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Phosphorus limitation in *Daphnia*: Evidence from a long term study of three hypereutrophic Dutch lakes

Abstract—The Loosdrecht lakes comprise three shallow, interconnected hypereutrophic lakes in The Netherlands. A lake restoration project conducted during the 1980s resulted in reduced phosphorus loading. However, no changes in phytoplankton abundance or species composition were noted, although seston carbon:phosphorus (C:P) ratios increased. Filamentous cyanobacteria and detritus continued to dominate the seston. Moreover, planktivorous fish were very abundant. Relationships between zooplankton abundance and seston abundance and stoichiometry (C:P and carbon:nitrogen ratios) were analyzed using data collected during 9 yr of intensive study of the three lakes. Analysis based on annual means shows surprisingly strong and consistent inverse relationships between *Daphnia* abundance and the seston C:P ratio for two of the three lakes. In these two lakes (Loosdrecht and Vuntus Lakes), the annual mean abundance of *Daphnia cucullata* ranged from 104 to 0.7 individuals L⁻¹ over a range in mean seston C:P ratios from about 250 to 500 (molar). In the third lake, Breukeleveen, *Daphnia* abundance was higher for a given seston C:P ratio, especially during the 2 yr following a biomanipulation project in this lake. Analysis of seston C:P ratios and *Daphnia* abundance in individual samples provides further evidence that high seston C:P ratios constrained *Daphnia* abundance in all three lakes. In contrast to *Daphnia*, the abundances of zooplankters with low P requirements, including *Bosmina* spp. and cyclopoid copepods, show little relationship to seston C:P ratios. These results suggest that the abundance of *Daphnia* in the Loosdrecht lakes was determined by the variable P content of their diets and consistently strong fish predation.

Zooplankton growth rates are strongly influenced by both food quantity and food quality. Recently, the possibility that zooplankton growth rates may be limited by the phosphorus (P) content of their diets has excited interest and controversy (reviews by Sterner and Hessen 1994; Brett and Müller-Navarra 1997; Gulati and DeMott 1997; Elser and Urabe 1999; Sterner and Schulz 1998). *Daphnia* has been a center of focus, in part because of its importance in lakes and in part because daphnids have high requirements for P as compared with other crustacean zooplankton. Thus far, the evidence for P limitation in *Daphnia* comes largely from laboratory growth experiments with P-deficient resources and from predictions of stoichiometric theory (Sterner and Hessen 1994). Assuming that resources are poor in quality only because of a P deficiency, stoichiometric theory can be used to calculate a resource threshold carbon:P (C:P) ratio where the growth of a zooplankton species becomes limited by P rather than by energy per se. Such threshold ratios depend not only on

the stoichiometry of the consumer but also on the concentration and digestibility of the resources (Urabe and Watanabe 1992; Sterner and Hessen 1994; Sterner 1997). Estimates of C:P thresholds for *Daphnia* usually range from 140 to 300 (molar units; Sterner and Hessen 1994), although values as low as 90 have been reported for fast-growing juvenile *Daphnia magna* (DeMott et al. 1998). Because seston C:P ratios >300 are common in lakes, surveys of seston C:P ratios result in the prediction that *Daphnia* should often experience P limitation in nature (Gulati et al. 1991; Hessen 1992; Elser and Hassett 1994). Because daphnids have higher P concentrations in their body tissues than do other freshwater zooplankton (Sterner and Hessen 1994), stoichiometric theory predicts that daphnids should be more sensitive to P limitation than should other taxa. For example, bosminids are predicted to be less sensitive than *Daphnia* to P limitation (Urabe and Watanabe 1992), and this prediction has recently been confirmed in laboratory growth experiments (Schultz and Sterner 1999).

