

Stoichiometric mismatch between littoral invertebrates and their periphyton food

Patrick Fink^{1, 2}, Lars Peters¹ and Eric Von Elert¹

Limnological Institute, University of Konstanz

With 6 figures and 3 tables

Abstract: Ecological stoichiometry is considered a key concept in understanding constraints in energy transfer at the plant-herbivore interface. However, whether this concept is relevant for benthic freshwater ecosystems is not fully known. Therefore, a field survey was conducted in 2003 during the growing season in the littoral zone of Lake Constance, a large pre-alpine lake in central Europe. The aim was to assess temporal variation in the elemental stoichiometric composition in both herbivorous macroinvertebrates and their food resource, the periphyton in two different lakes. The periphyton showed large temporal and spatial variation in carbon, nitrogen, and phosphorus content, with particularly high molar C:P ratios of up to 1225:1. Periphyton C:P and C:N ratios were often high and constantly above the Redfield ratio that is considered optimal for autotrophic growth. In contrast to the pronounced fluctuations in the nutrient ratios of their food resource, the herbivorous macroinvertebrates showed only very little variation in their nutrient ratios, which indicated that they are homeostatic, i.e., physiologically restricted to a comparatively narrow range of C:P and C:N ratios. Distinct species-specific C:P and C:N ratios were found for different taxonomic groups of macroinvertebrates, which indicated different requirements of optimal dietary C:P and C:N ratios and which might influence the ability of the taxa to compete for limiting elemental nutrients. Considering the temporally very high C:P and C:N ratios of the periphytic resource and the very low ratios of the consumer body tissue, this stoichiometric mismatch is likely to constrain growth and reproduction of these littoral invertebrates. Therefore, the concept of stoichiometric food quality limitation might also be applicable to the littoral food web in lakes.

Key words: benthos, C:N:P ratio, ecological stoichiometry, herbivory, homeostasis, lake littoral, macroinvertebrates, mismatch, nutrient ratios, phosphorus.

¹ **Authors' addresses:** Limnological Institute, University of Konstanz, Mainaustraße 252, D-78464 Konstanz, Germany.

² Corresponding author, present address: Max-Planck-Institute for Limnology, Department of Physiological Ecology, August-Thienemann-Straße 2, D-24306 Ploen, Germany; E-Mail: fink@mpil-ploen.mpg.de

Introduction

Elemental nutrients, especially phosphorus (P) and nitrogen (N), are considered to be key elements in determining ecosystem productivity as the availability of phosphorus and nitrogen often limits primary production (ELSER et al. 1990). The primary producers have evolved mechanisms to cope with the fluctuating availability of elemental nutrients in their environment: They are able to store excess elemental nutrients (DROOP 1974) and improve their uptake (FITZGERALD & NELSON 1966). Therefore, the elemental composition of primary producers is variable, depending on environmental conditions (ELSER et al. 2000). In contrast, herbivores are often homeostatic, i. e., the C:P and C:N ratios vary over a narrow range, as has been shown for a variety of zooplankton taxa (HESSEN 1990, ANDERSEN & HESSEN 1991).

The requirement of the herbivores for elemental nutrients and the varying availability of these essential resources in their food can constrain their growth (STERNER & SCHULZ 1998) and reproduction (FÆRØVIG & HESSEN 2003). Such stoichiometric mismatches between primary producers with often highly variable elemental composition and homeostatic herbivorous consumers are considered to be a key factor that leads to poor energy transfer at the plant-herbivore interface in food webs (STERNER & SCHULZ 1998). STERNER & ELSE (2002) recently summarized the current knowledge on the interaction of mineral nutrients with molecules, organisms, and ecosystems. However, in contrast to the vast number of studies on zooplankton stoichiometry (e. g., STERNER & ELSE 2002, and references therein), only little is known about the elemental composition of freshwater benthic invertebrates and their periphyton resource. In particular, the role of periphyton elemental nutrient stoichiometry as a food quality determinant for benthic freshwater invertebrates is not yet understood. To date, only two experimental studies have provided evidence for phosphorus limitation in mayflies (FROST & ELSE 2002a) and in a lentic prosobranch (STELZER & LAMBERTI 2002). However, no attempts have been made to determine the seasonal dynamics of the elemental nutrient stoichiometry of natural periphyton and associated invertebrates in a lake, which is a prerequisite to estimate the extent to which elemental nutrients might constrain food quality for herbivorous macroinvertebrates. To date, field studies have only analysed the elemental composition of either benthic invertebrates (FROST et al. 2003) or periphyton (KAHLERT 1998, KAHLERT et al. 2002), but have never considered both aspects simultaneously, which would allow hypotheses on possible stoichiometric limitations within the benthic food web to be formed. In a recent study, LIESS & HILLEBRAND (2005) reported nutrient ratios from littoral zone invertebrates and periphyton. However, they did not sample consumer and resource at the same dates and sites, which makes it difficult to relate the nutrient ratios of the periphyton to the elemental composition of the

invertebrates. Our aim was to simultaneously study the elemental compositions of periphyton and invertebrate grazers over an entire growth season. Thus, for the first time, we directly related the stoichiometry of benthic herbivorous consumers to the stoichiometry of their periphyton resource to investigate, whether a mismatch between demand and supply of elemental nutrients occurs in the field. We present data from a field survey that was conducted during the growing season (April–October) in 2003 in the littoral zone of Lake Constance, a large lake in southern Germany. The aim of the study was to assess both temporal and spatial variation in the elemental nutrient stoichiometry of the dominant benthic, herbivorous invertebrates and simultaneously in their natural food resource, the periphyton community. We specifically addressed the hypotheses that i) the elemental composition of periphyton in the littoral zone of Lake Constance is variable both in space and time, ii) benthic herbivorous invertebrates maintain homeostatic body C:N:P ratios independent of the elemental composition of their periphyton resource, and, iii) there are species-specific differences in the elemental composition between groups of macroinvertebrate taxa.

Methods

Study sites

The study was conducted during the summer season (April–October) in 2003 in Upper and Lower Lake Constance, two basins of a large, pre-alpine meso-oligotrophic lake in central Europe. Upper Lake Constance is large and deep (surface area 473 km², mean depth 101 m), whereas Lower Lake Constance is smaller and shallower (surface area 63 km², mean depth 14 m). The site in the Lower Lake (N 47° 41.10', E 9° 04.19') is close to an agricultural drainage inflow, which led to thick periphyton layers on the surrounding cobblestones. The periphyton layer at the Upper Lake Constance site (N 47° 41.47', E 9° 12.20') was thinner and comprised higher amounts of inorganic sediments.

Sampling design

Both sites were sampled on the 15th day of each month from April through October 2003 at 40-cm water depth. Four replicate samples of periphyton and of invertebrates were taken and analysed, thus resulting in 2 sites × 7 months × 8 taxa × 4 replicate samples. Only groups of organisms were chosen that occurred frequently enough to allow reliable determination of body elemental nutrient ratios and that are considered to be at least partially herbivorous (MOOG 1995). The collected taxa were: *Asellus aquaticus* L. (Isopoda, Asellidae), *Bithynia tentaculata* L. (Prosobranchia, Bithyniidae), *Gammarus roeseli* Gervais (Amphipoda, Gammaridae), and *Tinodes waeneri* L. (Trichoptera, Psychomyidae). When a quick determination of live samples to the species level was not possible or when certain species occurred only at few sampling dates,

groups of taxa were pooled at the level of orders or families. For example, all case-bearing Trichoptera, all Ephemeroptera, all Oligochaeta, and all Chironomidae were considered as groups dominated by herbivorous taxa in Lake Constance (BAUMGÄRTNER 2004) and analysed as groups. The sampled case-bearing Trichoptera were members of the Leptoceridae, Sericostomatidae, and Goeridae, the Ephemeroptera of the genera *Caenis* and *Baetis*. All groups were present at both sites, except for *A. aquaticus*, which occurred only in Lower Lake Constance.

