

Influence of zooplankton stoichiometry on nutrient sedimentation in a lake system

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Abstract

We explored rates and stoichiometry (C:N:P ratios) of sinking particles in a temperate reservoir during a 2-yr period. Plankton was sampled weekly, and a sediment trap placed below the metalimnion collected sinking particles. There were no significant relationships between the stoichiometry of entrapped material and seston or zooplankton stoichiometry. However the differences in the entrapped C:P and N:P ratios between consecutive trap samplings were negatively correlated with the time variations of the zooplankton C:P and N:P ratios. Zooplankton C:P and N:P ratios were positively correlated with the percentage of copepod biomass in total zooplankton biomass >250 μm and negatively correlated with the percentage of cladocerans. Zooplankton biomass >250 μm reduced the fraction of N and P primary production lost to sinking (export ratio). The residuals of the N export ratio versus zooplankton biomass relationship were negatively correlated with the zooplankton N:P ratio, whereas there was a positive relationship with the residuals of the P export ratio relationship. These observations support the hypothesis that the regulation of elemental homeostasis in the herbivorous zooplankton consumers occurs at least partly at the assimilation/egestion level. Elements ingested in excess—P for the herbivorous copepods and N for many cladocerans—are concentrated into sinking feces, whereas the deficient elements are captured into biomass.

In lakes, the vertical flux of small particulate matter essentially comprises two components: the sinking of ungrazed phytoplankton cells (e.g., Reynolds et al. 1982) and the sinking of the feces or fecal pellets of planktonic primary consumers (Sarnelle 1999). Both types of particles will settle from the upper layer below the thermocline if their sinking rates are higher than their mineralization rates. A simple heuristic model (Elser et al. 1995) explicitly represents the processes involved (Eq. 1).

$$S_x = r_{z,x}e_xgP_x + r_x s_x(1 - g)P_x \quad (1)$$

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S_x is the sedimentation rate of the element x ; $r_{z,x}$ is the fraction refractory to mineralization of the egested (e_x), grazed (g) fraction of the production rate of element x (P_x), and r_x is the corresponding refractory fraction for the sinking (s_x), ungrazed ($1 - g$) fraction of the production rate of element x . For any given time interval, we can express the sedimentation rate as a fraction of elements incorporated by autotrophic activity during that interval. This fraction is referred to as the “export ratio” (ER; Eppley and Peterson 1979) and can be defined for any element x (ER _{x} ; Elser et al. 1995).

$$\begin{aligned} \text{ER}_x &= (S_x P_x^{-1}) = r_{z,x} e_x g + r_x s_x (1 - g) \\ \Leftrightarrow \text{ER}_x &= r_x s_x + g(r_{z,x} e_x - r_x s_x) \end{aligned} \quad (2)$$

This expression defines a relationship between export ratio and grazing intensity (g) in which ER _{x} is a linear function of g with a y -intercept of $r_x s_x$ and a slope of $r_{z,x} e_x - r_x s_x$. The slope of this formula explicitly formalizes the influence of zooplankton on sedimentation of autotrophic production. Zooplankton, by its grazing activity, enhances the export ratio if the fraction of feces refractory to mineralization is

higher than the refractory fraction of sinking phytoplankton cells ($r_{z,x}e_x > r_x s_x$). However, in turn, zooplankton will decrease the export ratio if $r_{z,x}e_x < r_x s_x$. The direction of the relationship between zooplankton (via its grazing) and sedimentation of particulate matter is thus dependent on (1) lake morphometry, which determines the fraction of phytoplankton that directly sediments (s_x), (2) phytoplankton community characteristics (via r_x and s_x), and (3) zooplankton community characteristics (via $r_{z,x}$ and e_x ; Elser et al. 1995).

The egested fraction of an element x (e_x) is a function of the digestive ability of animals. Stoichiometric theory indicates that it could also be a function of consumer elemental needs (Sterner and Elser 2002). P-rich consumers, such as daphniids (Andersen and Hessen 1991; Hessen and Lyche 1991), have high demands of P from food; thus, the fraction of P egested from *Daphnia* must be lower than for genera with a lower P demand. In turn, copepods are rich in body nitrogen (N), and the fraction of N egested should be lower than for zooplankton species with a lower body N content. Moreover, apart from zooplankton stoichiometry, the fraction of feces refractory to mineralization ($r_{z,x}$) also depends on their structure. Copepods surround their feces with a peritrophic membrane, which largely increases fecal cohesion and therefore the probability of sinking out of the epilimnion. So we can predict that copepods are likely to increase the export ratio of P and decrease the export ratio of N. However, for daphniid species, their effect on nutrient sedimentation will depend on the cohesion of their feces. If the feces sink before remineralization, we can predict a positive effect of daphniids on N sedimentation and a negative effect on P sedimentation.

In this study, we monitored the elemental contents of seston, zooplankton, and settling particles in a mesoeutrophic lake during the annual period of stratification for 2 yr. We determined sinking of elements as a fraction of primary production to test the effects of zooplankton stoichiometry on vertical particulate-bound nutrient exports. Our results indicate that zooplankton biomass has a positive effect on N and P retention in the epilimnion and that zooplankton stoichiometry determines stoichiometry of settling particles.

Materials and methods

Field data acquisition—This field study was conducted in the Esch-sur-Sûre reservoir in Grand-Duchy of Luxembourg. A map and a summary of morphometric and ecological characteristics are provided in Fig. 1. According to the Organization for Economic Cooperation and Development (1982) classification, the reservoir is considered a mesoeutrophic waterbody (Dohet and Hoffmann 1995). The survey was conducted at a station (maximum depth 30 m) located in the middle of the lake, representative of whole lake conditions (Thys et al. 1998).

