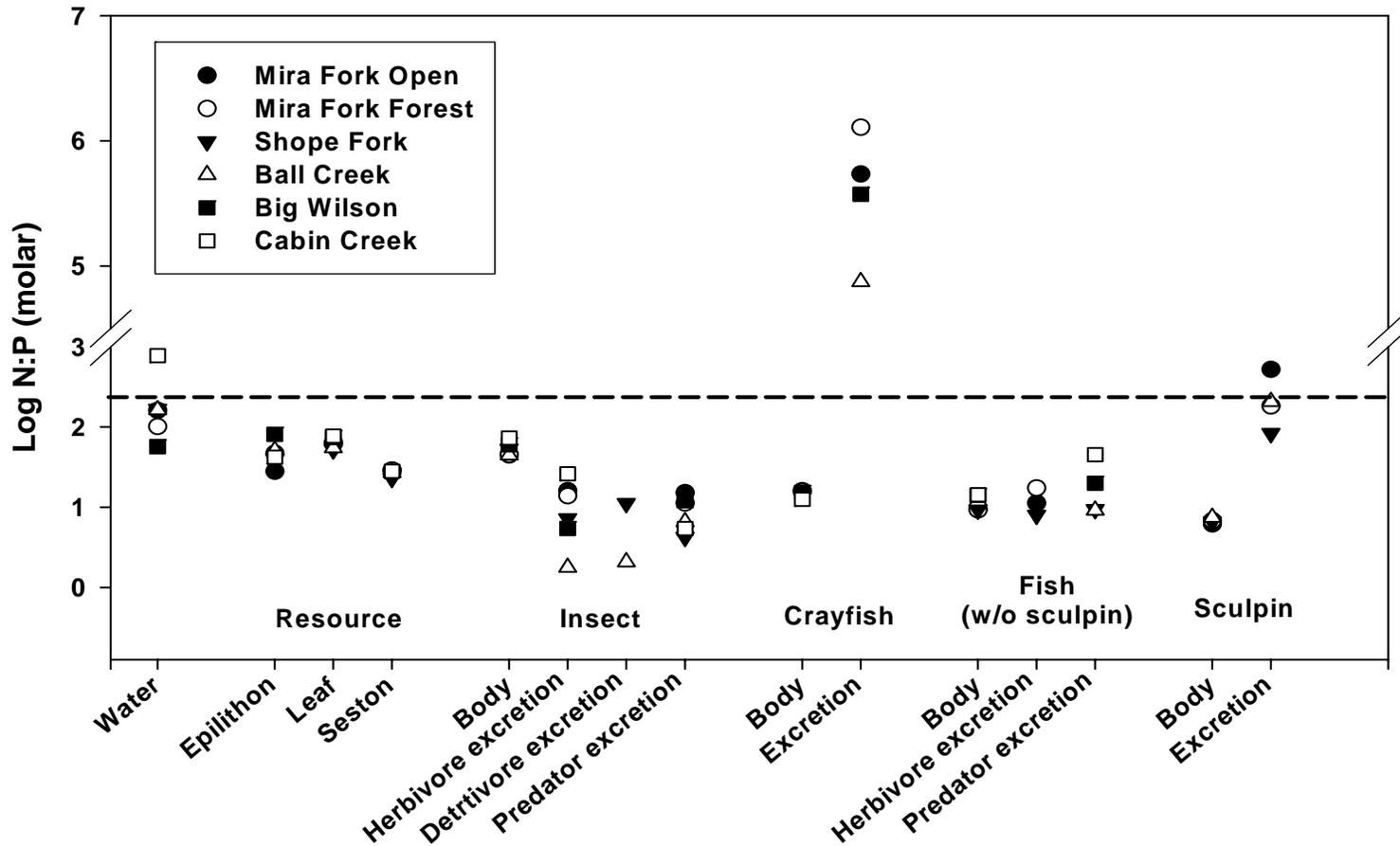


Figure 16. N:P ratios of various compartments of a stream food web. Each symbol represents the average ratio for each compartment for each stream.

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