Sample processing and analyses

Periphyton samples were taken with a syringe-brush sampler (PETERS et al. 2005), which scrapes a defined area (3.14 cm^2) off natural hard substrates (cobblestones). Macroinvertebrate samples were taken non-quantitatively by collecting substrate from a $30 \times 30 \text{ cm}$ (900 cm^2) area into a hand net. In addition, dissolved nutrients (soluble reactive phosphorus and nitrogen) in the water column approximately 10 cm above ground were analysed. All samples were stored at 4°C in the dark until processing within 24 h of sampling.

To assess whether Lake Constance invertebrates that graze on periphyton could be limited by the quantity of their resource, periphyton standing stocks, i.e., chlorophyll-a and ash-free dry mass, were determined as described by PETERS et al. (2005). The October samples for chlorophyll-a and ash-free dry mass were lost during processing in the laboratory; therefore, biomass parameters are only available for the period from April through September.

Aliquots of the periphyton samples were filtered on precombusted glass-fibre filters (Whatman GF/F, 25 mm diameter, Whatman, Maidstone, UK) and dried for subsequent analysis of particulate organic carbon and particulate organic nitrogen with an NCS-2500 analyser (Carlo Erba Instruments). For determination of particulate phosphorus (P_{part}), aliquots were filtered through acid-rinsed polysulfone membrane filters (HT-200, Pall, Ann Arbor, Mich., USA) and digested with a solution of 10 % potassium peroxodisulfate and 1.5 % sodium hydroxide at 121°C for 60 min, before soluble reactive phosphorus was determined using the molybdate-ascorbic acid method (GREENBERG et al. 1985).

Live macroinvertebrate samples were sorted. Snails and case-bearing caddisflies were removed from their shells and cases before analyses. Sorted invertebrates were stored frozen at -80°C until analysis. Prior to analysis, samples were freeze-dried and ground to a powder. Aliquots of freeze-dried and powdered invertebrate bodies (approx. 1 mg each) were placed either into tin cups (HEKAtech, Wegberg, Germany) for C/N analysis or into glass vials for P_{part} determination as described above.

Statistical analyses

Periphyton nutrient ratios were $\ln(x)$ transformed to obtain homogeneity of variances and compared with a two-way ANOVA with date and site as fixed factors and the nutrient ratio as dependent variable, followed by a post-hoc comparison with Tukey's HSD (with $\alpha = 0.05$). To test whether elemental nutrient ratios of invertebrates were dependent on the elemental nutrient ratio of their periphyton resource, nonparametric

correlations between the mean ratios for each sampling date and site were calculated, and the resulting Spearman rank correlation coefficients were tested for significance at $\alpha = 0.05$. The analyses of variance were performed using the GLM module of STATISTICA v.6 software package (STATISTICA, version 6, StatSoft, Inc. (2004), Tulsa, USA); Spearman's R in the correlations between periphyton and macroinvertebrate nutrient ratios was calculated with the nonparametric module of the same software.

An analysis of similarities (ANOSIM) test was applied to assess whether significant differences in the carbon, nitrogen and phosphorus composition of invertebrates between sites, sampling dates and species/groups were present. Similarity between samples was measured on matrices with relative (%) carbon, nitrogen and phosphorus content of invertebrates. Bray-Curtis coefficient S was used for computing similarity (with untransformed data), with a coefficient value of 100 % for completely similar samples. The ANOSIM procedure compares the ranked similarities for differences within and between groups. The resulting R -value usually lies between 0 and 1, but can lie within a range of -1 to $+1$. An R -value of approximately 0 suggests an acceptance of the null hypothesis, whereas large R -values indicate separation of the groups, and small values close to 0 imply little or no separation (CLARKE & WARWICK 2001). Here, an R -value of >0.6 was considered to be an indicator of pronounced difference between sample groups. In contrast to standard Z -type statistics, R has an absolute value interpretation of its value that is potentially more meaningful than its statistical significance. As with standard tests, R can be significantly different from 0 with a difference too small to be important, if there are enough replicates. There is a global R for the analyses according to the ANOVA result and for pairwise comparisons according to multiple post-hoc tests. This analysis was carried out using the PRIMER 5 software package [PRIMER version 5, PRIMER-E Ltd. (2001), Plymouth, United Kingdom].

Results

Periphyton biomass

Periphyton biomass at both sites was high in April, especially in Upper Lake Constance, and declined during May and June to $2.8 \mu\text{g}$ chlorophyll- $a \text{ cm}^{-2}$ and 0.4 mg ash-free dry mass cm^{-2} , respectively (Fig. 1). During late summer (July–September), periphytic biomass recovered in the Lower Lake and finally reached values similar to those in spring. In the Upper Lake, the biomass remained low throughout the rest of the sampling period (Fig. 1). At this site, the periphyton biomass does not recover earlier than in November, when the high abundances of herbivorous invertebrates, especially gastropods, decline owing to low temperatures in the littoral zone (data not shown) and the decreasing water level in the lake (BAUMGÄRTNER 2004). The periphyton community was dominated by diatoms. Green algae occurred frequently, but were usually not dominant, and cyanobacteria were only of minor abundance.

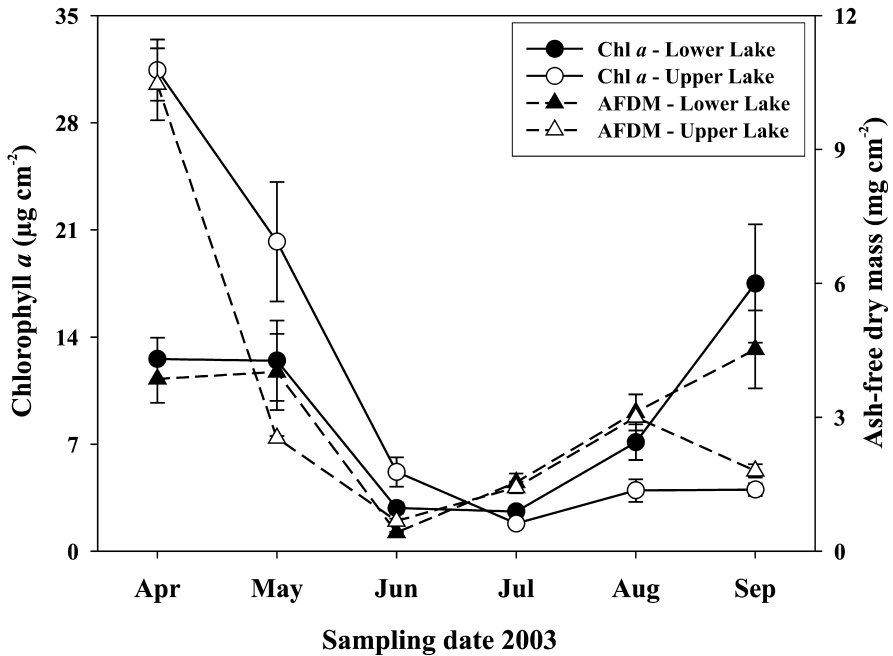


Fig. 1. Biomass of Lake Constance periphyton measured as chlorophyll-*a* (Chl-*a*, $\mu\text{g cm}^{-2}$, circles) and ash-free dry mass (AFDM, mg cm^{-2} , triangles). Open symbols, samples from the Upper Lake site; filled symbols, samples from the Lower Lake site. Error bars represent mean \pm 1 SE (of $n = 4$ replicates).