Seston and zooplankton were sampled weekly during the period of stratification (roughly from April to October) in 1999 and 2000. Lake stratification was determined weekly according to the temperature and oxygen vertical profiles obtained with a Hydrolab DS-4 multiprobe. The lower limit of the epilimnion was at 2–3 m in early May and continu-

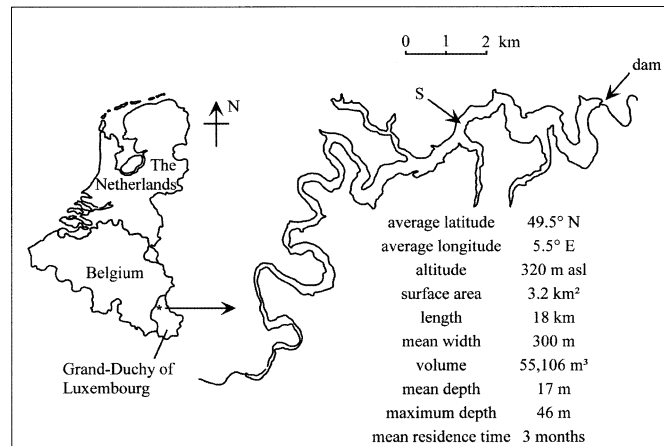


Fig. 1. Location of the Esch-sur-Sûre reservoir with morphometric and ecological summary information. The location of the sampling and sediment trap deployment station is indicated by S.

ously deepened until it was at 11–14 m in late September. The lower limit of the metalimnion started at 7–8 m in May and deepened to 17–20 m in September. Zooplankton was sampled with a 17-cm diameter, 250- μ m mesh net towed vertically in the epilimnion. Six zooplankton samples for elemental analysis were collected on each sampling occasion and immediately filtered on preignited (12 h at 500°C), pre-weighted Whatman GF/C filters and directly frozen in dry ice. Epilimnetic zooplankton was additionally sampled with a 50-cm diameter, 50- μ m mesh net to determine community composition and densities. Triplicate samples were collected and pooled to reduce heterogeneity in zooplankton horizontal distribution and sampling variability. The collected zooplankton was immediately narcotized in soda water, rinsed, and preserved within 4% formalin (Haney and Hall 1973). For seston analysis, a pooled sample was constituted on each sampling occasion from discrete samples (3 liters) collected with a Ruttner bottle and spaced every meter in the epilimnion. Another pool was constituted for the metalimnion. From each pool, one subsample of 1–2.5 liters were filtered on a Whatman GF/C filter and directly frozen in liquid nitrogen for pigment analysis by high-performance liquid chromatography (HPLC), and six subsamples of 0.15–0.5 liters were filtered on preignited Whatman GF/C filters and directly frozen in dry ice for elemental analysis.

A sediment trap was deployed at the top of the hypolimnion, and its deployment depth (8–21 m) was adjusted every 2 weeks according to the thermal and oxygen stratification of the water column. The trap consisted of one 15.4-cm-diameter, 133-cm-long polyvinyl chloride collection tube suspended from a floating pontoon. The trap was initially filled with GF/C-filtered lake water taken at the immersion depth of the trap. One liter of an inhibitory, high-density solution (180 μ mol L⁻¹ HgCl₂, 10% w/w NaCl) was added with the use of a small tube at the bottom of the trap. This solution inhibits the breakdown of entrapped material without catching swimmers (Lee et al. 1992; data not shown). The trap was recovered every 2 weeks, and the upper 4 liters were discarded. The remaining volume (~20 liters) was carefully poured into a large basin and gently mixed. The

water was filtered through a 250- μm Nitex screen, and six subsamples of particles $<250\ \mu\text{m}$ were collected for elemental analysis on preignited Whatman GF/C filters. In addition, lake water was collected with a Ruttner bottle at the same depth as the trap immersion depth and submitted to the same procedures to correct trap contents for ambient particulate matter.

Laboratory analyses—Phytoplankton pigments were extracted and analyzed following Descy et al. (1999) with the HPLC protocol of Wright et al. (1991). Chlorophyll *a* (Chl *a*) was detected by a Waters 996 PDA detector and a Waters 470 fluorescence detector, and calibration was achieved with external standards.

Zooplankton crustaceans were counted and measured under an inverted microscope (minimum 200 individuals of each species). Dry weight was estimated from body length from literature values (Bottrell et al. 1976), except for *Daphnia galeata* and *Eudiaptomus gracilis*, for which own length (*L*, μg)–weight (dry wt, mm) relationships were used (*D. galeata*: dry wt = $-2.26 + 1.3 \exp(1.66L)$; *E. gracilis*: dry wt = $8.08L^{2.33}$).

Six samples of zooplankton, seston, and sinking particles were collected on each sampling occasion for elemental analysis. Three were analyzed for particulate C and N content and three for particulate P content. Particulate C and N were analyzed with a Carlo-Erba NA1500 elemental analyzer. Total P was analyzed by spectrophotometric determination of phosphate after digestion with potassium persulfate and boric acid (Valderrama 1981). The elemental ratios were expressed as the ratio of the means in molar units corrected for the variance of the denominator (Dagnelie 1992).

Primary production measurements—Primary production was determined every 2 weeks from the incorporation of ^{14}C following Steeman-Nielsen (1952). Eighteen 100-ml glass bottles filled with water from a pooled epilimnion sample were incubated in situ just below the surface with $\text{NaH}^{14}\text{CO}_3$. The bottles were placed at 1200 h for 2–3 h in duplicate into a nine-cases surface incubator providing a range from 0–93% of light energy. Radioactivity was measured by a Beckman scintillation counter (LS 6000 SC) with Filter-Count (Packard) as scintillation cocktail and the external standard method for quench correction. A Li-Cor sensor measured in situ surface irradiance throughout the study. The vertical light extinction coefficient was estimated weekly by subsurface and submersible quantum Li-Cor sensors. Photosynthesis parameters and depth-integrated daily primary production ($\text{mg C m}^{-2} \text{d}^{-1}$) were determined as in Vollenweider (1974) with the equation of Smith (1936). N and P uptake rates ($\text{mg m}^{-2} \text{d}^{-1}$) were estimated by dividing C production by the weighted mean of measured epilimnetic and metalimnetic seston C:N and C:P ratios, respectively. Light extinction coefficient, Chl *a*, C:N and C:P ratios and photosynthesis–light parameters were linearly interpolated between (bi-)weekly measurements to obtain daily parameters. Daily production was calculated from daily parameters and continuous surface irradiance data.