The controversy over P limitation comes, in part, from difficulties in separating direct P limitation from alternative mechanisms for the poor quality of P-deficient algae, including fatty acid deficiencies (Müller-Navarra 1995) and digestion resistance (Van Donk and Hessen 1993; Van Donk et al. 1997). Cyanobacteria, for example, can be a poor quality food for a variety of reasons, including toxicity, digestion resistance, difficulties in handling and ingesting filaments and colonies, and nutritional inadequacy (reviews by Lampert 1987; de Bernardi and Giussani 1990; Christoffersen 1996). Ahlgren et al. (1990) noted that cyanobacteria are deficient in highly unsaturated fatty acids (HUFA) and hypothesized that HUFA deficiencies are a major cause of the poor food quality of cyanobacteria. In support of this HUFA hypothesis, DeMott and Müller-Navarra (1997) showed that the addition of a HUFA-rich emulsion to the cyanobacterium *Synechococcus* markedly improved the growth and reproduction of three species of *Daphnia*. Thus, several distinct mechanisms in addition to P deficiency could cause poor zooplankton growth in lakes dominated by cyanobacteria.

Lake surveys have supported the P-limitation hypothesis by showing that the abundance of *Daphnia* is positively correlated with seston particulate P concentration (Hessen 1992) and negatively correlated with the seston C:P ratio (Hassett et al. 1997). However, many factors that potentially influence *Daphnia* abundance, including predation and phytoplankton productivity and composition, vary markedly between lakes and may co-vary with P concentration. Thus, long-term studies in individual lakes with varying C:P ratios are needed

to complement survey studies. In this study, we reanalyzed the results of a long-term intensive study of three hypereutrophic Dutch lakes with phytoplankton dominated by filamentous cyanobacteria and a filamentous prochlorophyte. A lake restoration project resulted in reduced P loading and changes in seston C:P ratios, with little or no apparent change in the phytoplankton species composition or total seston concentration. Although the results of this large interdisciplinary project have been extensively reported in the proceedings of a symposium (Van Liere and Gulati 1992) and elsewhere, previous analyses were made before the possibility of P limitation in *Daphnia* was clearly understood. Thus, we focused our analysis on the relationship between seston P content (C:P ratios) and *Daphnia* abundance.

The three Loosdrecht lakes included in this study lie between Utrecht and Amsterdam and were originally formed by peat mining in the 17th century. All three lakes are shallow, highly eutrophic, and open to wind action. The main lake, Lake Loosdrecht, has an area of 979 ha and a mean depth of 1.85 m. The two smaller lakes lie on the northeast (Lake Vuntus: 88 ha, mean depth = 1.5 m) and southeast (Lake Breukeleveen: 179 ha, mean depth = 1.45 m) corners of the main lake. Each of the smaller lakes is connected to Lake Loosdrecht by small channels (see Gulati et al. 1992 for a map and further description). The phytoplankton of all three lakes were dominated by cyanobacteria, mainly *Oscillatoria limnetica* and the prochlorophyte *Prochlorothrix hollandica*. Together, these two filamentous forms comprised, on average, about 90% of the algal mass in suspension (Van Tongeren et al. 1992). However, about half to two-thirds of the seston (<150 μm) consisted of detritus, largely originating from *Oscillatoria* and *Prochlorothrix* (Otten et al. 1992). Secchi depth ranged from 30 to 50 cm. The zooplankton in all three lakes were dominated by *Bosmina* spp. (*Bosmina longirostris* and *Bosmina coregoni*), *Chydorus sphaericus*, cyclopoid copepods, and rotifers (Gulati et al. 1992). *Daphnia*, represented almost exclusively by the smallest Eurasian species (*Daphnia cucullata*), was highly variable in abundance between years. Fish were very abundant (about 300 kg/ha) and were dominated by stunted populations of planktivorous bream (*Abramis brama*; Van Densen et al. 1986; Lammens et al. 1992).