Periphyton nutrient ratios

Not only the biomass of the periphyton, but also the elemental composition of the periphyton showed temporal and spatial variation (Fig. 2). Molar C : N and N : P ratios varied significantly between sites as well as C : P, C : N and N : P ratios between sampled months at the same sites as revealed by a two-way ANOVA (Table 1). C : P ratios were especially high at the Upper Lake site during spring and early summer (April–June), reaching means of up to 1225 : 1 in June (Fig. 2 A). The values decreased during the summer months, and in late summer (August–September), the periphyton at the Lower Lake site had significantly higher C : P ratios than at the Upper Lake site (Fig. 2 A). The C : N ratios of the periphyton from the Upper Lake site were significantly higher than those of the periphyton from the Lower Lake site from early summer until autumn (June–October, Fig. 2 B). For all sites and dates, both C : P and C : N ratios were always well above the Redfield ratio (C : N : P = 106 : 16 : 1). The N : P ratio, which is considered as a measure to distinguish between nitrogen or phosphorus limitation in the periphyton (HILLEBRAND & SOMMER 1999), showed a less-consistent pattern (Fig. 2 C). During late summer (Au-

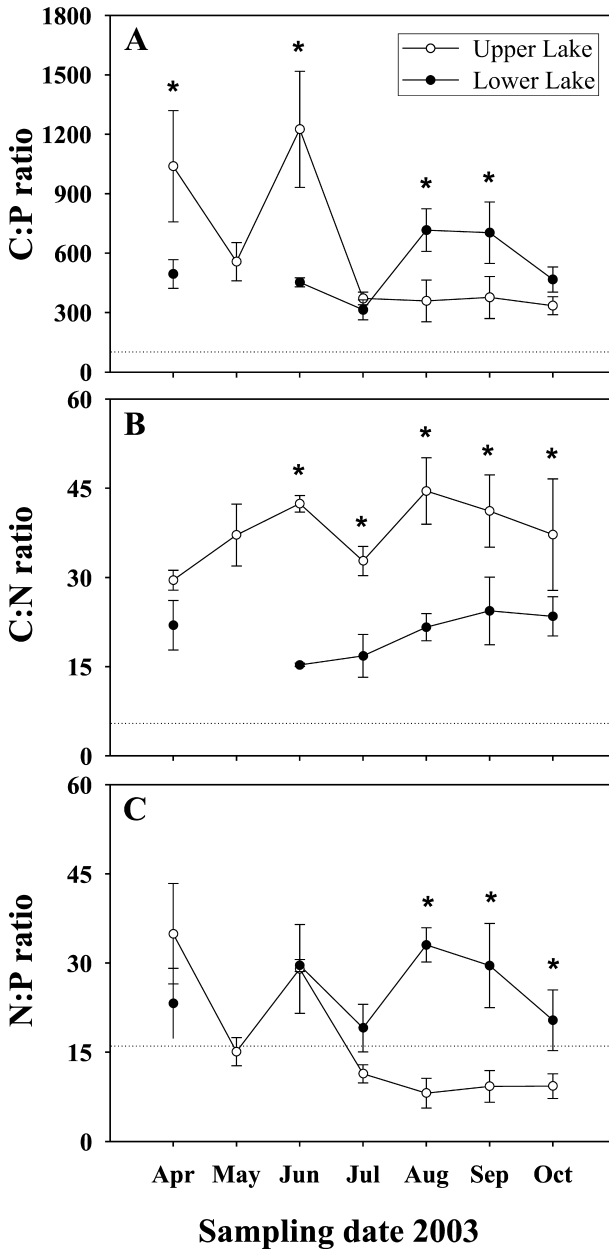


Fig. 2. Molar elemental nutrient ratios (mean \pm 1 SE) of Lake Constance periphyton during the 2003 sampling period. Open circles, samples from the Upper Lake site; filled circles, samples from the Lower Lake site; dotted lines, Redfield ratio. **A:** C : P ratio; **B:** C : N ratio; **C:** N : P ratio. Asterisks indicate significant differences between sites (multiple comparisons among means with Tukey's HSD test after two-way ANOVA).

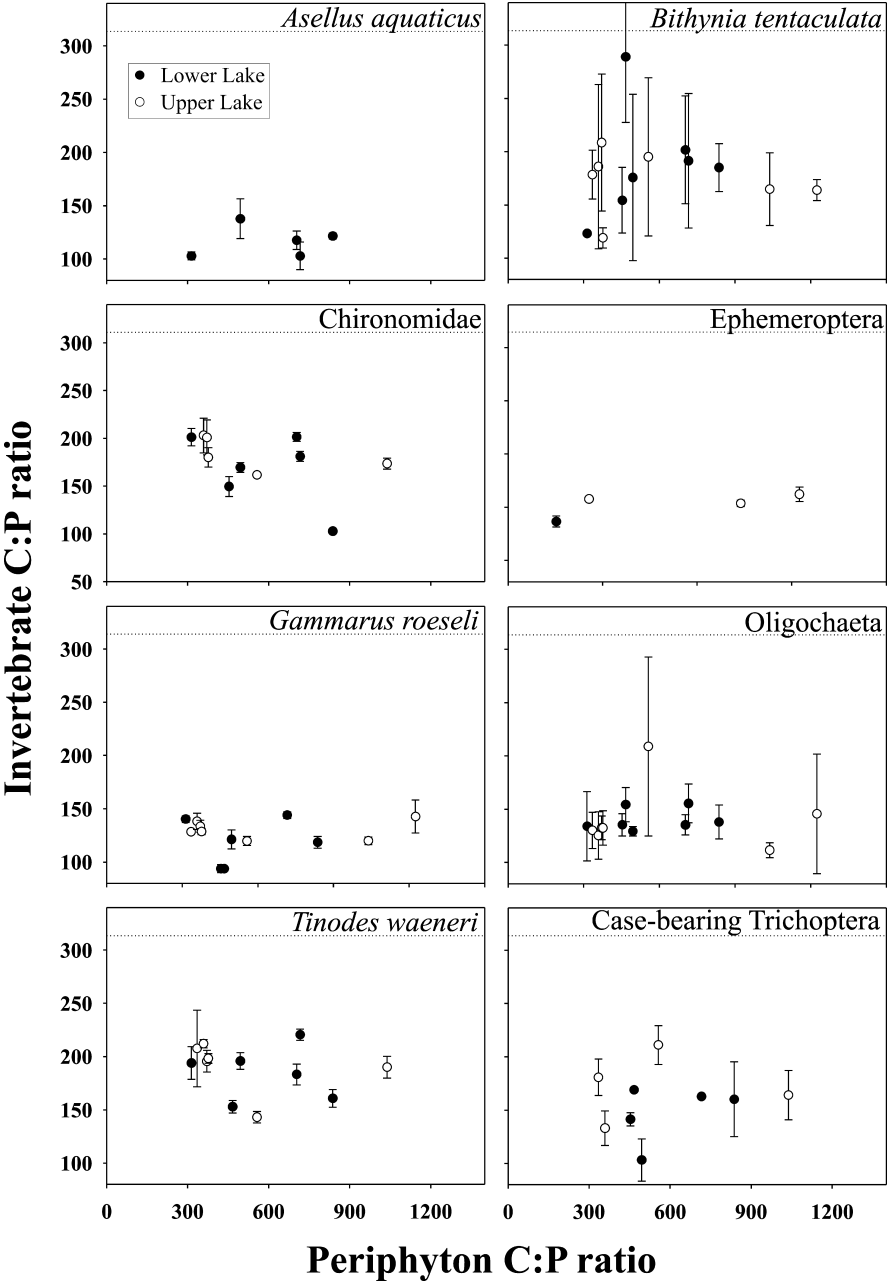


Fig. 3. Molar C : P ratios (mean \pm 1 SE) of Lake Constance herbivorous macroinvertebrates plotted against the molar C : P ratios of Lake Constance periphyton from the same samplings. Open circles, samples from the Upper Lake site; filled circles, samples from the Lower Lake site; dotted lines, lowest C : P ratio found in the periphyton samples (313.4).

gust–October), when the N:P ratios in the periphyton from the Upper Lake became very low (around 9:1), N:P ratios at the Lower Lake site were significantly higher than those in the Upper Lake. In general, N:P ratios of Lake Constance periphyton remained around the Redfield ratio of 16:1 (Fig. 2 C). Despite the pronounced fluctuations in the nitrogen and phosphorus content of the periphyton, these changes seemed to be largely independent from the availability of dissolved reactive phosphorus and nitrogen in the water column, as dissolved nutrients constantly remained at low levels close to the detection limit throughout the whole summer. Average concentrations (\pm SE) of dissolved reactive phosphorus and nitrogen were $7.6 (\pm 3.2)$ and $742.1 (\pm 72.4) \mu\text{g L}^{-1}$, respectively.