Calculations—Regression analyses were used to investigate the role of plankton variables on sedimentation variables. The sediment trap was removed every 2 weeks, and plankton was examined weekly. Because the sediment trap sample collected in 2 weeks is a conservative estimate, we calculated the weighted mean of days 0, 7, and 14 of plankton variables. The C:N:P ratios of sedimented particles $<250\ \mu\text{m}$ were tested against seston and zooplankton C:N:P ratios. The export ratio was calculated for C, N, and P. The export ratio is simply the fraction of production that sediments (Eq. 2). C, N, and P sedimentation rates of particles $<250\ \mu\text{m}$ were simply divided by the sum of daily C, N, and P production rates of the same period. C, N, and P export ratios were correlated with zooplankton biomass as an index of grazing. The residuals of these relationships were tested against zooplankton N:P ratio. All regressions were made with Statistica 5.5 software (StatSoft, Inc.).

Results

The major constituents of zooplankton biomass were the copepod *E. gracilis*, some cyclopoid copepods, and the cladocerans *Bosmina coregoni*, *Diaphanosoma brachyurum*, *D. galeata*, and *Daphnia cucullata*, which accounted for 29%, 24%, 16%, 11%, 11%, and 6%, respectively, of total annual dry weight biomass of zooplankton $>250\ \mu\text{m}$.

The C:P and N:P ratios of the zooplankton community ranged from 50 to 220:1 and from 10 to 40:1 (Fig. 2). There were strong positive relationships between zooplankton C:P ($r^2 = 0.18$, $n = 59$; $p < 0.001$) or N:P ratio ($r^2 = 0.07$, $n = 59$, $p = 0.042$) and the percentage of copepod biomass in total (copepods + cladocerans) zooplankton dry weight biomass (data not shown). Note that very logically the same but negative correlations were also observed with the percentage of cladoceran biomass.

The C:P and N:P ratios of seston were highly variable, ranging from 140 to 550:1 and from 15 to 72:1. Higher values were observed at the end of both growing seasons, with C:P ratios above 400. But in 1999, two first peaks were already observed in spring. The C:N:P ratios of entrapped particles $<250\ \mu\text{m}$ were also highly variable, but no clear seasonal trend was observed. The variability of the respective C:P and N:P ratios were nearly equivalent between seston, zooplankton, and sedimented particles (C.V. = 31–36%).

We tested the correlations between C:N:P ratios in the trap and weighted means of zooplankton and seston C:N:P ratios observed during each trap collecting period. No significant relationships were observed (Table 1). However, the differences between the consecutive trap samplings of the entrapped C:P ratio (Δ sedimented C:P) were significantly correlated with the differences of the zooplankton C:P ratio (Δ zooplankton C:P; Table 1; Fig. 3A). The negative relationship indicates that increases in the zooplankton C:P (Δ zooplankton C:P > 0) were accompanied by decreases in the C:P of sedimented matter (Δ sedimented C:P < 0) and vice versa. The differences in the sedimented N:P ratio were also significantly negatively correlated with the differences in the zooplankton N:P ratio (Fig. 3B). Note that there

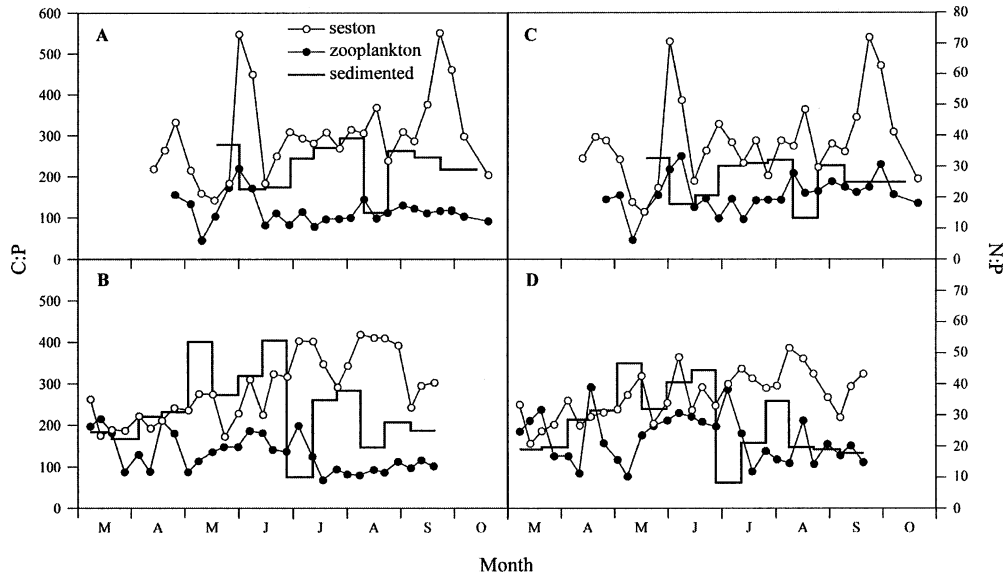


Fig. 2. C:P and N:P ratios of entrapped particles $<250 \mu\text{m}$, epilimnetic seston, and zooplankton $>250 \mu\text{m}$ in (A, C) 1999 and (B, D) 2000 in the Esch-sur-Sûre reservoir. All ratios are from atomic values.

were no significant relationships between the differences of the sedimented C:P or N:P ratios and the differences of the seston C:P or N:P ratios.

The C primary production rates, C sedimentation rates of particles $<250 \mu\text{m}$, and C export ratios are presented in Fig. 4. Except for two dates in early spring 2000 characterized by C export ratio > 1 , all rates seemed highly variable, and no clear seasonal trends were obvious. Both high values experienced in early spring 2000 were observed during the spring phytoplankton bloom characterized by high diatom biomass. Data from both dates were excluded from the rest of the analysis. According to Eq. 2, C, N, and P export ratios were correlated with the zooplankton biomass in the epilimnion as an index of grazing (Table 2). Both N and P export ratios were negatively correlated with the zooplankton biomass (Figs. 5A, 6A), but not C export ratios. Because variation in the export ratio can be driven by variation in sedimentation rate or by variation in production rate, Table 2 also indicates results of correlations between nutrient sedimentation and zooplankton biomass and between nutrient

production and zooplankton biomass. None of these relationships were significant, so zooplankton effects on nutrient export ratios were not due to separate effects on production or sedimentation rates. The main operating mechanism was presumably the retention in upper layers of a fraction of the primary production. Zooplankton by its grazing activity induced stronger nutrient turnover and retention of nutrients in heterotroph biomass.