The eutrophication of the Loosdrecht lakes was largely due to high external P loading. From 1976 onwards, two measures were taken to restore the lakes. The first measure was the construction of a sewage system, in the period from 1976 to 1986, for villages that had earlier discharged their sewage into the lakes. The second measure involved a change in the source of the water supply. Until 1984, lake water levels were maintained by allowing water from the highly polluted Vecht River to flow into Lake Loosdrecht. In 1984, river water was replaced by P-depleted water from the Amsterdam Rhine Canal. Together, these measures reduced external P loading from about 1.1 g P m⁻² yr⁻¹ to 0.35 g P m⁻² yr⁻¹ (Van Liere and Janse 1992). However, although seston particulate P slowly decreased from an average of 130 $\mu\text{g P L}^{-1}$ to about 80 $\mu\text{g P L}^{-1}$, extensive monitoring from 1981 through 1991 revealed that the restoration efforts were essentially unsuccessful (Van Liere and Janse 1992). Chlorophyll *a* and seston concentrations

showed small increases following the restoration efforts, and the total numbers of filamentous cyanobacteria and *Prochlorothrix hollandica* also appeared to increase slightly (Van Tongeren et al. 1992). *D. cucullata* declined to very low densities in Loosdrecht and Vuntus Lakes during the last 2–3 yr of the study but remained more abundant in Lake Breukeleveen. No other discernible patterns were noted in the species composition or abundance of the crustacean zooplankton (Gulati 1990; Gulati et al. 1992; Van Tongeren et al. 1992). Thus, the one notable impact of the restoration effort was an increase in the C:P ratio of the seston, especially in Lake Loosdrecht (Gulati et al. 1991; Janse et al. 1992).

In addition to reductions in P loading, a biomanipulation project was carried out in 1989 in Lake Breukeleveen. In March of that year, about two-thirds of the fish stock of Lake Breukeleveen were removed by netting in an attempt to increase the abundance of *Daphnia* and thereby improve water clarity (Van Donk et al. 1990). The lake was also stocked with fingerlings of a piscivorous fish (pike, *Esox lucius*) and with large-bodied *Daphnia* (*Daphnia pulex* and *Daphnia hyalina*). Although *D. cucullata* reached a late spring peak that was higher than that of previous years, the *Daphnia* population declined to very low numbers by midsummer and there was neither a discernible decline in chlorophyll concentration nor an improvement in water clarity.

Our analysis is based on samples of zooplankton and seston collected from all three lakes from 1983 through 1991. Methods for sampling and sample processing were presented by Gulati (1990) and Gulati et al. (1992). Lake water was sampled at 2-week intervals from March to October during 1983 through 1985 and at monthly intervals during winters and in the study period from 1986 to 1991. In 1991, samples were only collected in Lake Loosdrecht. Samples were collected using a 5-liter Friedinger sampler. At each station, six samples were collected at a depth of 10–70 cm from a drifting boat to make a 30-liter composite sample. Because of the shallowness of the lakes and the well-mixed nature of the water columns, this method gave representative samples. Samples were collected from two stations in Lake Loosdrecht and from one station in each of the smaller lakes. The two Lake Loosdrecht stations provided very similar estimates, and thus we calculated mean values for these two stations for each sampling date.

Seston (<150 μm) was routinely separated from the zooplankton using a 150- μm metal sieve. Observations with a microscope showed that the seston fraction contained copepod nauplii and rotifers, but these contaminants only comprised a small percentage of the total seston biomass. Moreover, nearly all of the filamentous procaryotes were collected in the <150- μm fraction. Thus, the <150- μm fraction provided unbiased estimates of seston abundance and stoichiometry. *Daphnia* readily ingests filaments of *Oscillatoria* 1 mm in length (DeMott and Moxter 1991). Moreover, laboratory experiments have shown that *Daphnia galeata* can grow on a diet of *Oscillatoria limnetica* alone (Repka 1997). Thus, using a smaller seston size fraction as food for *Daphnia*, such as <30 μm , would have excluded some of the filamentous procaryotes, an important food source in these lakes.

Table 1. Summary of the overall means, ranges, and variability of annual means of selected parameters from the seston (<150 μm) and zooplankton (>150 μm) of three hypereutrophic lakes. Data set includes 9 yr of growing season data (May–October) for Lake Loosdrecht and 8 years for both Vuntus and Breukeleveen lakes. Variability is expressed as the coefficient of variation (%CV; $100 \times$ standard deviation/mean).