Invertebrate nutrient ratios

The variation in the elemental composition of the invertebrates was lower than that of the periphyton, especially for the groups where a determination to the species level was possible. When the standard deviation of the C:P ratio of the respective taxonomic group was normalized to the standard deviation of the C:P ratio of the periphyton, the variability in the invertebrate composition was only 8.2 % (*A. aquaticus*), 6.2 % (*G. roeseli*), and 9.6 % (*T. waeneri*) of the variation found in the periphyton. In some cases, the variability was found to be higher for groups with a lower taxonomic resolution (Oligochaeta, 18.3 %; and case-bearing Trichoptera, 15.0 %). However, there was no clear connection between the observed variability in the invertebrates' C:P ratios and the taxonomic resolution, as the Chironomidae (9.1 %) and the Ephemeroptera (4.2 %) showed very low variability in C:P ratios despite the lower taxonomic resolution. The prosobranch snail *B. tentaculata*, with a comparatively high respective variability of 30.9 %, was the only exception to this pattern.

The mean values of the C:P and C:N ratios of all analysed taxonomic groups were consistently lower than any of the C:P and C:N ratios found in the periphyton (Figs. 3, 4). The elemental composition of the periphyton was not reflected in the elemental composition of the invertebrates. Using a linear model, analyses between elemental nutrient ratios of periphyton and invertebrates revealed no significant correlations, neither for the C:P nor for the C:N ratios. For the N:P ratios (Fig. 5), only the correlation between the N:P ratio of the periphyton and the caseless trichopteran *T. waeneri* was significantly negative ($R = -0.62$, $p < 0.05$). Nevertheless, there was no positive correlation between the elemental nutrient ratios of resource and consumer.

When the mean C:P ratio of the invertebrates was plotted against the mean C:N ratio, it became apparent that there were pronounced differences between the analysed taxonomic groups (Fig. 6). The observation of species-specific differences was supported by an analysis of similarity (ANOSIM) between the

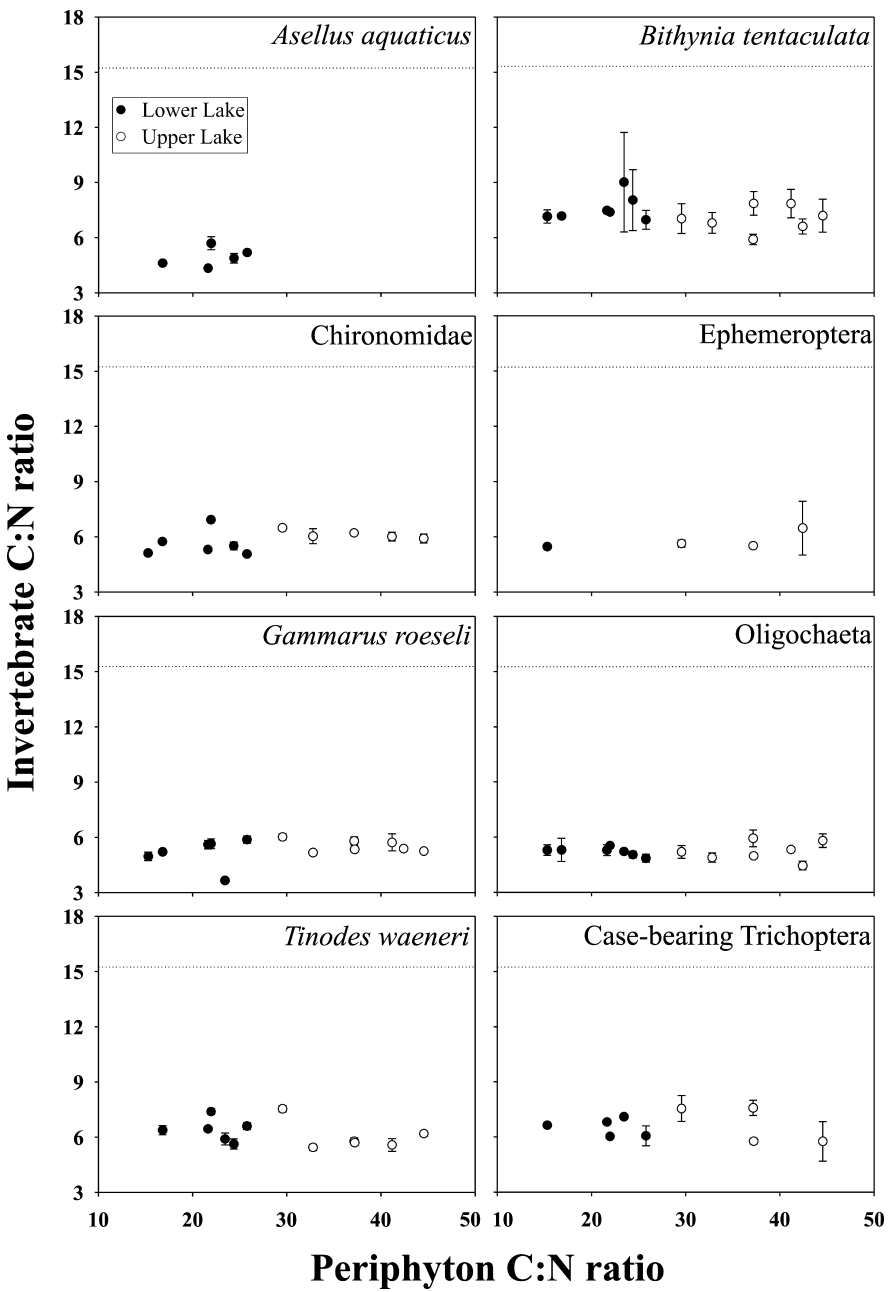


Fig. 4. Molar C : N ratios (mean \pm 1 SE) of Lake Constance herbivorous macroinvertebrates plotted against the molar C : N ratios of Lake Constance periphyton from the same samplings. Open circles, samples from the Upper Lake site, filled circles, samples from the Lower Lake site; dotted lines, lowest C : N ratio found in the periphyton samples (15.3).