The part of export ratio variance not explained by zooplankton biomass was still large (see Figs. 5A, 6A). We calculated the residuals of export ratios versus zooplankton biomass relationships. They were regressed against the zooplankton N:P ratio (Table 2). The residuals of N and P export ratios relationships were highly significantly correlated with the zooplankton N:P ratio (Figs. 5B, 6B). Note that the slope of the relationship for the N export ratio was negative, whereas it was positive for the P export ratio. These figures illustrate the role of zooplankton stoichiometry on sedimentation rates. A zooplankton community dominated by N-rich, P-poor species (i.e., herbivorous copepods)

Table 1. Results of regressions between the C:P and N:P ratios of sinking particles $<250 \mu\text{m}$ (and their differences between consecutive trap samplings indicated by Δ) and C:P and N:P ratios of seston and zooplankton (and their differences). Only parameters for regressions with $p < 0.05$ (bold) are given.

Variable		r^2	p	n	Intercept \pm SE	Slope \pm SE
Dependent	Independent					
Sedimented C:P	Seston C:P	0.04	0.32	24		
	Zooplankton C:P	0.00	0.94	24		
Sedimented N:P	Seston N:P	0.02	0.52	24		
	Zooplankton N:P	0.02	0.53	24		
Δ sedimented C:P	Δ seston C:P	0.07	0.25	22		
	Δ zooplankton C:P	0.21	0.03	22	-24 ± 23	-1.84 ± 0.79
Δ sedimented N:P	Δ seston N:P	0.06	0.28	22		
	Δ zooplankton N:P	0.30	0.01	22	-0.8 ± 2.3	-1.29 ± 0.44

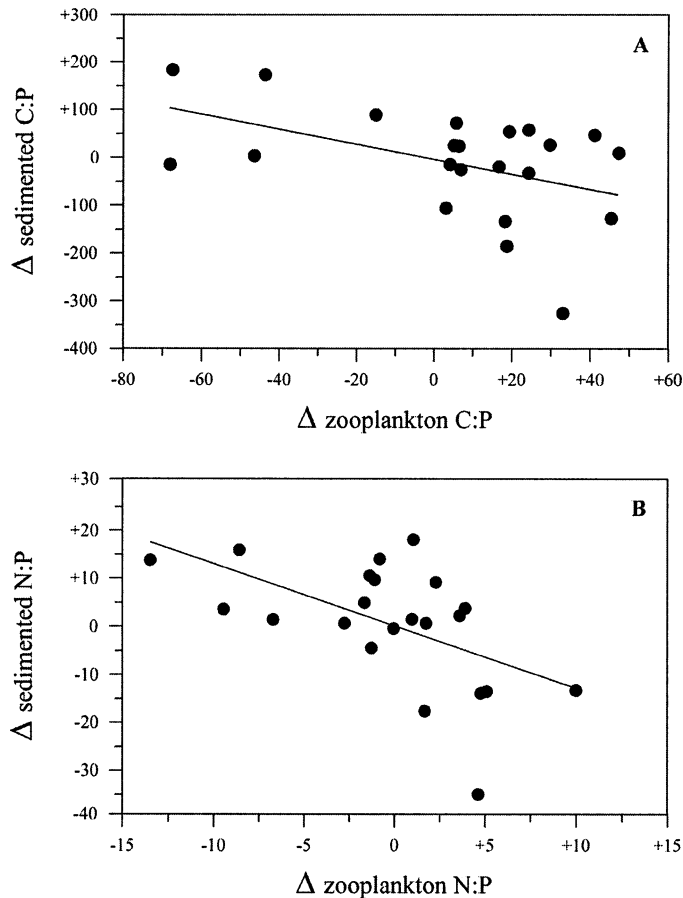


Fig. 3. Relationships between time variations (Δ) of C:P and N:P ratios in sedimented matter and zooplankton in the Esch-sur-Sûre reservoir. Regression details are given in Table 1.

decreased the sedimentation of N (residuals < 0) and increased the sedimentation of P (residuals > 0) compared with a community dominated by N-poor, P-rich species, such as *Daphnia*.

Discussion

In the Esch-sur-Sûre reservoir, zooplankton had significant effects on N and P export ratios (Figs. 5A, 6A). Export ratios are simply the fraction of nutrients assimilated by autotrophs that sediment below the thermocline. These ratios were obtained by dividing respective nutrient sedimentation rates by the sum of daily N or P production rates occurring during the same period. This calculation assumes that the material sinking during a given time interval was produced during the interval. However, accumulation of phytoplankton biomass in the epilimnion before sedimentation might lead to situations in which materials sinking during an interval were produced during previous intervals. The long time deployment (2 weeks) of the trap should reduce the effect of this time lag. Moreover, because of the continuous horizontal movement downstream of the water body, the trap was likely to collect particles produced upstream. It can produce a constant under- or overestimation of calculated export ratios.

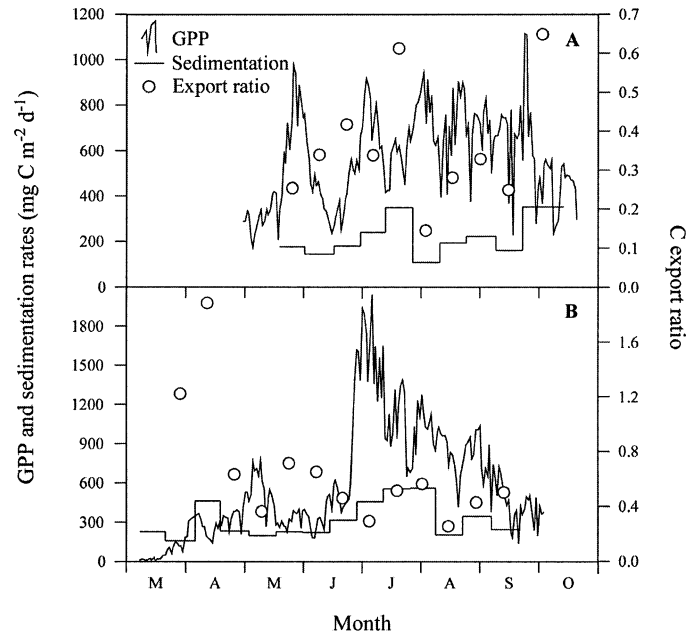


Fig. 4. Daily gross primary production (GPP) rates, C sedimentation rates of particles $< 250 \mu\text{m}$, and C export ratios in (A) 1999 and (B) 2000 in the Esch-sur-Sûre reservoir. Note the differences in scales between 1999 and 2000.

