

Taxonomic and regional patterns in benthic macroinvertebrate elemental composition in streams

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SUMMARY

1. Ecological stoichiometry has been used to better understand dynamics in consumer growth and the role of consumer-recycled nutrients because it focuses on more than one element. Most research has focused on pelagic rather than benthic consumers. Variation in elemental composition among benthic consumer taxa would suggest that taxa differ in their susceptibility to nutrient limitation or in their role in recycling nutrients.
2. We collected benthic macroinvertebrates from streams in two regions (Indiana–Michigan and Wisconsin, U.S.A.) to examine taxonomic and regional variation in benthic macroinvertebrate body carbon (C), nitrogen (N), and phosphorus (P) concentrations and ratios.
3. Elemental composition varied little within taxa common to both regions. In contrast, elemental composition differed greatly among taxa and appeared to be related to phylogeny. The elemental composition of macroinvertebrates clustered into three distinct groups: insects, mollusks, and crustaceans. To a lesser extent, insects and mollusks also differed in elemental composition among genera.
4. Functional feeding groups (FFGs) differed in elemental composition, with predators having a higher N content than other groups. Substantial elemental imbalances between C and N were found between most primary consumers and their likely food sources, and the magnitude of the imbalance depended in part on the FFG.
5. Our results support an assumption of most ecological stoichiometry models that, within a species, the elemental composition of aquatic invertebrates is relatively constant. Variation in elemental composition among taxa at various higher taxonomic levels suggests that susceptibility of stream invertebrates to nutrient limitation and their role in nutrient cycling will strongly depend on phylogeny.

Keywords: carbon, crustacean, insects, mollusk, nitrogen, nutrient content, phosphorus, stoichiometry, stream

Introduction

Ecological stoichiometry addresses the consequences and constraints of mass balance of multiple chemical elements in ecological interactions (Reiners, 1986; Sterner & Elser, 2002). Trophic interactions and those between organisms and their abiotic environment can

be influenced by the elemental requirements of those organisms relative to elemental supply in their environment (Elser & Urabe, 1999). Ecological stoichiometry theory has enhanced our understanding of two main areas in aquatic sciences: food quality effects on consumer growth (Sterner & Hessen, 1994; Frost, Elser & Turner, 2002a; Stelzer & Lamberti, 2002) and consumer-driven nutrient recycling (Sterner, Elser & Hessen, 1992; Elser *et al.*, 1995; Evans-White & Lamberti, 2005). Ecologists are just beginning to apply this theory to the benthic ecology of lakes (Frost

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& Elser, 2002; Frost *et al.*, 2002b, 2005; Vanni *et al.*, 2002) and streams (Cross *et al.*, 2003; Bowman, Chambers & Schindler, 2005). To facilitate hypothesis generation and to bound parameters in stoichiometry models, ecologists need more descriptive information on the amount of variation in benthic macroinvertebrate elemental composition and the possible factors driving this variation (Frost *et al.*, 2002b, 2003). Information is also needed on the magnitude of elemental imbalances between food sources and consumers to predict where and when consumers may be limited by essential elements and the rates and ratios at which consumers cycle nutrients.

Recent studies have contributed information on factors affecting variation in benthic macroinvertebrate elemental composition. Significant taxonomic variation in benthic macroinvertebrate elemental composition has been described in lakes (Frost *et al.*, 2003; Liess & Hillebrand 2005) and streams (Cross *et al.*, 2003; Bowman *et al.*, 2005). Both lake studies found significant taxonomic variation in littoral benthic macroinvertebrates. While Frost *et al.* (2003) found little variation in macroinvertebrate elemental composition across lakes, Liess & Hillebrand (2005) found significant seasonal variation in benthic macroinvertebrate elemental composition and variation in that composition among sites within the same lake. The stream studies have predominantly compared insect macroinvertebrates sampled from a small number of streams within the same region (Cross *et al.*, 2003; Bowman *et al.*, 2005). To date, no comprehensive assessment of variation in elemental composition among broad taxonomic groups of macroinvertebrates (i.e. insects, crustaceans, mollusks) from streams exists.

Some evidence suggests that benthic macroinvertebrate elemental composition may track the elemental composition of their food source. Cross *et al.* (2003) sampled benthic insects from two similar forested streams, one that was amended with nutrients [nitrogen and phosphorus (N+P)] and one that was not, and found that P content and the carbon (C) : P and N : P ratios of some insects (e.g. Trichoptera) tracked changes in the per cent P and N : P of food sources. However, Frost *et al.* (2003) found no such pattern in benthic macroinvertebrates collected from lakes encompassing a trophic gradient. In fact, the C, N, and P content of benthic macroinvertebrates varied little among lakes. In addition Bowman *et al.* (2005)

also found that benthic macroinvertebrate elemental composition upstream and downstream of nutrient point sources (e.g. wastewater treatment plants) changed little relative to changes in epilithon elemental composition. It would be useful to explore whether the relationships between macroinvertebrate elemental composition and food source elemental composition observed by Cross *et al.* (2003) exist at a regional scale in streams.

The stoichiometry of consumer-driven nutrient recycling and of nutrient limitation of animal growth is most relevant when an elemental imbalance occurs in trophic interactions. An elemental imbalance is a measure of how dissimilar the relative supply of one or more elements is between an organism and its food (Sterner & Elser, 2002). Elemental imbalances between consumers and food resources are common in pelagic and terrestrial ecosystems (Elser *et al.*, 2000). Cross *et al.* (2003) reported large elemental imbalances between detritivorous insects and leaf detritus, and between herbivorous insects and epilithon in streams. However, ecologists need more information about the magnitude of elemental imbalances from a wide variety of stream ecosystems and various taxa of benthic insects, mollusks, and crustaceans to more accurately assess the prevalence of elemental imbalances in stream ecosystems. Such information may allow us to predict where and when consumer-driven nutrient recycling and nutrient-limited growth in consumers will occur.

The main objective of our study was to determine if stream benthic macroinvertebrate elemental composition varied spatially and with phylogeny. We hypothesised that phylogeny would explain more variance in benthic macroinvertebrate elemental composition than spatial factors in accordance with previous studies of pelagic zooplankton (Andersen & Hessen, 1991) and lake benthic macroinvertebrates (Frost *et al.*, 2002a). We also collected potential food sources to estimate the degree of consumer-resource elemental imbalances and to determine if benthic macroinvertebrate elemental composition tracked spatial differences in food source elemental composition. We expected that the elemental composition of some macroinvertebrates might track that of their potential food source given evidence from a previous study (Cross *et al.*, 2003). Our results should better define the functional roles of macroinvertebrates in stream ecosystems by determining which taxa are likely to

control the relative supply of N and P in streams. Furthermore, information on macroinvertebrate elemental composition could indicate which taxa may be more susceptible to nutrient limitation. Finally, knowledge of within-taxon variation in elemental composition can be used to assess the homeostasis assumption of most ecological stoichiometry models.