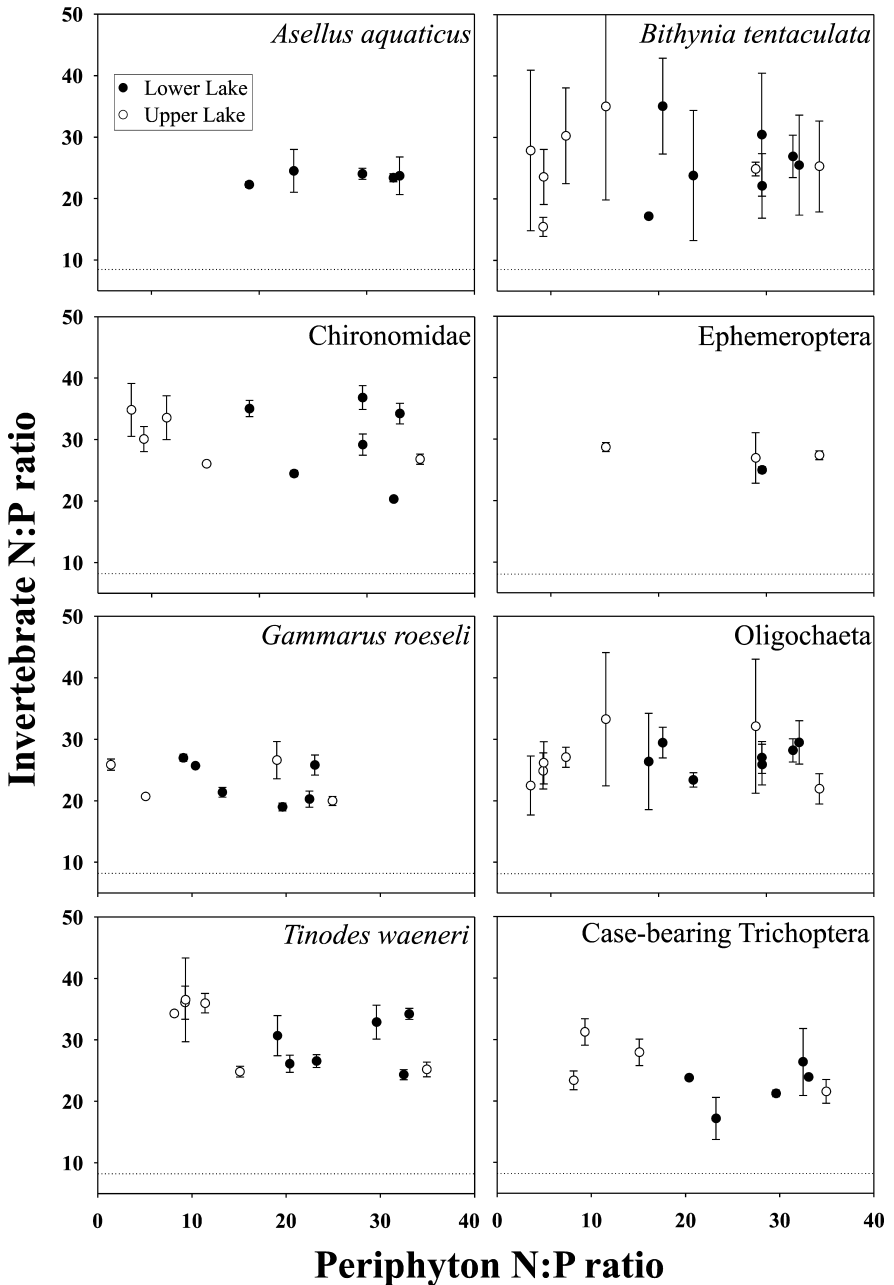


Fig. 5. Molar N:P ratios (mean \pm 1 SE) of Lake Constance herbivorous macroinvertebrates plotted against the molar N:P ratios of Lake Constance periphyton from the same samplings. Open circles, samples from the Upper Lake site; filled circles, samples from the Lower Lake site; dotted lines, lowest N:P ratio found in the periphyton samples (8.1).

Table 1. Results of two-way ANOVA on the mean molar elemental nutrient ratios of Lake Constance periphyton with sampling date and site as factors.

Factor	d.f.	C:P			C:N			N:P		
		MS	F	p	MS	F	p	MS	F	p
Date	6	0.649	16.83	<0.001	0.102	4.19	<0.01	0.720	14.039	<0.001
Site	1	0.012	0.32	0.57	3.751	154.99	<0.001	4.195	81.785	<0.001
Date × Site	6	0.791	20.50	<0.001	0.102	4.22	<0.01	0.749	14.606	<0.001
Error	37	0.039			0.024			0.051		

Table 2. ANOSIM test (2-way crossed) for differences in carbon, nitrogen and phosphorus composition of invertebrates between sites (Upper and Lower Lake) and sampled taxa/groups (*Asellus aquaticus*, *Bithynia tentaculata*, Chironomidae, Ephemeroptera, *Gammarus roeseli*, Oligochaeta, *Tinodes waeneri* and Trichoptera). Pronounced differences ($R > 0.6$) are in bold. The R -value and the significance level (*, **, ***, with $p < 0.05$, 0.01, 0.001) are given for each combination.

Between Sites Upper vs. Lower Lake (Global $R = 0.027^*$)
Between Taxa/Groups (Global $R = 0.38^{***}$)

	<i>A. aquaticus</i>	<i>B. tentaculata</i>	Chironomidae	Ephemeroptera	<i>G. roeseli</i>	Oligochaeta	<i>T. waeneri</i>
<i>B. tentaculata</i>	0.531***						
Chironomidae	0.060 n.s.	0.39***					
Ephemeroptera	0.754***	0.642***	0.067 n.s.				
<i>G. roeseli</i>	0.023 n.s.	0.612***	0.279***	0.647***			
Oligochaeta	0.108 n.s.	0.513***	0.086**	−0.059 n.s.	0.175***		
<i>T. waeneri</i>	0.906***	0.815***	0.421***	0.289**	0.834***	0.383***	
Trichoptera	0.846***	0.679***	0.241**	0.279**	0.734***	0.238**	0.049 n.s.

C, N and P contents (as % of dry weight) of the different invertebrate taxa and taxonomic groups (Table 2). This multivariate analysis revealed pronounced differences between some, but not all of the analysed taxa. However, two 1-way ANOSIMs (one for each site) did not indicate any effects of the sampling date (season) on the invertebrates’ nutrient contents, as indicated by the low “Global R ” values (Upper Lake Global $R = 0.061$ $p < 0.05$; Lower Lake Global $R = 0.015$ n.s.). Even though there were significant differences in the periphyton stoichiometry between the two sites (Table 1), no pronounced differences between the Upper and the Lower Lake site were found within the invertebrates (Table 2). Especially *G. roeseli*, *T. waeneri* and the Chironomidae showed almost constant body C:P and C:N ratios (Fig. 6). While the Ephemeroptera showed only little variation in their body C:P ratio, they seemed to be less constrained in their body C:N ratio (Fig. 6). In contrast to the mayflies, the Oligochaeta seemed to be more constrained in their body C:N ratio than in their C:P ratio (Fig. 6). Some of the invertebrates, such as the prosobranch snail *B. tentaculata*, exhibited a broader range in their body elemental nutrient ratios than those mentioned above, but even *B. tentaculata* was still a rather

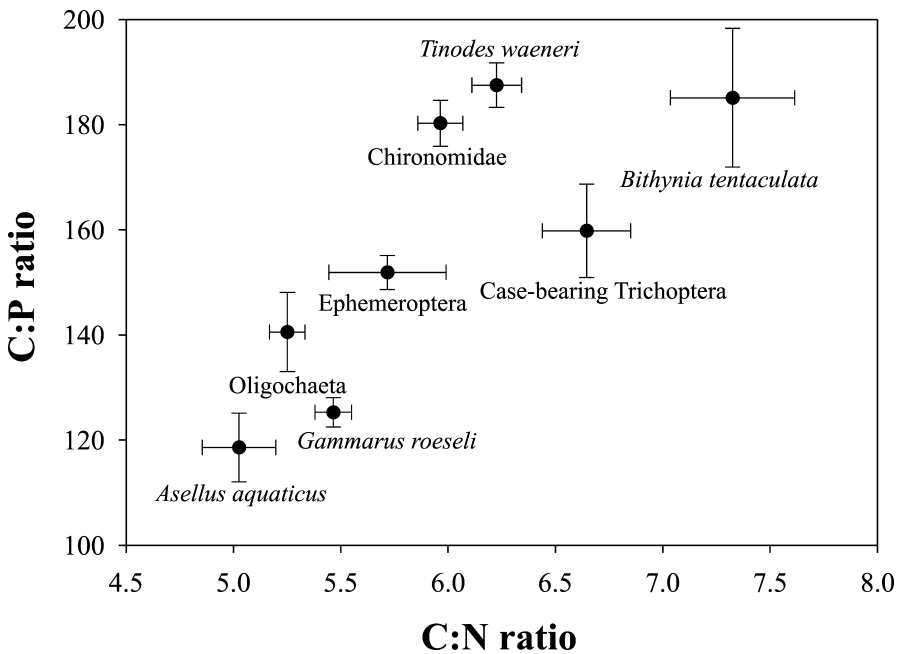


Fig. 6. Molar C : N ratios of Lake Constance macroinvertebrates plotted against their molar C : P ratios. Data points represent means over all samplings ± 1 SE.