	Loosdrecht			Vuntus			Breukeleveen		
	Mean	Range	%CV	Mean	Range	%CV	Mean	Range	%CV
Seston									
POC (mg L^{-1})	9.9	8.0–11.7	11.9	11.0	8.9–12.1	8.2	12.9	11.2–15.2	9.3
N (mg L^{-1})	1.30	1.12–1.52	13.7	1.47	1.17–1.75	11.2	1.63	1.39–1.91	11.7
P ($\mu\text{g L}^{-1}$)	80	52–107	22.1	72	57–92	15.4	82	64–93	14.1
C:N ratio	9.0	8.0–10.1	7.7	8.9	8.1–9.7	7.1	9.4	8.4–10.8	8.6
C:P ratio	335	247–488	22.3	408	333–500	13.6	415	351–497	12.5
Zooplankton									
C ($\mu\text{g L}^{-1}$)	369	272–567	23.8	299	173–462	31.0	433	331–533	6.0
<i>Daphnia</i> (L^{-1})	26.5	0.7–104	112	7.31	0.7–20.5	97.5	25.9	4.9–43.9	52.5
<i>Bosmina</i> (L^{-1})	232	129–315	23.5	170	70.9–333	47.3	245	54.3–523	52.7
<i>Chydorus</i> (L^{-1})	115	47.0–221	90.6	104	25.1–378	110	110	0.2–54.3	56.7
<i>Cyclops</i> (L^{-1})	148	79.0–203	31.1	196	131–256	17.7	143	86.7–178	20.0

The carbon concentration of the seston (<150 μm) and zooplankton (>150 μm) fractions was derived from chemical oxygen demand measurements, using a modified procedure involving digestion of organic matter at 140°C (Gulati et al. 1982). Particulate P was measured in seston samples (50–100 ml) concentrated on Whatman GF/F filters that were then dried and ashed at 550°C for 2 h. The ashed filters were digested at 100°C, and P was measured according to Murphy and Riley (1962). Samples for measuring nitrogen (N) were first centrifuged at 2,000 rpm and then freeze-dried. Seston N was determined using a Perkin Elmer N analyzer (model 240) up to 1985 and a Carbo Erba Analyzer (model 1106) thereafter. Counting techniques for estimating zooplankton abundance and species composition were presented by Gulati (1990).

Poor food conditions, such as a P deficiency, should first cause reduced growth and reproduction. However, if mortality rates remain largely unchanged, reduced abundance should result. Thus, we used simple correlations to test for relationships between zooplankton abundance (individuals per liter) and seston parameters. We focused on *Daphnia* abundance and C:P ratios. However, because stoichiometric theory predicts that other zooplankton taxa should be less sensitive than *Daphnia* to P limitation, we also calculated correlations for other zooplankton taxa and other seston parameters as a basis for comparisons. Because *Daphnia* was scarce or absent in winter and early spring, our analysis was restricted to the May–October growing season. Data were analyzed for two levels of temporal aggregation. At one extreme, we compared growing season means. Here, each annual mean for each lake provided one data point. We tested for correlations for each lake individually and for all three lakes combined. At the opposite time scale, we compared zooplankton abundance with the seston C:P ratio from individual samples. Zooplankton densities were log transformed. Because autocorrelation is a concern in time-series data, we tested for autocorrelations in the annual data for *Daphnia* abundance and seston C:P ratios.

Table 1 summarizes the overall means and between-year variability of seston parameters and zooplankton abundance.

The three lakes showed only modest variation in seston biomass (particulate organic carbon [POC]) between years. POC was modestly higher in Lake Breukeleveen than in the other two lakes. Seston P and C:P ratios were more variable than seston N and C:N ratios. The range of seston C:P ratios was greater in Lake Loosdrecht than in the two smaller lakes. This was probably a consequence of the diversion of P-rich water from the River Vecht in 1985, which earlier had passed through a sluice directly into Lake Loosdrecht.