Methods

Study sites

We sampled benthic macroinvertebrates from 35 streams located in two main regions: Indiana–Michigan border region (IN–MI) and central Wisconsin (WI) (Appendix). In addition, a gradient of dissolved P concentrations, measured as soluble reactive phosphorus (SRP; range: 3–99 $\mu\text{g}\cdot\text{L}^{-1}$), existed among the streams. The IN–MI streams and rivers ($n = 7$) were located within the St Joseph River basin, which occupies mostly glacial till and outwash plains. Catchments of individual streams contained a mix of agriculture (predominant), urban, and forested areas. They were relatively low gradient, had sandy substrate, and varied in size (4 to >30 m in width; 22 to >93 cm depth). Streams and rivers sampled in Wisconsin ($n = 27$) were located in the Fox, Wolf, and Wisconsin River basins. Catchments of individual streams contained a mix of hardwood forests and row-crop and feedlot agriculture. They were all low-gradient but varied considerably in size (1–20 m in width; 15–150 cm in depth). Sand was the predominant substrate in most streams, but several streams contained abundant cobbles and boulders. Woody debris was common in many of the smaller streams.

Sampling procedures

Benthic macroinvertebrates were collected by hand and with dip nets at each site in April and July of 2000 for IN–MI sites and during June, July, and August of 2002 for Wisconsin sites. We sampled macroinvertebrates from three sites, which were approximately 50–100 m apart, in IN–MI streams and at one site in WI streams. The number of individuals collected per taxon at each site ranged from one individual for larger taxa (e.g. crayfish) to up to 150 individuals for smaller taxa (e.g. *Baetis* mayflies). In general, at least two to three and 15–30 individuals per large and small

taxa, respectively, were sampled per site. Individuals of small taxa were pooled (i.e. two to three pooled samples per stream for IN–MI sites and one pooled sample per stream for WI sites) to obtain enough material for elemental analysis. All macroinvertebrates were transported to the laboratory on the same day of collection, kept at 4 °C overnight to allow clearance of guts, identified to the lowest taxonomic level possible (typically genus or species) using a dissecting microscope, stored in a freezer (–20 °C), and then either freeze-dried (IN–MI; VirTis Freeze-Mobile 12SL, VirTis, Gardner, NY, U.S.A., <–40 °C, 1 Pa) or oven-dried (WI; 45 °C). We removed shells from all mollusks sampled in both regions prior to analysis, but all body parts were analysed for crustaceans and insects. Mollusk shells were not included. Some macroinvertebrates from IN–MI were measured and sorted into size categories before drying and elemental analysis. We measured *Orconectes propinquus* (Girard) carapace length (CL), and the shell length of *Elimia livegens* (Menke) and *Corbicula fluminea* (Muller). *Gammarus pseudolimnaeus* (Holsinger) were sorted into the following size categories before analysis of nutrient composition: <5, 5–10, and >10 mm total length. *Hydropsyche* spp. were sorted into the following size categories: 5–10 and >10 mm total length.

We sampled epilithon (i.e. biofilm attached to rocks), fine particulate organic matter (FPOM), and submersed fallen leaves from various locations of streams in both IN–MI and WI to determine the degree of elemental imbalances between benthic macroinvertebrates and their potential food sources. We scraped epilithon from cobbles using a brush and collected FPOM from the benthos using a turkey baster. Leaves and filamentous green algae were collected when available by hand from various spots in the stream. For WI, seston samples were also collected from the thalweg of each stream in 1- or 2-L acid-washed bottles. We transported all samples to the laboratory on ice for processing. For IN–MI, we filtered epilithon and FPOM slurries through a 500- μm sieve to remove macroinvertebrates and then allowed filtered slurries to settle before decanting off excess water. Samples were then freeze-dried and stored in a desiccator until they could be further processed. For WI, FPOM slurries were passed through a 500- μm sieve, filtered onto GF/F filters (Whatman Inc., Florham Park, NJ, U.S.A.) under

vacuum, and dried (45 °C) until they could be processed for elemental analysis. After removing macroinvertebrates by visual inspection, epilithon slurries were filtered onto GF/F filters as described for FPOM. In both regions, leaves and filamentous green algae were picked free of macroinvertebrates and dried as described above for each region. For WI, seston was retained on GF/F filters by vacuum filtration and then dried (45 °C).

Nutrient analyses

To prepare for elemental analysis, most dried macroinvertebrate and food source samples were ground to a fine powder in a Wig-L-Bug® grinder. Large macroinvertebrates (e.g. crayfish) were homogenised in either a coffee grinder or with a mortar and pestle. Subsamples were analysed for total C and N in a CHN elemental analyser (Carlo Erba EA1108 for IN–MI and a Carlo Erba 1500 for WI; Thermo Electron Corp., Milan, Italy) and for total P according to Rosemond (1993) which included digestion of invertebrate and food samples by combustion at 500 °C, followed by addition of 1 N HCl. After digestion, P was analysed by the ascorbic acid method. Total C, N, and P are reported as a percentage of dry mass. Elemental ratios (C : P, C : N, and N : P) are reported on a molar basis. Apple leaf standards (US National Institute of Standards and Technology, US Department of Commerce) were included with each set of samples. Per cent recovery of P and N from the apple leaf standards was typically 99–102%.

Statistical analyses

We analysed relationships between body length and per cent P, C : P, and N : P for *O. propinquus*, *E. livescens*, and *C. fluminea* using linear regression. Replicates in regression analysis were individual *O. propinquus* or the average of individual *E. livescens* and *C. fluminea* within the same 1-mm shell-length categories. Multivariate analysis of variance (MANOVA) was used to test for differences in nutrient composition among size categories of *G. pseudolimnaeus*. MANOVA significance was determined by Wilks' likelihood ratio test. Variables within significant multivariate tests were subsequently analysed with single-factor ANOVAs. One-tailed *t*-tests were used to test for differences in per cent P, N : P, and C : P

among the two size classes of *Hydropsyche* spp. sampled because our *a priori* hypothesis was that per cent P should decrease with size, and that N : P and C : P should increase with size. To assess variation in macroinvertebrate elemental composition at multiple spatial scales, a coefficient of variation (CV) for each elemental content and ratio was calculated among pooled samples taken from different locations within streams (IN–MI only), among streams within regions, and between regions for each insect macroinvertebrate sampled from both regions ($n = 7$). A two-way ANOVA was used to assess variation in the CV among elemental composition and spatial categories followed by a Tukey's HSD multiple comparison test when a factor was significant.

Individual streams served as replicates for each species sampled in all other statistical analyses. All variables (%C, %N, %P, C : N, C : P, and N : P) were tested for normality and homogeneity of variance before statistical analysis and %C, %N, and %P were arc-sine transformed and C : P and N : P were logarithm transformed. Two-tailed *t*-tests were used to test for seasonal (paired *t*-test) and regional (independent *t*-test) differences in macroinvertebrate nutrient content and ratio. We determined statistical significance based upon a Bonferroni-corrected alpha (Zar, 1999). We used MANOVA to determine differences in nutrient content and ratio among insects, mollusks, and crustaceans, and species differences within taxonomic groups to protect against experiment-wise error rate. Tukey's HSD multiple comparison test was used to test for differences among the elemental composition of insects, mollusks, and crustaceans when single-factor ANOVAs were significant.