Table 3. Carbon, nitrogen, and phosphorus content of the bodies of herbivorous macroinvertebrates in Lake Constance. Values are given as means (\pm SE) of weight normalized to dry mass of animals pooled over all samplings.

Taxonomic group		% C	% N	% P
<i>Asellus aquaticus</i>	(n = 14)	39.7 \pm 0.7	9.3 \pm 0.2	0.89 \pm 0.03
<i>Bithynia tentaculata</i>	(n = 48)	26.4 \pm 1.1	4.6 \pm 0.3	0.43 \pm 0.02
Chironomidae	(n = 38)	39.7 \pm 1.6	7.8 \pm 0.3	0.59 \pm 0.03
Ephemeroptera	(n = 15)	47.1 \pm 1.1	9.9 \pm 0.4	0.81 \pm 0.03
<i>Gammarus roeseli</i>	(n = 44)	39.3 \pm 0.4	8.5 \pm 0.1	0.83 \pm 0.02
Oligochaeta	(n = 52)	40.7 \pm 1.1	9.2 \pm 0.3	0.79 \pm 0.03
<i>Tinodes waeneri</i>	(n = 45)	51.1 \pm 0.5	9.7 \pm 0.2	0.72 \pm 0.01
Case-bearing Trichoptera	(n = 25)	49.6 \pm 1.4	8.9 \pm 0.3	0.85 \pm 0.04

homeostatic consumer compared to the high variation in its periphyton food resource.

In concordance with the observed differences in the C : P and C : N ratios of the invertebrates (Table 2, Fig. 6), the carbon, nitrogen, and phosphorus contents expressed as the percent of the body dry weight were also quite different (Table 3). In particular, *B. tentaculata* had lower carbon, nitrogen, and phos-

phorus contents (in its soft body) relative to the dry mass than any other taxon or group.

Discussion

Periphyton biomass

The biomass of Lake Constance periphyton showed considerable temporal and spatial variability. Therefore, it is important to disentangle possible effects of food quantity and food quality on the grazers. However, this is difficult, as the quantitative food requirements of many benthic invertebrates are not known and the functional responses of their food uptake have not been experimentally determined. In the littoral zone of Lake Constance, the periphyton biomass never was lower than 0.4 mg ash-free dry matter cm^{-2} (Fig. 1). We can use this for some indirect estimate using evidence from the literature. For example, when gastropods, which graze on periphyton very efficiently, were offered limiting quantities of periphyton, only 0.1 mg ash-free dry matter cm^{-2} remained (STELZER & LAMBERTI 2002), which suggested that the 0.4 mg ash-free dry matter cm^{-2} found in this study indicates that food quantity was not limiting. Furthermore, the observed periphyton biomass of 0.4 mg ash-free dry matter cm^{-2} is similar to the food quantities (0.15 mg C cm^{-2}) that proved to be sufficient to detect significant food quality effects on the growth of larvae of the mayfly *Caenis* when fed algae with a high and a low C:P ratio (FROST & ELSER 2002 a). Hence, it can be assumed that during the entire sampling season, food quantity was sufficiently high in Lake Constance to rule out limitation of invertebrate growth by low food quantity.

Periphyton elemental nutrient ratios

Not only the biomass, but also the elemental nutrient ratios of the periphyton showed high temporal and spatial variation (Fig. 2), thus supporting our first hypothesis. The mean C:P ratio of periphyton at the Upper Lake site reached 1225:1 during June 2003, which is even above the maximum values that FROST & ELSER (2002 b) found in an ultraoligotrophic lake and much higher than values reported in the review of KAHLERT (1998) on freshwater periphyton nutrient ratios. Compared to the optimal C:N:P ratio for benthic algae of 119:17:1 (HILLEBRAND & SOMMER 1999), the mean ratio of Lake Constance periphyton of 589:22:1 suggests a nutrient limitation of the autotrophic community. However, compared to carbon, both nitrogen and phosphorus contents were low; a distinction between nitrogen or phosphorus limitation according to HILLEBRAND & SOMMER (1999) is difficult. The photoautotrophs in the periphyton might have been co-limited by nitrogen and phosphorus. It should

be noted that periphyton samples taken with the brush sampler can contain varying proportions of living components, such as photoautotrophs (algae and cyanobacteria), meiofauna organisms (rotifers, nematodes, etc.), and dead organic matter (detritus) as already discussed by KAHLERT (1998). A variable proportion of detritus versus autotrophic components might have further contributed the extremely high variation in periphyton nutrient ratios. However, for the scraper and collector taxa studied here, the entire periphyton community sampled by the brush sampler is probably similar to what the invertebrates collected as their food, provided that the invertebrates did not actively select for nutrient-rich particles.

Invertebrates

The elemental nutrient ratios of the invertebrate bodies varied only within very small ranges, which was in remarkable contrast to the highly variable elemental nutrient ratios found in the samples of their periphyton food source from the same sites. The only study to date in which the elemental nutrient stoichiometry of the body of an herbivorous invertebrate and its periphyton food were analysed simultaneously revealed a similar discrepancy: FROST et al. (2002) found caddisfly larvae to have a body C:P ratio of 171:1 at a mean C:P ratio of the epilithic community of 820:1. Here, we present data that extend the findings of FROST et al. (2002) to a broad range of benthic metazoan grazers. Admittedly, direct comparisons of resource and consumer stoichiometry might not equally apply to all taxonomic groups, considering possible species-specific bioenergetics. However, as there are no data available on metabolic rates of most benthic invertebrates, we found this straightforward approach of direct comparisons justified. The small variation observed in all the analysed groups of invertebrates indicates that these organisms maintain stoichiometric homeostasis of their body tissue. We cannot exclude the possibility that the invertebrates feed selectively on resources with a constant elemental composition, and therefore reflect this (constant) elemental composition in their body tissue without actively maintaining homeostasis. However, this seems unlikely, as all the analysed taxonomic groups belong to the functional groups of either scrapers, brushers, and/or shredders and are all considered to be rather unselectively ingesting the whole periphyton community (e. g., BARNESE et al. 1990, DIAZ VILLANUEVA et al. 2004).