Because our findings result from regression between export ratios and plankton biomass, we believe these artifacts do not interfere with the main conclusions of the study.

Two dates were characterized by unrealistically high export ratios (> 1). They were experienced during the spring phytoplankton bloom of the year 2000. At these dates, high diatom densities were observed in the reservoir (Darchambeau and Thys 2005) and the zooplankton density was still low. A large fraction of these rapidly sinking algae (Reynolds and Wiseman 1982; Poister and Armstrong 2003) were probably ungrazed and sank. Previously presented artifacts might help explain why these export ratios are > 1 .

Mathematically, the zooplankton negative effect on both N and P export ratios can be caused by an increase of the primary production with constant sedimentation, by a decrease of the sedimentation with constant production, or by simultaneous effects on both production and sedimentation resulting in the decrease of the export ratio. Both first mechanisms seem nonexistent in the reservoir because no significant regressions between zooplankton biomass and primary production or sedimentation rates were observed (Table 2). So, effects on primary production or sedimentation are not self-sufficient to explain the observed zooplankton effect on the fraction of primary production that is exported.

The y -intercept of the export ratios versus zooplankton biomass relationship was ~ 0.5 for N and ~ 0.9 for P (Table 2). From Eq. 2, we observe that this intercept corresponds to r_{x,s_x} (i.e., the refractory fraction of the sinking fraction of phytoplankton). Thus, in absence of grazing by zooplankton $> 250 \mu\text{m}$, $\sim 50\%$ of N and $\sim 90\%$ of P assimilated by phytoplankton should sediment below the thermocline in the Esch-sur-Sûre reservoir. Simply, grazing by zooplankton modifies the fate of a fraction of primary production: instead

Table 2. Results of regressions of C, N, and P export ratios, production, and sedimentation rates ($\text{mg m}^{-2} \text{d}^{-1}$) with zooplankton biomass ($\mu\text{g C L}^{-1}$). Residuals of the regressions between export ratios and zooplankton biomass were also regressed against zooplankton N:P ratio. $p < 0.05$ are in bold. $n = 20$.

Variable						
Relationship	Dependent	Independent	r^2	p	Intercept \pm SE	Slope \pm SE
	Export ratio	Zooplankton biomass				
1.	C		0.03	0.488	0.490 ± 0.080	-0.0007 ± 0.0010
2.	N		0.21	0.044	0.494 ± 0.060	-0.00166 ± 0.00077
3.	P		0.23	0.034	0.89 ± 0.13	-0.0038 ± 0.0016
	Production rate	Zooplankton biomass				
	C		0.02	0.597	746 ± 156	-1.1 ± 2.0
	N		0.01	0.632	104 ± 19	-0.12 ± 0.24
	P		0.00	0.986	5.6 ± 1.0	0.000 ± 0.013
	Sedimentation rate	Zooplankton biomass				
	C		0.07	0.249	343 ± 62	-0.94 ± 0.79
	N		0.08	0.233	43.9 ± 7.3	-0.114 ± 0.092
	P		0.10	0.166	5.5 ± 1.5	-0.027 ± 0.019
	Residuals	Zooplankton N:P				
	Relationship 1		0.00	0.926	-0.01 ± 0.15	0.0006 ± 0.0069
	Relationship 2		0.33	0.008	0.269 ± 0.092	-0.0128 ± 0.0043
	Relationship 3		0.29	0.015	-0.54 ± 0.20	0.0255 ± 0.0094

of sinking as algae, they are captured into zooplankton mass or recycled to a dissolved form.

The negative influence of zooplankton on nutrient export ratios in the Esch-sur-Sûre reservoir was qualitatively similar to that found in low-productivity Lake 110 but inverse to that of low-productivity Lake 240 of the Experimental Lakes Area (Elser et al. 1995). Results from this study and those from Elser et al. (1995) are compared in Fig. 7. In Lake 240, zooplankton negatively influenced both sedimentation and production rates, but with greater effects on production than on sedimentation, resulting in a net increase of the export ratio (Sarnelle 1999, with data from Elser et al. 1995). In this lake, the zooplankton community was dominated by cladoceran species (Elser et al. 1998). In Lake 110, there was no significant effect of zooplankton on primary production, but had a negative effect on sedimentation, resulting in a net decrease of the export ratio (Sarnelle 1999, with data from Elser et al. 1995). The zooplankton community was dominated by calanoid copepods (Elser et al. 1998). We believe that zooplankton community composition has a major influence on the zooplankton effect on vertical nutrient exports (Fig. 7). Grazing by the fast-growing cladocerans presumably can reduce the phytoplankton biomass sufficiently to induce a decrease of primary production in Lake 240. But in Lake 110, the slow-growing copepods don't graze the autotrophs sufficiently to reduce their production significantly, whereas the ingested materials are egested under the form of fast-sinking fecal pellets. In the Esch-sur-Sûre reservoir, the zooplankton community was more variable, with, for example, the percentage of copepods in zooplankton biomass varying from 13% to 100% during the study period. Consequently, major effects of zooplankton on sedimentation or production rates are undetectable, and values of

slopes between export ratios and zooplankton fall between the values of Lake 110 and Lake 240 (Fig. 7).