Results

Because body size varied within species of benthic macroinvertebrates collected and because we sampled the same species in multiple months, we determined if size and season explained a significant amount of variation in macroinvertebrate elemental composition. In general, body size explained little variation in macroinvertebrate %C, %N, %P, C : N, C : P, or N : P. There were no significant relationships between shell length and the nutrient composition of *E. livescens* (%C $P = 0.14$, %N $P = 0.25$, %P $P = 0.28$, C : N $P = 0.08$, C : P $P = 0.92$, N : P $P = 0.07$, $n = 23$, shell length range: 8.2–22.7 mm) or *C. fluminea* (%C

$P = 0.06$, %N $P = 0.34$, %P $P = 0.08$, C : N $P = 0.32$, C : P $P = 0.27$, N : P $P = 0.21$, $n = 22$, shell length range: 6.5–20.0 mm). Similarly, size categories of *G. pseudolimnaeus* (MANOVA, Wilks' $\lambda = 1.24$, $P = 0.36$, $n = 23$) and *Hydropsyche* spp. ($P = 0.21$, $n = 9$) did not differ in elemental composition. However, *O. propinquus* CL was negatively related to %P (%P = $-0.011\text{CL} + 1.15$, $P = 0.007$, $r^2 = 0.24$, $n = 29$, CL range = 3.5–33.5 mm, %P range = 0.62–2.00) and positively related to C : P (C : P = $1.16\text{CL} + 79.0$, $P = 0.001$, $r^2 = 0.33$, C : P range = 45.3–133.9) and N : P (N : P = $0.15\text{CL} + 16.50$, $P = 0.04$, $r^2 = 0.15$, N : P range = 10.1–27.6). Although regressions between *O. propinquus* CL and %P, C : P, and N : P were significant, the amount of variance explained was small. Therefore, other factors are likely to be more important in explaining variability in %P, N : P, and C : P among *O. propinquus*. Only two taxa, *G. pseudolimnaeus* ($n = 6$) and *C. fluminea* ($n = 4$), were sampled in multiple streams in both April and July from IN–MI. Per cent C, %N, %P, C : N, C : P, and N : P ratios did not differ significantly between April and July for these taxa. Therefore, we pooled April and July samples from the same streams in IN–MI.

The elemental composition of benthic insects ($P > \alpha = 0.05$ per seven spp. = 0.007) and crustaceans ($P > \alpha = 0.05$ per two spp. = 0.025) generally did not differ significantly among regions (Fig. 1). However, *Helicopsyche borealis* (Hager) sampled from IN–MI (%C = 49.8 ± 1.7 ; mean ± 1 SD) had a lower %C ($P = 0.0004$) than those sampled from WI (%C = 53.2 ± 1.2). Moreover, *H. borealis* sampled in IN–MI (%N = 8.6 ± 0.4 , %P = 0.85 ± 0.09) had a higher %N ($P = 0.0011$) and %P ($P = 0.005$) than those sampled from WI (%N = 8.4 ± 0.1 , %P = 0.39 ± 0.1). *Helicopsyche borealis* in WI tended to have a higher C : P and N : P ratio but differences were not significant (Fig. 1). The elemental composition of epilithon, a potential food source for *H. borealis*, was not significantly different among sites where *H. borealis* was sampled in both IN–MI and WI. In addition, the elemental composition of potential food sources never significantly correlated with the elemental composition of macroinvertebrates. However, it must be borne in mind that food source samples are bulk measures of nutrient composition and may not reflect what is actually being eaten and assimilated by specific macroinvertebrates. Within the insect taxa sampled from both regions, the CV for %C, %N, %P, C : N,

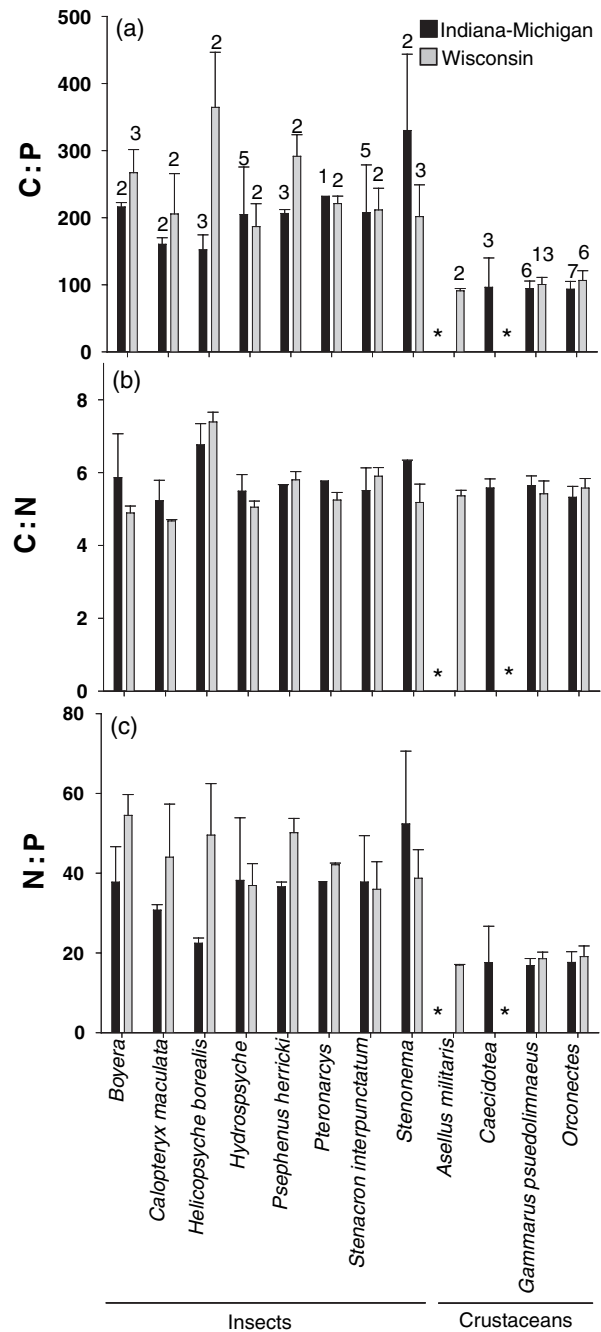


Fig. 1 Elemental composition (mean + 1 SD) of benthic insects and crustaceans, ordered alphabetically, sampled in both Indiana–Michigan and Wisconsin streams. The number of streams represented is indicated at the top of each bar in panel (a). (a) C : P molar ratio; (b) C : N molar ratio; (c) N : P molar ratio. * = no data.

C : P, and N : P of macroinvertebrates were similar regardless of the spatial scale considered (Fig. 2; $P = 0.75$). Variability was higher for %P than for %C and %N ($P < 0.0001$). This translated into a