The analysed taxonomic groups showed differences in the degree to which their elemental nutrient ratios varied, and, in contrast to the findings of HESSEN & LYCHE (1991) for freshwater zooplankton, not only the C:P ratios, but also the C:N ratios of the benthic invertebrates differed between the taxonomic groups. These differences in (homeostatically fixed) body nitrogen and phosphorus content probably reflect species-specific physiological require-

ments of the different taxonomic invertebrate groups. These different requirements are likely to influence their susceptibility to competitive exclusion under elemental nutrient limitation, as it has been found for freshwater zooplankton (STERNER 1990). This might be especially interesting since, in contrast to zooplankton (HESSEN & LYCHE 1991), apparently not only the phosphorus content, but also the nitrogen content (relative to carbon) differs between investigated taxa, which suggests that competition for the two nutrients might have important consequences for the community structure of benthic herbivores. In particular, further investigations might reveal if the growth of certain invertebrate taxa feeding on the more nitrogen-rich periphyton at the Lower Lake site is limited by the availability of phosphorus alone, while the invertebrates might be co-limited by both nutrients at the Upper Lake site, where both phosphorus and nitrogen are equally low in the periphyton. Different requirements for phosphorus and nitrogen might also contribute to the observed changes in the composition of the Lake Constance macroinvertebrate community in both season and depth (BAUMGÄRTNER 2004). For example, the relatively high abundance of the caseless caddisfly *Tinodes waeneri* compared to the case-bearing Trichoptera (BAUMGÄRTNER 2004) might be partly due to their observed lower P requirements (Fig. 6). However, this remains to be resolved by further experimental studies on the competitive abilities of these taxa under different nutrient availabilities. The higher variation in the elemental nutrient ratios in the prosobranch gastropod *B. tentaculata* than in the other groups of invertebrates might be explained by the comparatively long life expectancy of several years of *B. tentaculata* (BREDELBERGER 1997), and therefore, probably several age-classes of this taxon were pooled in the analyses. Probably, a sorting of *B. tentaculata* size classes would have reduced this variation. For other taxa (e. g., copepods, CARRILLO et al. 1996), ontogenetic shifts in the elemental composition of the body occur with the age of the individual. This might have also been the case for *B. tentaculata*, thus leading to a larger variation in the elemental nutrient ratios of the body tissues. Furthermore, a relatively high variability in body stoichiometry relative to other taxa might be a competitive advantage, if high C:N:P ratios in the periphyton resource are common. The fact that *B. tentaculata* was the least homeostatic taxon in our study might therefore, at least in part, explain why it is one of the most abundant benthivorous invertebrates in Lake Constance (BAUMGÄRTNER 2004).

The choice of macroinvertebrate groups for this study was constrained by several factors. Four taxonomic groups were not sorted to the species level since either only single specimens from different species were found at most of the sampling dates (Trichoptera, Ephemeroptera) or a determination of live samples to the species level was not possible (Oligochaeta, Chironomidae). No Ephemeroptera were found later than June, which can be explained by

emergence. Emergence during the sampling period also occurred for *T. waeneri* (June), the case-bearing Trichoptera, and parts of the chironomid population. Therefore, for some of the insect larvae, it was not possible to obtain samples of these taxa in all the sampled months.

The analysed invertebrates can all be considered as at least partially herbivorous (MOOG 1995), i.e., they rely on periphyton as food source. Furthermore, there were no leaves present as an alternative food source for shredders, such as *G. roeseli* and *A. aquaticus*, at either of the two sites during the sampling period. Therefore, it can be assumed that the invertebrates had to rely largely on periphyton as the food source. Detritus, which can make up a large proportion of periphyton assemblages, is known to be even more depleted in P and N than benthic algae (KAHLERT 1998), so that this study might even have underestimated the effective stoichiometric mismatch for selectively detritivorous taxa. Other macroinvertebrates, e.g., the zebra mussel (*Dreissena polymorpha*), leeches (Hirudinea), and tricladids (Turbellaria), occur frequently in Lake Constance (BAUMGÄRTNER 2004), but these were not analysed since they are either predominantly carnivorous (MOOG 1995) or active filter-feeders of phytoplankton, and therefore, do not fit in the context of a possible coupling of periphyton and benthic grazer stoichiometry.

Possible consequences of stoichiometric mismatch

Mismatches between resource and consumer nutrient stoichiometry will likely result in strong effects on growth and reproduction of the invertebrate consumers, which in turn will influence important ecosystem processes such as nutrient turnover. Such consequences are especially likely to occur when species with high somatic growth rates are affected. The “growth rate hypothesis” states that species with higher specific growth rates need a higher percentage of P-rich RNA in their body tissue than slower-growing species (ELSER et al. 1996). Therefore, their demand for phosphorus in the diet is higher (a lower C:P ratio in the food is needed), and low food quality in terms of high food C:P ratios will affect the growth of those fast-growing species more than the growth of species with a lower growth rate. For the species-specific growth rates of the taxonomic groups analysed here, there is no data available in the literature except for *B. tentaculata*, with maximum somatic growth rates of 0.033 d^{-1} (FINK & VON ELERT, in press) and the mayfly *Caenis* with somatic growth rates of up to 0.04 d^{-1} (FROST & ELSER 2002 a). In the future, it will be interesting to test (i) whether the growth rate hypothesis can be applied to macroinvertebrates, i.e., whether species with a high phosphorus content grow faster than species with a low phosphorus content, (ii) whether fast-growing benthic invertebrates are more susceptible to phosphorus limitation than taxa with lower growth rates, and (iii) whether the correlation between body tissue

phosphorus content and growth rate can be broken by nitrogen limitation, as has been suggested by ACHARYA et al. (2004).

A dietary mismatch between grazers and periphyton not only affects growth and reproduction of the grazers, but also affects grazer-mediated nutrient regeneration and thus has whole-ecosystem effects. Several studies have investigated this interaction (e. g., HILLEBRAND & KAHLERT 2001, FROST, et al. 2002, HILLEBRAND et al. 2004). However, until now, the ecological importance of these effects was unclear owing to the lack of field data on the elemental nutrient stoichiometry of both herbivorous invertebrates and periphyton. The results of this study allow grazer-mediated nutrient regeneration to be related to species-specific differences in invertebrate elemental nutrient content and to periphyton elemental nutrient ratios actually occurring in the field.

It should be noted that the magnitude of effects of stoichiometric mismatch on growth and reproduction of consumers and on nutrient recycling would be affected by compensatory feeding behaviour of the invertebrates, as has been found for marine amphipods (CRUZ-RIVERA & HAY 2000), daphnids (PLATH & BOERSMA 2001) and freshwater gastropods (FINK & VON ELERT unpubl.). By ingesting more of a low-quality food (e. g., periphyton with a high C:P ratio), consumers could still satisfy their need for limiting essential nutrients, thus dampening the effects of dietary mismatch on growth and reproduction. On the other hand, an increase in grazing activity might enhance recycling of nutrients that are not limiting for the grazer and could thereby lead to shifts in the kind of nutrient limitation in other members of the benthic invertebrate community. Hence, a mismatch between the elemental composition of primary producers and herbivores can hamper the energy transfer at the plant-herbivore interface, influence competition between grazers, and alter the regeneration of limiting nutrients. It is high time that these concepts, extensively studied in freshwater plankton, are also tested for their applicability in benthic systems. The confirmation of grazer homeostasis and of highly variable elemental nutrient ratios of the periphytic resource by the data presented in this study allows further testing of hypotheses on the role of food quality limitation of herbivore growth and reproduction, grazing-mediated nutrient recycling, and whole-ecosystem effects of changing nutrient availability in freshwater benthic ecosystems owing to eutrophication (nitrogen, phosphorus) or climatic change (carbon).

Acknowledgements

We thank C. GEBAUER and O. WALENCIAK for excellent technical assistance, K. BRUNE for editing the English, and N. SCHEIFHACKEN and M. KORN for help with the identification of invertebrate taxa. Two anonymous reviewers gave helpful comments that improved the manuscript considerably. This study was supported by the German

Research Foundation (DFG) within the Collaborative Research Centre SFB 454 "Littoral Zone of Lake Constance".