Interestingly, the part of N and P export ratio variance not explained by zooplankton biomass was largely explained by the zooplankton N:P ratio. Positive residuals of the N export ratio versus zooplankton biomass relationship were explained by N-poor, P-rich zooplankton species, whereas negative residuals were explained by N-rich, P-poor species (Fig. 5B). Very convincingly, the opposite correlation was found for the residuals of the P export ratio relationship (Fig. 6B). So, zooplankton stoichiometry does influence vertical particulate-bound nutrient fluxes. Note that the zooplankton N:P ratio is a signature of the zooplankton community composition. The significant positive correlations observed between zooplankton C:P–N:P ratios and percentage of copepods in total zooplankton biomass (or negative with percentage of cladocerans) are not surprising. The four cladoceran species dominant in the reservoir, *D. galeata*, *D. cucullata*, *B. coregoni*, and *D. brachyurum*, are known to have body C:P < 160 and N:P < 25, whereas adult calanoid and cyclopoid copepods have C:P > 190 and N:P > 25 (Sterner and Elser 2002). The low fraction of variance of zooplankton C:N:P ratios not explained by the percentage of copepods is not surprising given the still large variability of C:N:P ratios between copepods and cladoceran species. Moreover, some intraspecific variation in body P content and N:P ratio might occur (Main et al. 1997).

Effects of zooplankton stoichiometry on nutrient sedimentation also were observed in the sedimented C:N:P ratios. When the zooplankton was composed of species with lower P content (i.e., copepods), the C:P and N:P ratios of sedimented small particles decreased (Fig. 3). These observations are in good agreement with previous results of Elser

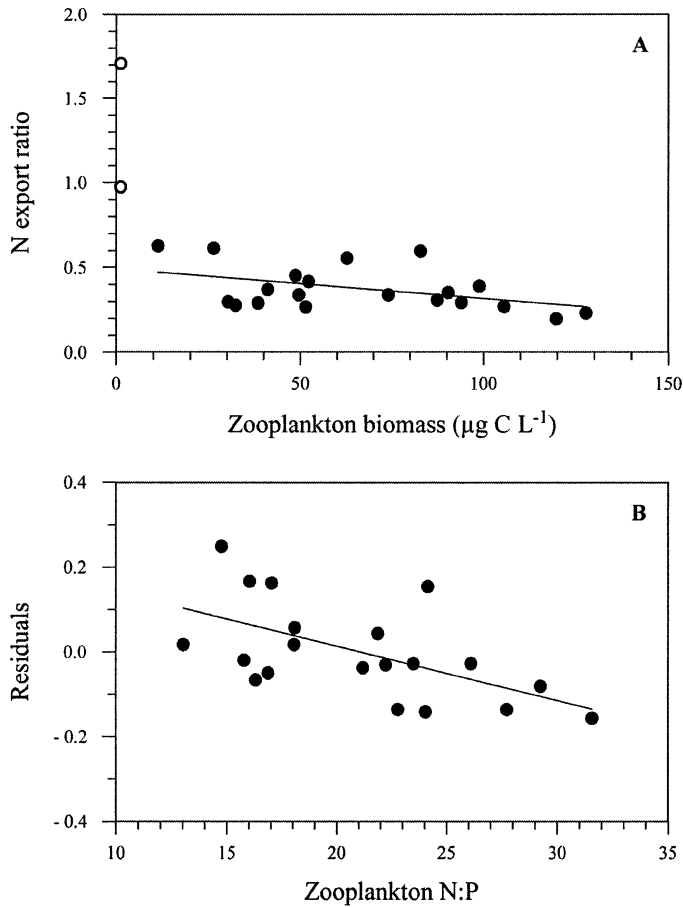


Fig. 5. (A) Relationship between N export ratio and zooplankton biomass in the Esch-sur-Sûre reservoir. Data represented by open circles were excluded from the relationship; see text for explanations. (B) Relationship between the residuals of N export ratio versus zooplankton biomass and zooplankton N:P ratio. The regression details are given in Table 2.

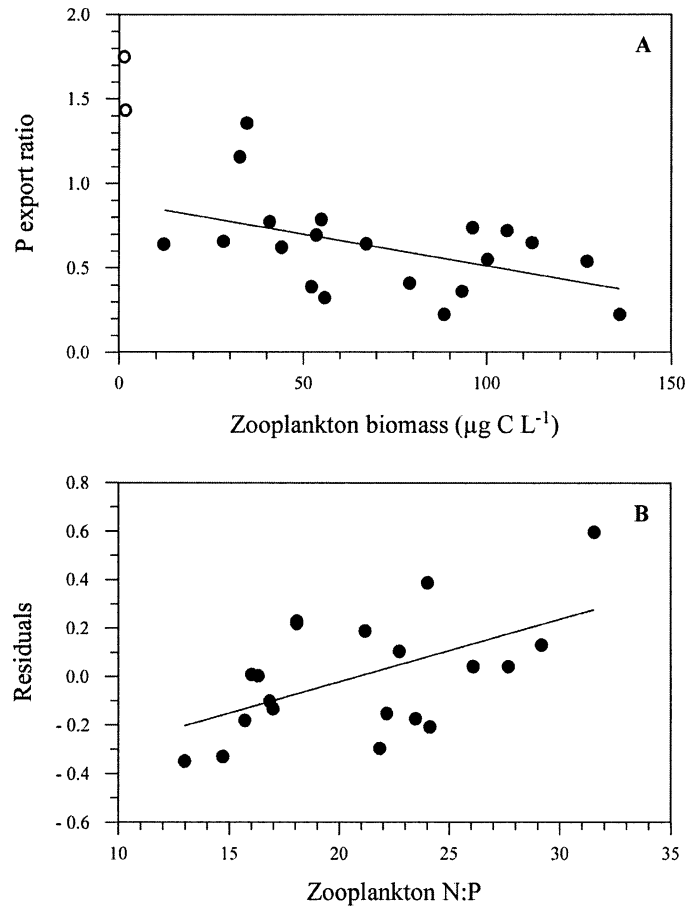


Fig. 6. (A) Relationship between P export ratio and zooplankton biomass in the Esch-sur-Sûre reservoir. Data represented by open circles were excluded from the relationship; see text for explanations. (B) Relationship between the residuals of P export ratio versus zooplankton biomass and zooplankton N:P ratio. The regression details are given in Table 2.

and Foster (1998). They surveyed 12 lakes located at the Experimental Lakes Area in Ontario and found a significant negative relationship between sedimented N:P and zooplankton N:P, which in that study was related to *Daphnia* contribution to total zooplankton biomass. Both variables were measured once in each lake during thermal stratification. So, the variance of N:P ratios in the study of Elser and Foster (1998) was between lakes, unlike this study, which focuses on variation over time in a single lake. To our knowledge, our study demonstrates for the first time the effects of intralake seasonal zooplankton succession on elemental stoichiometry of vertical export fluxes.