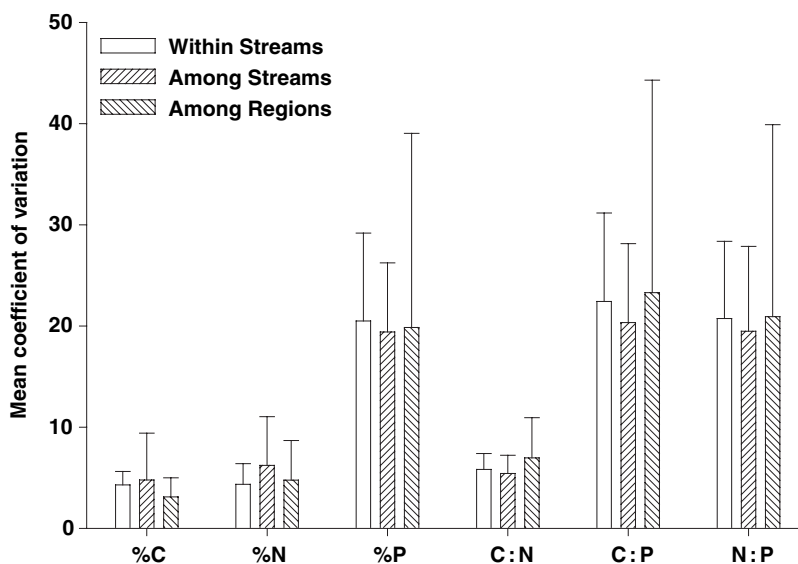


Fig. 2 Mean coefficient of variation (CV + 1 SD) for %C, %N, %P, C : N, C : P, N : P of benthic insect macroinvertebrates sampled in both Indiana–Michigan (IN–MI) and Wisconsin streams ($n = 7$). Within streams represents the mean CV for each species sampled from two to three sites (approximately 50–100 m apart) within each stream (IN–MI only). Among streams represents the mean CV for each species across streams within a region. Among regions represents the mean CV for each species across the two regions.

higher variation in ratios containing P than for those without P.

Scatterplots of %N versus %P (Fig. 3a) and C : N ratio versus C : P (Fig. 3b) for all macroinvertebrate samples revealed three major taxonomic clusters: insects, mollusks, and crustaceans. Per cent C, %N, %P, C : N, C : P, and N : P differed among the major taxonomic groups of benthic macroinvertebrates (Wilks' $\lambda = 39.2$, $P = 0.0001$) (Table 1). Insects had the highest %C followed by mollusks and then crustaceans. A similar pattern was observed for %N except that mollusks did not differ from either insects or crustaceans. Crustaceans had the highest %P followed by mollusks and then insects. Both C : P and N : P were highest for insects followed by mollusks and then crustaceans. Insects had a slightly higher C : N ratio than did mollusks.

Significant variation in macroinvertebrate elemental composition also existed at a finer taxonomic scale. MANOVA indicated significant differences in nutrient content and ratio variables (Table 2) among insect taxa (Figs 1 & 4), crustaceans (Fig. 1), and mollusks (Fig. 5). Per cent C did not differ among insect taxa (Table 2). However, we observed significant variation in %N and %P leading to differences in C : N, C : P, and N : P among insect species (Figs 1 & 4). Overall, C : N ratio was more consistent across insect taxa than either C : P or N : P ratios. Crustaceans varied only in %C (Table 2). Mollusks varied significantly in %C and %N, but not in %P (Table 2). Ratios with C varied significantly but N : P ratio did not (Fig. 5).

Macroinvertebrate functional feeding groups (FFGs) differed significantly in nutrient content and ratio (MANOVA, Wilks' $\lambda = 2.7$, $P = 0.0003$), but did not differ in P content ($P = 0.22$) or in C : P ratio ($P = 0.16$; Fig. 6). Predator N content was higher ($F = 7.3$, $P = 0.0003$) than that of scrapers and collector-gatherers (Fig. 6a). Filterers had a significantly higher N content than collector-gatherers. Shredders had a significantly higher C : N ratio ($F = 3.2$, $P = 0.03$) than predators (Fig. 6b). Predators also had a significantly higher N : P ratio ($F = 3.5$, $P = 0.02$) than scrapers and collector-gatherers.

Imbalances between food sources and benthic macroinvertebrate FFGs were more pronounced for nutrient content than for nutrient ratio (Fig. 6). Material at the base of stream food webs (e.g. algae, leaves and FPOM) contained a much lower amount of N and P than did consumers (Fig. 6a). Accordingly, macroinvertebrate FFGs had a lower C : N and C : P than their potential food sources (Fig. 6b). Imbalances in C : N between FFGs and potential food sources were more prominent than those found for C : P. Shredders had much lower body C : N and C : P than leaves, their potential food source. Because means from pooled data were plotted (Fig. 6), the patterns obscure larger (or smaller) imbalances between some invertebrate taxa and their food sources. For example, some taxa of grazing snails, such as *Physa gyrina*, had C : P ratios of about 100. Therefore, these snails have a larger elemental imbalance with epilithon than did inver-

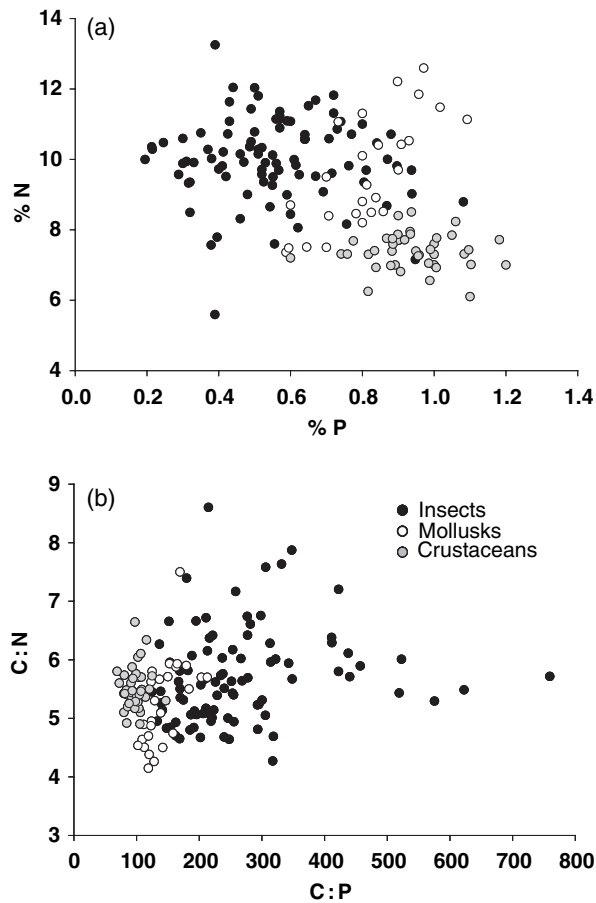


Fig. 3 Elemental composition of benthic insects, mollusks, and crustaceans from Indiana–Michigan and Wisconsin streams. Each point represents a taxon from a particular stream. Insects ($n = 93$) are represented by 29 taxa in 25 streams, mollusks ($n = 26$) by 11 taxa in 13 streams, and crustaceans ($n = 42$) by four taxa in 25 streams. (a) %N versus %P per unit dry mass; (b) molar C : N versus C : P ratio.

tebrate grazers collectively (mean C : P ratio of 175). Scrapers, collector-gatherers, and filterers often had a higher N : P ratio than did epilithon, filamentous green algae, seston, and FPOM food sources (Fig. 7). Shredders had a slightly lower mean N : P ratio than did leaves.

Discussion

Spatial patterns

The degree of homeostasis of animal elemental composition is a central question in ecological stoichiometry theory. There were no significant differences in nutrient ratios within taxa collected from two different regions. In fact, the magnitude of variation in elemental composition of benthic macroinvertebrates was similar within streams, among streams within regions, and between regions. The results of this study and others (Andersen & Hessen, 1991; Hessen & Lyche, 1991; Frost *et al.*, 2003) suggest that the elemental ratios of specific invertebrates sampled from a variety of environments are relatively stable. However, homeostasis of elemental composition may not be as strong in some taxa. Cross *et al.* (2003) observed variation in Trichoptera elemental composition that appeared to track food sources. We also observed differences in nutrient content within one trichopteran, *H. borealis*, but its nutrient content did not track epilithon nutrient content. It is possible that our measure of the elemental composition of epilithon does not adequately represent the food source eaten by *H. borealis*. For example, our sampling technique included everything attached to cobbles in the stream, yet *H. borealis* may be more selectively feeding on only some portions of the epilithon. It is also possible that some other factor (e.g. temperature, which can control growth rate and therefore possibly P content) was responsible for the observed differences.