References

- ACHARYA, K., KYLE, M. & ELSE, J. J. (2004): Biological stoichiometry of *Daphnia* growth: An ecophysiological test of the growth rate hypothesis. – *Limnol. Oceanogr.* **49**: 656–665.
- ANDERSEN, T. & HESSEN, D. O. (1991): Carbon, nitrogen, and phosphorus content of freshwater zooplankton. – *Limnol. Oceanogr.* **36**: 807–814.
- BARNESE, L. E., LOWE, R. L. & HUNTER, R. D. (1990): Comparative Grazing Efficiency of Pulmonate and Prosobranch Snails. – *J. N. Amer. Benthol. Soc.* **9**: 35–44.
- BAUMGÄRTNER, D. (2004): Principles of macroinvertebrate community structure in the littoral zone of Lake Constance. – PhD Thesis, University of Konstanz, Germany.
- BRENDELBERGER, H. (1997): Contrasting feeding strategies of two freshwater gastropods, *Radix peregra* (Lymnaeidae) and *Bithynia tentaculata* (Bithyniidae). – *Arch. Hydrobiol.* **140**: 1–21.
- CARRILLO, P., RECHE, I. & CRUZ-PIZARRO, L. (1996): Intraspecific stoichiometric variability and the ratio of nitrogen to phosphorus resupplied by zooplankton. – *Freshwat. Biol.* **36**: 363–374.
- CLARKE, K. R. & WARWICK, R. M. (2001): Changes in marine communities: an approach to statistical analysis and interpretation. – PRIMER-E, Plymouth, UK.
- CRUZ-RIVERA, E. & HAY, M. E. (2000): Can quantity replace quality? Food choice, compensatory feeding, and fitness of marine mesograzers. – *Ecology* **81**: 201–219.
- DIAZ VILLANUEVA, V. D., ALBARINO, R. & MODENUTTI, B. (2004): Grazing impact of two aquatic invertebrates on periphyton from an Andean-Patagonian stream. – *Arch. Hydrobiol.* **159**: 455–471.
- DROOP, M. R. (1974): The nutrient status of algal cells in continuous culture. – *J. mar. biol. Assoc. U. K.* **54**: 825–855.
- ELSER, J. J., DOBBERFUHL, D. R., MACKAY, N. A. & SCHAMPEL, J. H. (1996): Organism size, life history and N:P stoichiometry – Toward a unified view of cellular and ecosystem processes. – *BioScience* **46**: 674–684.
- ELSER, J. J., FAGAN, W. F., DENNO, R. F., DOBBERFUHL, D. R., FOLARIN, A., HUBERTY, A., INTERLANDI, S., KILHAM, S. S., MC CAULEY, E., SCHULZ, K. L., SIEMANN, E. H. & STERNER, R. W. (2000): Nutritional constraints in terrestrial and freshwater food webs. – *Nature* **408**: 578–580.
- ELSER, J. J., MARZOLF, E. R. & GOLDMAN, C. R. (1990): Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: a review and critique of experimental enrichments. – *Can. J. Fish. Aquat. Sci.* **47**: 1468–1477.
- FÆRØVIG, P. J. & HESSEN, D. O. (2003): Allocation strategies in crustacean stoichiometry: the potential role of phosphorus in the limitation of reproduction. – *Freshwat. Biol.* **48**: 1782–1792.
- FINK, P. & VON ELERT, E. (in press): Food quality of algae and cyanobacteria for the freshwater gastropod *Bithynia tentaculata*: the role of polyunsaturated fatty acids. – *Verh. Internat. Verein. Limnol.* **29**.

- FITZGERALD, G. P. & NELSON, T. C. (1966): Extractive and enzymatic analyses for limiting or surplus phosphorus in algae. – J. Phycol. **2**: 32–37.
- FROST, P. C. & ELSEY, J. J. (2002a): Growth responses of littoral mayflies to the phosphorus content of their food. – Ecol. Lett. **5**: 232–240.
- (2002b): Effects of light and nutrients on the net accumulation and elemental composition of epilithon in boreal lakes. – Freshwat. Biol. **47**: 173–183.
- FROST, P. C., ELSEY, J. J. & TURNER, M. A. (2002): Effects of caddisfly grazers on the elemental composition of epilithon in a boreal lake. – J. N. Amer. Benthol. Soc. **21**: 54–63.
- FROST, P. C., TANK, S. E., TURNER, M. A. & ELSEY, J. J. (2003): Elemental composition of littoral invertebrates from oligotrophic and eutrophic Canadian lakes. – J. N. Amer. Benthol. Soc. **22**: 51–62.
- GREENBERG, A. E., TRUSSEL, R. R. & CLESCERI, L. S. (1985): Standard methods for the examination of water and wastewater. – APHA, Washington D. C., USA.
- HESSEN, D. O. (1990): Carbon, nitrogen and phosphorus status in *Daphnia* at varying food conditions. – J. Plankton Res. **12**: 1239–1249.
- HESSEN, D. O. & LYCHE, A. (1991): Inter- and intraspecific variations in zooplankton element composition. – Arch. Hydrobiol. **121**: 343–353.
- HILLEBRAND, H., DE MONTPELLIER, G. & LIESS, A. (2004): Effects of macrograzers and light on periphyton stoichiometry. – Oikos **106**: 93–104.
- HILLEBRAND, H. & KAHLERT, M. (2001): Effect of grazing and nutrient supply on periphyton biomass and nutrient stoichiometry in habitats of different productivity. – Limnol. Oceanogr. **46**: 1881–1898.
- HILLEBRAND, H. & SOMMER, U. (1999): The nutrient stoichiometry of benthic microalgal growth: Redfield proportions are optimal. – Limnol. Oceanogr. **44**: 440–446.
- KAHLERT, M. (1998): C : N : P ratios of freshwater benthic algae. – Arch. Hydrobiol. Spec. Issues Advanc. Limnol. **51**: 105–114.
- KAHLERT, M., HASSELROT, A. T., HILLEBRAND, H. & PETTERSSON, K. (2002): Spatial and temporal variation in the biomass and nutrient status of epilithic algae in Lake Erken, Sweden. – Freshwat. Biol. **47**: 1191–1215.
- LIESS, A. & HILLEBRAND, H. (2005): Stoichiometric variation in C : N, C : P, and N : P ratios of littoral benthic invertebrates. – J. N. Amer. Benthol. Soc. **24**: 256.
- MOOG, O. (1995): Fauna aquatica austriaca – Einstufungskatalog benthischer Invertebraten Österreichs – Österreichisches Bundesministerium für Land- und Forstwirtschaft, Wien, Austria.
- PETERS, L., SCHEIFHACKEN, N., KAHLERT, M. & ROTHHAUPT, K.-O. (2005): A precise in situ method for sampling periphyton communities in lakes and streams. – Arch. Hydrobiol. **163**: 133–141.
- PLATH, K. & BOERSMA, M. (2001): Mineral limitation of zooplankton: Stoichiometric constraints and optimal foraging. – Ecology **82**: 1260–1269.
- STELZER, R. S. & LAMBERTI, G. A. (2002): Ecological stoichiometry in running waters: periphyton chemical composition and snail growth. – Ecology **83**: 1039–1051.
- STERNER, R. W. (1990): The ratio of nitrogen to phosphorus resupplied by herbivores: zooplankton and the algal competitive arena. – Amer. Nat. **136**: 209–229.

- STERNER, R. W. & ELSER, J. J. (2002): Ecological stoichiometry: the biology of elements from molecules to the biosphere. – Princeton University Press, Princeton, USA.
- STERNER, R. W. & SCHULZ, K. L. (1998): Zooplankton nutrition: Recent progress and a reality check. – *Aquat. Ecol.* **32**: 261–279.

Submitted: 25 July 2005; accepted: 8 November 2005.