Observed effects of zooplankton stoichiometry on vertical export fluxes are explained by the general stoichiometric theory about nutrient recycling of homeostatic consumers (see Elser and Urabe [1999] for a review). Because of their lower body N:P ratio than many other zooplankton species (Andersen and Hessen 1991; Hessen and Lyche 1991), *Daphnia*, *Bosmina*, and *Diaphanosoma* species incorporate more efficiently ingested P than P-low species and release N ingested in excess to maintain their bodily elemental homeostasis. In turn, N-rich copepod species, retain more

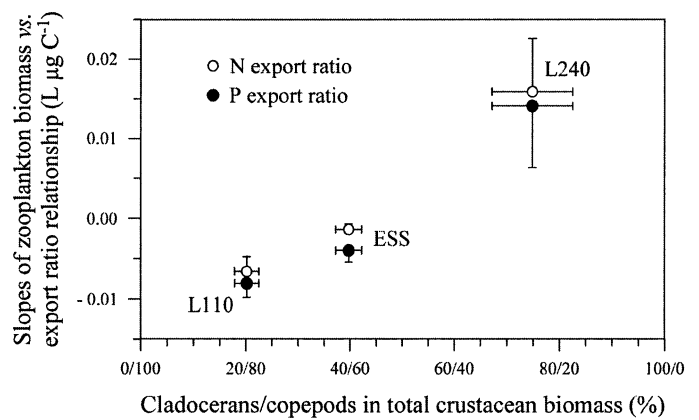


Fig. 7. Comparison of the effect of zooplankton biomass on N and P export ratios. Lake 110 (L110) and Lake 240 (L240) slopes are from Elser et al. (1995). Lake 110 and Lake 240 zooplankton community composition are the means between monthly June–August 1992 data from Elser et al. (1998). Esch-sur-Sûre reservoir (ESS) data are from this study. Vertical and horizontal bars represent 1 SE.

efficiently ingested N, and dispose of P ingested in excess. The disposal of the element ingested in excess can be driven by its nonassimilation, and thus by its final egestion, or, if assimilated, by its metabolization and its final excretion. The relative importance of both processes remains difficult to estimate, but most probably, each one plays a significant role in the maintenance of elemental homeostasis. We believe the relative enrichment in P of sinking particles we observed when copepods dominated most probably was due to the egestion of excess P into highly sinking fecal pellets. As a corollary, the relative enrichment in N was observed when the zooplankton community was dominated by cladocerans. Unfortunately, we cannot statistically distinguish the respective role of copepods and cladocerans in these dynamics because all effects are relative. Because cladoceran feces disrupt to flocculent particles after excretion (Peters 1987) and are therefore probably largely recycled in the epilimnion, we believe the enrichment in P and depletion in N of copepod fast-sinking fecal pellets were very likely the main operating mechanism. Our data convincingly demonstrates that at least a significant fraction of nutrients ingested in excess is not assimilated through the gut wall and can be concentrated into sinking feces. So, we can conclude that part of the physiological regulation leading to elementary homeostatic growth occurs at the assimilation/egestion level.

If the stoichiometric effect of zooplankton grazing on elemental sedimentation is widespread, it stresses the importance of separating excretion from egestion processes in the study of the effects of zooplankton-driven nutrient recycling on phytozooplankton interactions. Indeed, these two types of loss differ in physical form: excreted nutrients are solutes, and egested nutrients are solid or semisolid. The processes also likely differ in the timescale over which nutrients become available for reuptake, and egested material has higher probability to sediment out of the water column. If the P ingested in excess by some copepods is egested instead of excreted, it will be depleted rapidly in the upper layers. As we demonstrated, zooplankton species with a relatively low demand in P, such as herbivorous calanoid copepods, produce fecal pellets with low N:P ratio and act as sinks for P relative to N, which could favor P limitation of phytoplankton in the upper layers. We predict that zooplankton might limit phytoplankton growth not by elements retained in consumer biomass but by elements in excess. This view is the opposite of the usual explicitly stoichiometric nutrient recycling theory that does not distinguish egestion from excretion and therefore considers that all resupplied nutrients are rapidly bioavailable (e.g., Sterner 1990; Sterner et al. 1992). In these theories, elements are conservative in the studied system, and a higher elemental demand of one trophic level (e.g., herbivorous consumers) induces a limitation of growth to other trophic levels (e.g., autotrophs) because of deficiency in the element. Consequently, the growth of the consumer is negatively affected by the consecutive deficiency of its food in the highly requested element. This negative feedback between consumers and producers leads to multiple stable and unstable equilibrium points determined by the initial conditions (Andersen 1997). When systems are uncoupled (e.g., an upper productive layer and a lower detrital layer collecting egested material from the up-

per layer, with nonconservative budgets of elements in each layer), we observe that the phytoplankton–grazer stoichiometric interactions could lead to a putative positive feedback loop in the upper layer. Whether there are multiple or single equilibrium points in these conditions, and if so, where they are situated, are stimulating questions still unresolved. So, although based on simple observations, ecological stoichiometry reveals complex patterns, allowing a better understanding of the dynamics observed at the largely unpredictable phytoplankton–grazer interface (Harris 1994; Sterner and Elser 2002).