Taxonomic patterns

Macroinvertebrate elemental content and ratio depended strongly on broad taxonomic identity. Crustaceans, mollusks, and insects had distinctive nutrient contents and ratios. These three groups differed most in %C and %P, which led to distinct differences in

Table 1 ANOVA results and the mean \pm 1 SD of %C, %N, %P, C : N, C : P, and N : P for insects, mollusks, and crustaceans

Taxon or statistic	%C	%N	%P	C : N	C : P	N : P
Insects	48.3 \pm 3.6 ^a	10.0 \pm 1.2 ^a	0.56 \pm 0.18 ^a	5.7 \pm 0.8 ^a	263 \pm 113 ^a	46 \pm 20 ^a
Mollusks	42.2 \pm 3.6 ^b	9.6 \pm 1.6 ^{a,c}	0.81 \pm 0.13 ^b	5.2 \pm 0.7 ^b	142 \pm 30 ^b	27.3 \pm 4.3 ^b
Crustaceans	34.8 \pm 1.7 ^c	7.4 \pm 0.5 ^c	0.94 \pm 0.12 ^c	5.5 \pm 0.3 ^{a,b}	98 \pm 15 ^c	17.5 \pm 3.4 ^c
F-value	262	85	78	5.3	145	134
P-value	<0.0001	<0.0001	<0.0001	0.006	<0.0001	<0.0001

Different superscript letters indicate significant differences among means.

Table 2 MANOVA results assessing patterns in variation among %C, %N, %P, C : N, C : P and N : P within insects, mollusks, and crustaceans

Taxon	MANOVA		%C		%N		%P		C : N		C : P		N : P	
	Wilks' λ	P-value	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value
Insects	2.01	0.0001	1.27	0.21	4.13	0.0001	2.49	0.0012	5.11	0.0001	3.80	0.0001	4.27	0.0001
Mollusks	4.50	0.0001	11.0	0.0002	9.63	0.0005	2.45	0.09	8.32	0.001	4.24	0.02	1.98	0.15
Crustaceans	2.11	0.011	3.62	0.02	1.93	0.14	0.32	0.81	0.43	0.73	0.40	0.75	0.18	0.91

MANOVA results were significant in all cases and were followed by individual one-way ANOVA's for each variable. These ANOVA results are also shown.

C : P and N : P ratios. The elemental contents of mollusks in this study only reflect composition of the soft body tissue (i.e. shell free). Mollusk shells, which often make up a large proportion of the body mass, primarily contain inorganic CaCO_3 and their inclusion in elemental analysis would have markedly reduced the overall percentage of C, N and P in mollusk bodies (Arnott & Vanni, 1996) and exacerbated the differences in elemental composition between the major taxonomic groups. Therefore, the observed elemental differences between mollusks, insects, and crustaceans may be more interesting because they primarily reflect physiologically active tissues affecting the cycling of nutrients and growth. However, because mollusk shells also contain a certain amount of the protein concholin, some organic C and N was not included in this analysis. The relative usefulness of shell-free mollusk tissue or whole mollusks including shells in predicting where and when mollusks may be limited by essential elements and the rates and ratios at which they cycle nutrients should be explored.

Previously, the average C : P, C : N, and N : P of crustaceans and insects had been considered similar (Elser *et al.*, 2000; Frost *et al.*, 2003). However, evidence from our study and from Cross *et al.* (2003) suggest that stream insect larvae have higher C : P and N : P than do terrestrial insects, which Elser *et al.* (2000) used for their crustacean-insect comparison. Cross *et al.* (2003) suggested that nutritional constraints in detrital-based streams may be severe enough to cause evolutionary selection for lower P content in detritivores because of the existence of large imbalances between food sources and consumers in these systems. Although Cross *et al.* (2003) found higher mean C : P and N : P for aquatic insects than we observed, a pattern of higher C : P and N : P in aquatic insects compared with terrestrial insects appears to be widespread. Evolutionary and ecolog-

ical factors that could potentially lead to a difference in the C : P and N : P of aquatic versus terrestrial insects should be explored further. Additionally, large differences in the elemental composition found between crustaceans and insects could result in large differences in elemental demand and cycling by these two taxonomic groups.

Why do crustaceans generally have a higher %P and a lower N : P and C : P than insects and mollusks? Relative proportions of major biomolecules contributing to body P pools may differ among these groups. Ribosomal RNA (rRNA) has been implicated as the biomolecule most likely to contribute to differences in invertebrate P content (Sterner & Elser, 2002). Therefore, benthic crustaceans may have an inherently higher rRNA content than do benthic mollusks and insects. It is also possible that P is associated with the calcium (Ca) in benthic crustacean carapaces and may account for the higher %P, and lower C : P and N : P of crustaceans when compared with insects and mollusks. Vrede, Andersen & Hessen (1999) showed that P complexed with Ca within the carapace of *Daphnia* contributed up to 14% of whole-body P. If P-Ca complexes in the carapace of crustaceans represents a significant P pool, then one might expect N : P to be negatively associated with the relative contribution of carapace to macroinvertebrate body mass. In addition to their carapace, crayfish contain other calcified structures within their body (e.g. the gastric mill and the entire exoskeleton) that may also contain elevated concentrations of P. More research is needed to identify the major pools of P-rich biomolecules in crustaceans, mollusks, and aquatic insects and the role of linkages among P and other elements (e.g. Ca) in driving differences in elemental composition among these groups.

Elemental composition varied among genera of insects and mollusks but not among crustacean