The annual means of zooplankton biomass ($\mu\text{g C L}^{-1}$) were mostly stable (Table 1). Mean zooplankton biomass was highest in Lake Breukeleveen, lowest in Lake Vuntus, and intermediate in Lake Loosdrecht. Among individual taxa, the densities of *Bosmina* and *Cyclops* showed low variability across lakes and years, whereas the densities of *Daphnia* and *Chydorus* were more variable. *Daphnia* was especially variable in Loosdrecht Lake, where the mean annual density ranged from 0.7 to 104 individuals L^{-1} . The overall mean abundance of *Daphnia* was lower in Lake Vuntus than in the other two lakes.

Figure 1 shows the time course of the annual means of the seston C:P ratio and *Daphnia* abundance for each lake. Detailed seasonal data for *Daphnia* abundance and seston C:P ratios for Lake Loosdrecht have been reported by Gulati et al. (1992). Initially, before the change in water supply, the seston C:P ratio was low in Loosdrecht Lake and high in both of the smaller lakes. The two smaller lakes showed a decline in mean seston C:P ratios during the first 3 yr of the study, whereas the seston C:P ratio in Lake Loosdrecht remained low with little variability during the same period. The lowest mean C:P ratio was found in 1985 in Lake Loosdrecht and in 1987 in both small lakes. All three lakes experienced increased seston C:P ratios during the last few years of the study. Leakage of P-rich water into the lakes through a sluice in 1987 may have delayed the trend for increased seston C:P ratios during 1987 and 1988. For each lake, mean seston C:P ratio and mean *Daphnia* abundance show opposite trends (Fig. 1).

Simple correlations between seston quantity and stoichiometry and zooplankton abundance are summarized in Table

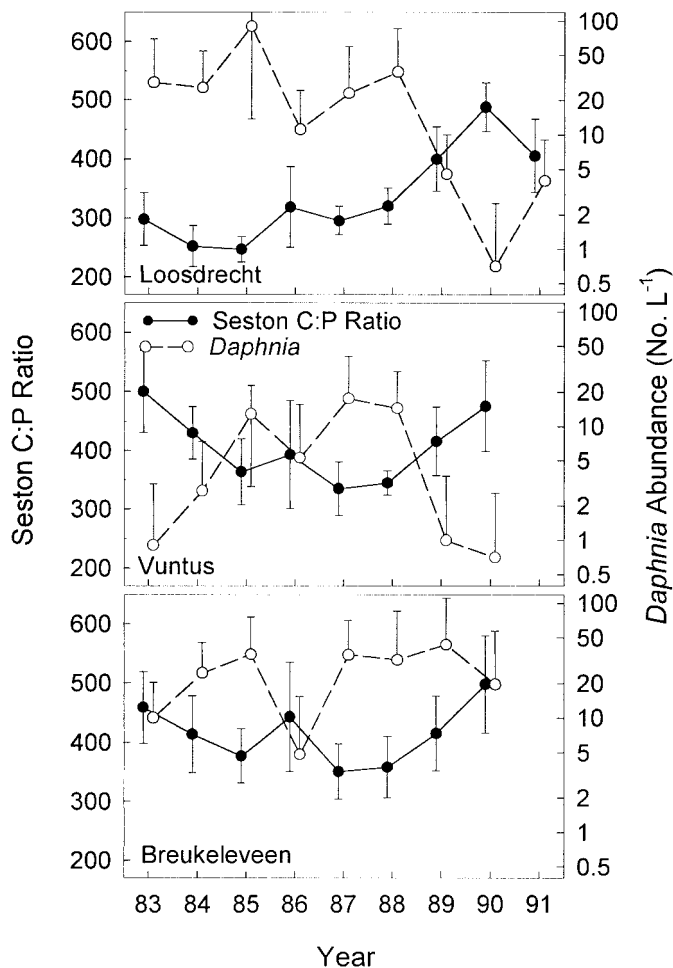


Fig. 1. Annual means of the seston C:P ratio (●) and *Daphnia* abundance (○) for three hypereutrophic lakes. Each data point is a growing season mean (May–October) \pm 1 SD. Note log scale for *Daphnia* abundance. Curves are offset for clarity.