References

- ANDERSEN, T. 1997. Pelagic nutrient cycles: Herbivores as sources and sinks. *Ecological studies*, v. 129. Springer-Verlag, Berlin.
- , AND D. O. HESSEN. 1991. Carbon, nitrogen, and phosphorus content of freshwater zooplankton. *Limnol. Oceanogr.* **36**: 807–814.
- BOTTRELL, H. H., AND OTHERS. 1976. A review of some problems in zooplankton production studies. *Nor. J. Zool.* **24**: 419–456.
- DAGNELIE, P. 1992. *Statistique théorique et appliquée—tome 1: Les bases théoriques*. Les Presses Agronomiques de Gembloux, Belgium.
- DARCHAMBEAU, F., AND I. THYS. 2005. In situ filtration responses of *Daphnia galeata* to changes in food quality. *J. Plankton Res.* **27**: 227–236.
- DESCY, J.-P., T. M. FROST, AND J. P. HURLEY. 1999. Assessment of grazing by the freshwater copepod *Diaptomus minutus* using carotenoid pigments: A caution. *J. Plankton Res.* **21**: 127–145.
- DOHET, A., AND L. HOFFMANN. 1995. Seasonal succession and spatial distribution of the zooplankton community in the reservoir of Esch-sur-Sûre (Luxembourg). *Belg. J. Zool.* **125**: 109–123.
- ELSER, J. J., T. H. CHRZANOWSKI, R. W. STERNER, AND K. H. MILLS. 1998. Stoichiometric constraints on food-web dynamics: A whole-lake experiment on the Canadian Shield. *Ecosystems* **1**: 120–136.
- , AND D. K. FOSTER. 1998. N:P stoichiometry of sedimentation in lakes of the Canadian Shield: Relationships with seston and zooplankton elemental composition. *Ecoscience* **5**: 56–63.
- , ———, AND R. E. HECKY. 1995. Effects of zooplankton on sedimentation in pelagic ecosystems: Theory and test in two lakes of the Canadian Shield. *Biogeochemistry* **30**: 143–170.
- , AND J. URABE. 1999. The stoichiometry of consumer-driven nutrient recycling: Theory, observations, and consequences. *Ecology* **80**: 745–751.
- EPPLEY, R. W., AND B. J. PETERSON. 1979. Particulate organic matter flux and planktonic new production in the deep ocean. *Nature* **282**: 677–680.
- HANEY, J. F., AND D. J. HALL. 1973. Sugar-coated *Daphnia*: A preservation technique for Cladocera. *Limnol. Oceanogr.* **18**: 331–333.
- HARRIS, G. P. 1994. Pattern, process and prediction in aquatic ecology. A limnological view of some general ecological problems. *Freshw. Biol.* **32**: 143–160.
- HESSEN, D. O., AND A. LYCHE. 1991. Inter- and intraspecific variations in zooplankton elemental composition. *Arch. Hydrobiol.* **121**: 343–353.
- LEE, C., J. I. HEDGES, S. G. WAKEHAM, AND N. ZHU. 1992. Effectiveness of various treatments in retarding microbial activity in sediment trap material and their effects on the collection of swimmers. *Limnol. Oceanogr.* **37**: 117–130.
- MAIN, T. M., D. R. DOBBERFUHL, AND J. J. ELSER. 1997. N:P stoichiometry and ontogeny of crustacean zooplankton: A test

- of the growth rate hypothesis. *Limnol. Oceanogr.* **42**: 1474–1478.
- [OECD] ORGANIZATION FOR ECONOMIC COOPERATION AND DEVELOPMENT. 1982. Eutrophication of waters: monitoring, assessment and control. OECD.
- PETERS, R. H. 1987. Metabolism in *Daphnia*. Mem. Ist. Ital. Idrobiol. "Dott. Marco de Marchi" **45**: 193–243.
- POISTER, D., AND D. E. ARMSTRONG. 2003. Seasonal sedimentation trends in a mesotrophic lake: Influence of diatoms and implications for phosphorus dynamics. *Biogeochemistry* **65**: 1–13.
- REYNOLDS, C. S., J. M. THOMPSON, A. J. D. FERGUSON, AND S. W. WISEMAN. 1982. Loss processes in the population dynamics of phytoplankton maintained in closed system. *J. Plankton Res.* **4**: 561–600.
- , AND S. W. WISEMAN. 1982. Sinking losses of phytoplankton in closed limnetic systems. *J. Plankton Res.* **4**: 489–522.
- SARNELLE, O. 1999. Zooplankton effects on vertical particulate flux: Testable models and experimental results. *Limnol. Oceanogr.* **44**: 357–370.
- SMITH, E. L. 1936. Photosynthesis in relation to light and carbon dioxide. *Proc. Natl. Acad. Sci. USA* **22**: 504–511.
- STEEMAN-NIELSEN, E. 1952. The use of radio-active carbon (^{14}C) for measuring organic production in the sea. *J. Cons. Perm. Int. Expl. Mer* **18**: 117–140.
- STERNER, R. W. 1990. The ratio of nitrogen to phosphorus resupplied by herbivores: Zooplankton and the algal competitive arena. *Am. Nat.* **136**: 209–229.
- , AND J. J. ELSER. 2002. Ecological stoichiometry: The biology of elements from molecules to the biosphere. Princeton Univ. Press.
- , ———, AND D. O. HESSEN. 1992. Stoichiometric relationships among producers, consumers and nutrient cycling in pelagic ecosystems. *Biogeochemistry* **17**: 49–67.
- THYS, I., A. DOHET, H.-M. CAUCHIE, AND L. HOFFMANN. 1998. Multivariate analysis of the longitudinal variation of the zooplankton community structure in a riverine reservoir. *Int. Rev. Hydrobiol.* **83**: 191–198.
- VALDERRAMA, J. C. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. *Mar. Chem.* **10**: 109–122.
- VOLLENWEIDER, R. A. 1974. A manual on methods for measuring primary production in aquatic environments. IBP Handbook, v. 12, 2nd ed. Blackwell Scientific.
- WRIGHT, S. W., S. W. JEFFREY, R. F. C. MANTOURA, C. A. LLEWELLYN, T. BJORNLAND, D. REPETA, AND N. WELSCHMEYER. 1991. Improved HPLC method for the analysis of chlorophylls and carotenoids in marine phytoplankton. *Mar. Ecol. Prog. Ser.* **77**: 183–196.

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