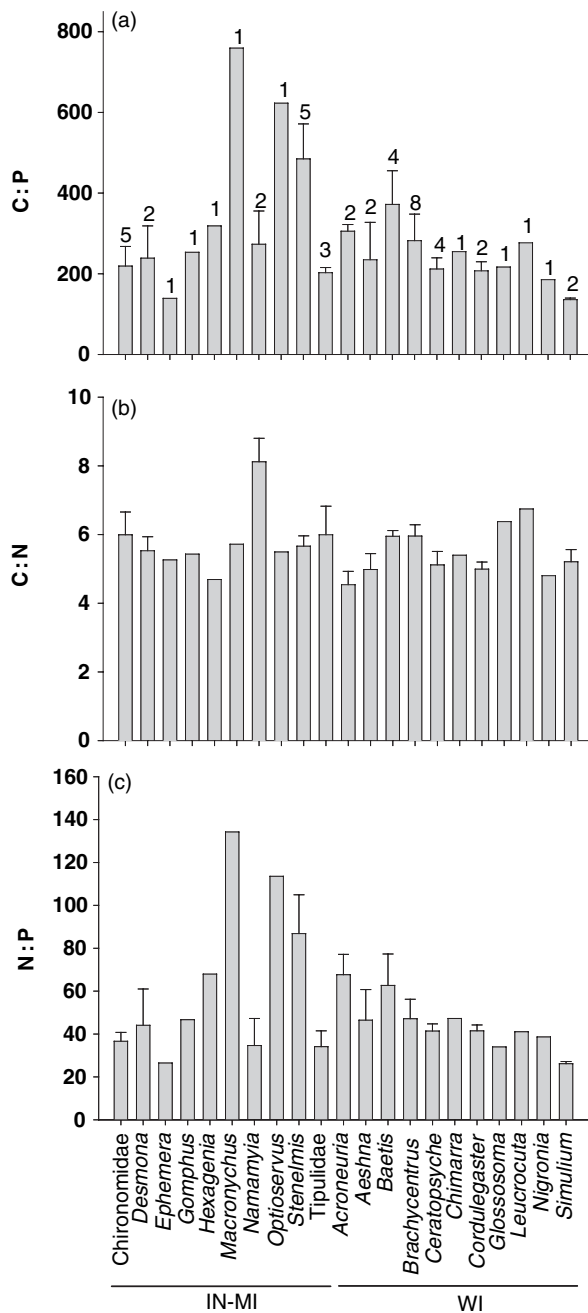


Fig. 4 Elemental composition (mean + 1 SD) of benthic insects, ordered alphabetically, sampled from Indiana–Michigan or Wisconsin streams. The number of streams is indicated at the top of each bar in panel (a). (a) molar C : P ratio; (b) molar C : N ratio; (c) molar N : P ratio.

genera. The smaller number of crustacean taxa tested (four) may have contributed to the lack of differences found within this group. Significant variation among insect and mollusk genera suggests that future studies examining patterns in invertebrate elemental compo-

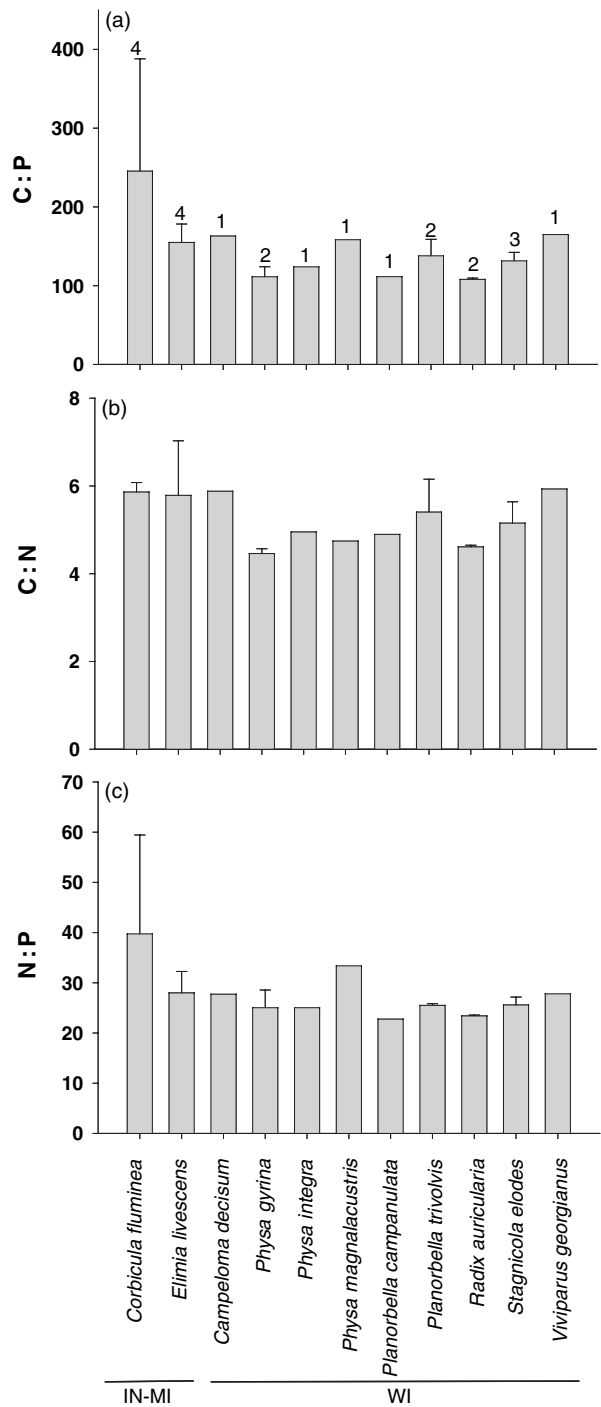


Fig. 5 Elemental composition (mean + 1 SD) of mollusks, ordered alphabetically, sampled from Indiana–Michigan or Wisconsin streams. The number of streams is indicated at the top of each bar in panel (a). (a) molar C : P ratio; (b) molar C : N ratio; (c) molar N : P ratio.

sition should identify invertebrates to the lowest taxonomic level possible. Based on our data, the mixture of biomolecules needed by an insect may be

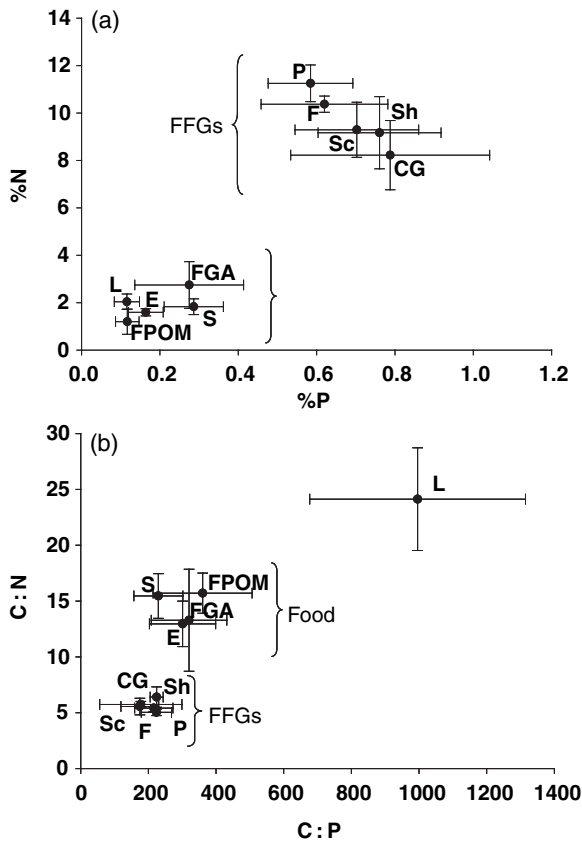


Fig. 6 Elemental composition (mean \pm 1 SD) of macroinvertebrate functional feeding groups (after Merritt & Cummins, 1996) and their potential food sources sampled from Indiana–Michigan and Wisconsin streams. Abbreviations are as follows: FFGs, functional feeding groups; CG, collector-gatherers; F, collector-filterers; P, predators; Sc, scrapers; Sh, shredders; E, epilithon; FGA, filamentous green algae; FPO, fine particulate organic matter; L, leaves; S, seston. (a) %N versus %P per unit dry mass; (b) molar C : N versus C : P ratio.