2. Strong negative correlations were found between annual mean *Daphnia* abundance and the annual mean seston C:P ratio for both Loosdrecht and Vuntus Lakes. The correlation was weaker and not significant for Lake Breukeleveen. None of the other zooplankton taxa showed a significant correlation with the seston C:P ratio for any of the individual lakes. Combining data from all three lakes, the correlation between abundance and seston C:P was strong for *Daphnia* and weaker but significant for *Bosmina* (Table 2). There was also an inverse relationship between total zooplankton biomass and seston C:P ratio for the combined data from all three lakes.

Daphnia abundance in Lake Loosdrecht showed an autocorrelation with a time lag of 1 yr ($r = 0.62$), whereas no such autocorrelation was noted for Breukeleveen and Vuntus Lakes ($r = -0.13$ and -0.17 , respectively). Thus, the significance values for correlations involving *Daphnia* abundance in Lake Loosdrecht may be overstated. We also found an autocorrelation in the annual C:P ratios in Lake Loosdrecht ($r = 0.44$) but not in Breukeleveen and Vuntus Lakes ($r = -0.42$ and -0.08 , respectively). Thus, the autocorre-

Table 2. Correlations between seston quantity and stoichiometry and zooplankton abundance in three hypereutrophic lakes. Each data point (n) is based on the annual mean for a growing season (May–October). Pearson correlation coefficients were calculated for each lake and for the combined data from all three lakes. The abundance of each species (L^{-1}) and the total biomass ($\mu g C L^{-1}$) were log transformed.

Genus	Loosdrecht (n = 9)	Vuntus (n = 8)	Breukele- veen (n = 8)	Combined (n = 25)
<i>Daphnia</i>				
C:P	-0.95****	-0.92**	-0.59	-0.77****
C:N	-0.22	0.00	-0.37	0.22
POC	-0.24	-0.18	-0.77*	-0.03
<i>Bosmina</i>				
C:P	-0.41	-0.56	-0.18	-0.40*
C:N	-0.10	-0.10	-0.77*	-0.13
POC	0.03	-0.07	-0.42	-0.07
<i>Chydorus</i>				
C:P	0.23	-0.28	0.24	0.00
C:N	0.64	0.28	-0.04	0.00
POC	-0.01	0.00	0.16	-0.10
<i>Cyclops</i>				
C:P	-0.64	-0.49	-0.47	-0.26
C:N	-0.20	-0.09	-0.31	-0.31
POC	0.44	0.28	-0.05	0.14
Biomass				
C:P	-0.54	-0.69	-0.49	-0.46*
C:N	-0.53	-0.07	-0.40	0.20
POC	-0.36	0.15	-0.18	0.13

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

lation in the *Daphnia* abundance data for Lake Loosdrecht may have been driven by corresponding autocorrelated changes in the seston C:P ratio. *Daphnia* declines to undetectable densities during the winter in these lakes and can show explosive growth during spring. Thus, unlike the situation with long-lived organisms, where abundance in 1 yr depends on survivorship from the previous year, *Daphnia* abundance for a particular year should be determined primarily by conditions in the lake during that year.

Figure 2 shows scatter plots and regression lines for annual means of *Daphnia* abundance versus seston C:P ratios. Data for Loosdrecht and Vuntus Lakes show a similar relationship, whereas Lake Breukeleveen exhibited consistently greater mean *Daphnia* abundance for a given mean seston C:P ratio. Elevated mean values of *Daphnia* abundance were found in Lake Breukeleveen during 1989, the year of the biomanipulation experiment, and during the following year, 1990 (Fig. 2). A significant decrease in predation should allow a higher abundance of *Daphnia* for a given resource-limited rate of individual growth and reproduction. Removing data for 1989 and 1990 substantially improves the *Daphnia* versus seston C:P ratio correlation for Lake Breukeleveen ($n = 6$, $r = -0.85$, $P < 0.05$).