less constrained than it is for other taxonomic groups. The range of N : P for benthic insects in this study was greater than that found for any other taxonomic group examined in the literature, including zooplankton and fishes (Fig. 8). Consequently, benthic insects may display greater variation in nutrient excretion than other benthic macroinvertebrates and changes in insect species composition could have substantial effects on aquatic nutrient dynamics. Benthic macroinvertebrates can provide nutrients to stream epilithon via excretion (Grimm, 1988; Mulholland *et al.*, 1991), which could also be important in lakes that have high benthic macroinvertebrate production (Vadeboncoeur, Vander Zanden & Lodge, 2002; Vander Zanden & Vadeboncoeur, 2002). Research on

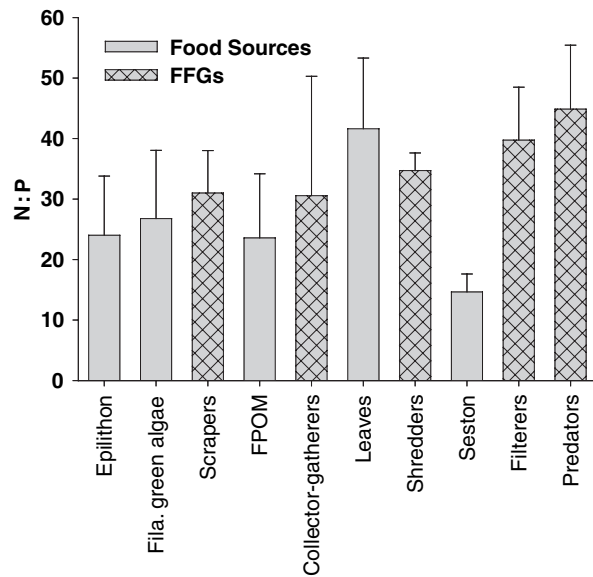


Fig. 7 Molar N : P ratio (mean + 1 SD) of macroinvertebrate functional feeding groups (FFGs) and their potential food sources from streams in Indiana–Michigan and Wisconsin.

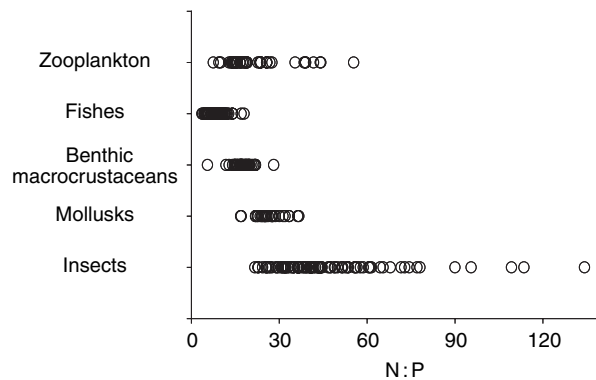


Fig. 8 Compilation of molar N : P ratios of insects, mollusks, benthic macrocrustaceans, fishes, and zooplankton. Insect, mollusk, and benthic crustacean data are from the present study of streams in Indiana–Michigan and Wisconsin. Each point represents a taxon from a particular stream. Fish data are from Davis & Boyd (1978); Penczak (1985); Tanner, Brazner & Brady (2000), and Sterner & George (2000). Zooplankton data are from Andersen & Hessen (1991) and the Stoichiometry Working Group at the National Center for Ecological Analysis and Synthesis (NCEAS) (<http://www.nceas.ucsb.edu/ecostoichiometry>).

the importance of benthic consumers to nutrient recycling using the framework of ecological stoichiometry should be pursued in streams as well as lakes and other aquatic ecosystems (e.g. Evans-White & Lamberti, 2005).

Functional feeding group patterns and elemental imbalances

Previous studies have found higher %N in predators relative to herbivores (Fagan *et al.*, 2002; Cross *et al.*, 2003; but see Bowman *et al.*, 2005). Our study similarly found that predators had a higher %N than shredders or collector-gatherers. Unfortunately, all predators in our study were odonates and it is unclear whether the higher %N in odonates is a FFG pattern or a taxonomic pattern (Fagan *et al.*, 2002). In addition, scrapers and collectors, which had a lower %N than predators, included mollusks and crustaceans, which had a lower %N than insects. Fagan *et al.* (2002) proposed that herbivores might have a lower %N content than predators including that herbivorous insects have evolved a lower dependence on N because of the chronic existence of low dietary N. If this hypothesis is correct, benthic herbivores should also have a lower P content than predators, because plants or algae often contain a lower amount of P than the food of predators (Fig. 6a). Cross *et al.* (2003) found that predators had a higher P content than other FFGs, but our results do not support this pattern.

In our study, elemental imbalances in %N and %P in stream food webs were larger than for carbon : nutrient ratios. Large imbalances in nutrient percentages between primary consumers and their food resources may also result in mineral limitation of consumers, which would not be predicted by ecological stoichiometry theory (but see Sterner & Elser, 2002), and therefore warrants further investigation. Elemental imbalances in nutrient ratios between collectors and scrapers and their food sources were not as great as those found in previous studies (Cross *et al.*, 2003; Frost *et al.*, 2003), whereas imbalances between shredders and leaves were substantial and similar to previous reports (Cross *et al.*, 2003). Such elemental imbalances between shredders and their food suggest that imbalanced nutrient recycling and mineral limitation of growth may be stronger in detritus-based streams than in primarily autotrophic streams. In general, elemental imbalances between FFGs and food sources were larger for C : N than for C : P and N : P, indicating that macroinvertebrates are more likely to be N-limited and therefore more retentive of N. However, combining diverse taxa into FFGs resulted in large variances and considerable

overlap with elemental ratios of food resources. Therefore, elemental imbalances may be best considered on a taxon-specific basis. For example, crustaceans had considerably lower C : P and N : P ratios than their primary food resources (i.e. algae and leaves). Therefore, crustaceans may be more conservative of P, release P back to the environment more slowly than other benthic macroinvertebrate taxa, and are more likely to be P-limited than other taxa.

There are limitations to using FFGs to estimate elemental imbalances between benthic macroinvertebrates and their food sources. FFGs predominantly sort macroinvertebrates by the mode of feeding, not necessarily by the food they eat (Merritt & Cummins, 1996). In addition, our bulk samples of potential food sources may not actually represent what is eaten by stream macroinvertebrates (e.g. Ledger & Hildrew, 2000; Dangles, 2002), and elemental imbalances should be considered cautiously. For example, macroinvertebrates that eat leaf litter may depend more on fungal species associated with the leaves than they depend on the leaf material itself (e.g. Arsuffi & Suberkropp, 1985).

Conclusions

Our study is the first to examine macroinvertebrate elemental composition from a large number of streams and to assess diverse taxa of benthic insects, mollusks, and crustaceans. Our results indicate that specific invertebrate taxa in streams are relatively invariable in their elemental composition, which supports the assumption of homeostasis in some stoichiometric models. However, considerable differences were found among taxa. Phylogeny explained more variation in benthic macroinvertebrate elemental composition than did regional or seasonal variation. The significant variation observed among insect, mollusk, and crustacean elemental composition may result in broad differences in mineral limitation and nutrient recycling among these groups. Additionally, elemental composition varied considerably among genera within these groups. Because elemental composition differed at various taxonomic levels, broad generalisations about animal elemental composition may lead to errors in predictions of animal mineral limitation or animal-mediated nutrient cycling. Furthermore, a strong linkage may exist between phylogeny and the ecosystem function of benthic

macroinvertebrates (e.g. their role in biogeochemical cycling) as has been suggested for stream vertebrates (Vanni *et al.*, 2002).

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Appendix Elemental composition for invertebrate taxa from streams in Indiana or Michigan (IM) and Wisconsin (W)

Taxa	Region	Percent phosphorus		Percent nitrogen		Percent carbon		n
		Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	
<i>Acroneuria</i>	W	0.42 (0.03)	0.39–0.44	12.65 (0.85)	12.04–13.25	49.09 (0.87)	48.47–49.71	2
<i>Aeshna</i>	W	0.58 (0.20)	0.43–0.72	11.45 (0.53)	11.08–11.82	48.81 (2.28)	47.20–50.43	2
<i>Asellus militaris</i>	W	0.98 (0.04)	0.96–1.01	7.51 (0.36)	7.26–7.76	34.56 (2.63)	32.70–36.41	2
<i>Baetis</i>	W	0.38 (0.09)	0.30–0.51	10.18 (0.29)	9.94–10.59	51.86 (0.58)	51.04–52.39	4
<i>Boyeria</i>	IM, W	0.53 (0.09)	0.43–0.67	11.10 (0.92)	9.52–11.80	49.77 (3.05)	47.33–54.82	5
<i>Brachycentrus</i>	W	0.48 (0.09)	0.32–0.61	9.85 (0.32)	9.35–10.33	50.25 (1.53)	47.70–52.40	8
<i>Caecidotea</i>	IM	0.99 (0.32)	0.63–1.20	6.75 (0.56)	6.12–7.17	32.23 (1.52)	30.53–33.46	3
<i>Calopteryx maculata</i>	IM, W	0.68 (0.12)	0.50–0.77	10.98 (1.02)	9.61–12.03	46.33 (1.10)	45.50–47.88	4
<i>Campeloma decisum</i>	W	0.59		7.36		37.10		1
<i>Ceratopsyche</i>	W	0.57 (0.06)	0.51–0.64	10.56 (0.46)	10.12–11.14	46.23 (2.50)	44.15–49.67	4
<i>Chimarra</i>	W	0.50		10.78		49.86		1
Chironomidae	IM	0.70 (0.22)	0.54–1.08	9.12 (0.41)	8.65–9.56	46.40 (4.37)	41.35–50.36	5
<i>Corbicula fluminea</i>	IM	0.79 (0.10)	0.68–0.91	9.39 (0.80)	8.24–10.10	45.50 (3.79)	40.47–48.89	4
<i>Cordulegaster</i>	W	0.61 (0.05)	0.57–0.65	11.38 (0.21)	11.23–11.52	48.68 (1.16)	47.86–49.51	2
<i>Desmona</i>	IM	0.47 (0.12)	0.38–0.56	7.58 (0.02)	7.56–7.60	48.81 (3.17)	46.56–51.05	2
<i>Elimia livescens</i>	IM	0.74 (0.09)	0.62–0.82	9.10 (1.61)	7.53–11.35	42.74 (2.33)	39.48–44.68	4
<i>Ephemera</i>	IM	0.81		9.10		43.69		1
<i>Gammarus pseudolimnacus</i>	IM, W	0.90 (0.13)	0.78–1.10	7.52 (0.45)	6.92–8.50	35.49 (1.54)	32.24–38.03	19
<i>Glossosoma</i>	W	0.59		8.99		49.14		1
<i>Gomphus</i>	IM	0.47		9.92		46.16		1
<i>Helicopsyche borealis</i>	IM, W	0.67 (0.27)	0.32–0.94	8.53 (0.33)	8.16–9.02	51.19 (2.31)	48.28–54.06	5
<i>Hetaerina</i>	IM	0.73		10.86		49.46		1
<i>Hexagenia</i>	IM	0.35		10.75		43.22		1
<i>Hydropsyche</i>	IM, W	0.67 (0.17)	0.43–0.90	10.31 (0.68)	9.35–11.08	47.62 (1.64)	44.41–49.11	7
<i>Leucrocota</i>	W	0.48		9.00		52.03		1

Appendix (Continued)

Taxa	Region	Percent phosphorus		Percent nitrogen		Percent carbon		n
		Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	
<i>Macronychus</i>	IM	0.22		10.29		50.38		1
<i>Namamyia</i>	IM	0.67 (0.39)	0.40–0.95	7.47 (0.45)	7.15–7.79	51.68 (1.22)	50.82–52.55	2
<i>Nigronia</i>	W	0.67		11.68		48.10		1
<i>Optioserous</i>	IM	0.19		9.99		47.01		1
<i>Orconectes propinquus</i>	IM	0.99 (0.11)	0.87–1.18	7.62 (0.45)	7.01–8.45	34.59 (1.92)	31.92–37.45	7
<i>Orconectes rusticus</i>	W	0.85 (0.12)	0.74–1.08	7.20 (0.50)	6.25–7.75	34.37 (1.54)	32.38–36.50	6
<i>Physa gyrina</i>	W	1.02 (0.10)	0.96–1.09	11.49 (0.50)	11.13–11.84	43.88 (0.83)	43.30–44.47	2
<i>Physa integra</i>	W	0.93		10.53		44.69		1
<i>Physa magnalacustris</i>	W	0.73		11.06		44.99		1
<i>Planorbella campanulata</i>	W	0.83		8.49		35.64		1
<i>Planorbella trivolis</i>	W	0.73 (0.12)	0.65–0.81	8.39 (1.25)	7.51–9.27	38.47 (0.39)	38.19–38.75	2
<i>Psephenus herricki</i>	IM, W	0.54 (0.09)	0.41–0.64	10.08 (0.42)	9.69–10.57	49.32 (2.04)	46.61–51.29	5
<i>Pteronarcys</i>	IM, W	0.70 (0.21)	0.57–0.94	10.77 (0.40)	10.33–11.09	49.89 (1.25)	48.47–50.82	3
<i>Radix auricularia</i>	W	0.87 (0.05)	0.84–0.90	9.21 (0.42)	8.91–9.51	36.40 (1.38)	35.42–37.38	2
<i>Simulium</i>	W	0.88 (0.01)	0.87–0.88	10.35 (0.50)	10.00–10.71	46.14 (0.94)	45.48–46.81	2
<i>Stagnicola elodes</i>	W	0.85 (0.06)	0.78–0.91	9.76 (1.13)	8.45–10.42	42.82 (2.30)	41.07–45.43	3
<i>Stenacron interpunctatum</i>	IM, W	0.61 (0.17)	0.41–0.89	9.69 (0.77)	8.44–10.58	46.78 (2.88)	43.94–52.30	6
<i>Stenelmis</i>	IM	0.29 (0.06)	0.21–0.37	10.12 (0.37)	9.57–10.47	48.94 (1.20)	47.01–49.98	5
<i>Stenonema</i>	IM, W	0.54 (0.16)	0.32–0.77	10.09 (0.90)	9.33–11.36	48.33 (2.54)	44.39–50.82	5
Tipulidae	IM	0.63 (0.01)	0.62–0.64	9.73 (1.45)	8.06–10.57	49.35 (3.36)	45.48–51.29	3
<i>Viviparus georgianus</i>	W	0.59		7.48		38.03		1

SD = standard deviation; n = number of streams

(Manuscript accepted 15 September 2004)