Plots of seasonal variation in seston C:P ratios and *Daphnia* abundance in Lake Breukeleveen provide further support for the hypothesis that seston C:P ratios constrained *Daphnia* abundance (Fig. 3). During 1987 and 1988, years of consistently low seston C:P ratios in Lake Breukeleveen, *Daphnia* remained abundant from spring through late sum-

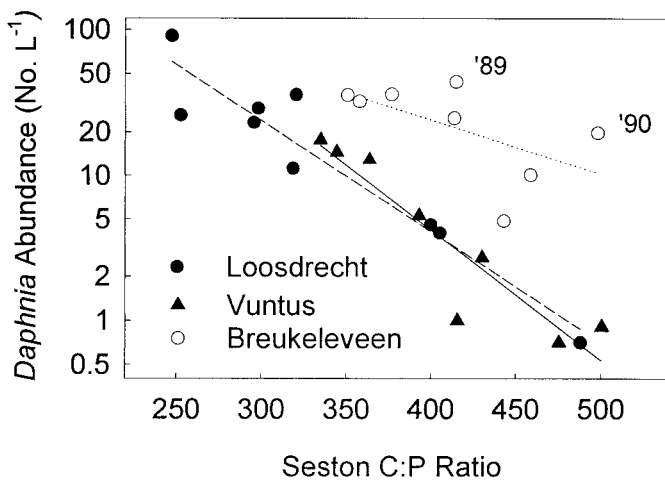


Fig. 2. Relationship between mean *Daphnia* abundance and mean seston C:P ratio. Each data point is the mean for a growing season (May–October). Curves are least-square regression lines. Labeled points for Lake Breukeleveen indicate the year of fish removal (1989) and the following year (1990). Note the log scale for *Daphnia* abundance.

mer. However, in both 1989 and 1990, following the fish reduction, *Daphnia* reached high late spring peaks but declined to undetectable densities after seston C:P ratios exceeded 500. In 1989, 1990, and 1986, *Daphnia* declined to undetectable levels within one sampling period (4 weeks) after peaks in the seston C:P ratio.

Daphnia can act as either a sink or a source for P and can thereby directly affect the seston C:P ratio (Urabe 1995; Elser and Urabe 1999). When *Daphnia* acts as a significant sink for P, a positive correlation should be expected between *Daphnia* abundance and the seston C:P ratio. When *Daphnia* grazing strongly depresses seston abundance, it may cause a reduction in the seston C:P ratio by increasing the per capita P availability for phytoplankton and bacteria. This circumstance could lead to a negative correlation between *Daphnia* abundance and the seston C:P ratio. However, the seasonal data for Lake Breukeleveen show that the seston concentration remained very high throughout the growing season, usually between 10 and 15 mg C L⁻¹, despite large fluctuations in *Daphnia* (Fig. 3). Thus, the seasonal data from Lake Breukeleveen do not support the hypothesis that negative correlations between *Daphnia* abundance and the seston C:P ratio were due to an effect of *Daphnia* on the seston rather than an effect of food quality on *Daphnia*.

Plots of zooplankton abundance versus the seston C:P ratio for each sample also show an inverse relation between *Daphnia* abundance and seston C:P ratios (Fig. 4, upper left). Although low and even zero *Daphnia* densities occurred over the full range of seston C:P ratios, high *Daphnia* densities were consistently found in samples with lower seston C:P ratios. This apparent constraint on *Daphnia* abundance was especially strong for Loosdrecht and Vuntus Lakes. For example, for these two lakes, *Daphnia* abundance was >30 L⁻¹ in 26 of 94 samples with a seston C:P ratio of <400, but *Daphnia* abundance did not exceed 30 L⁻¹ in any of 44 samples with a seston C:P ratio of >400. Again,

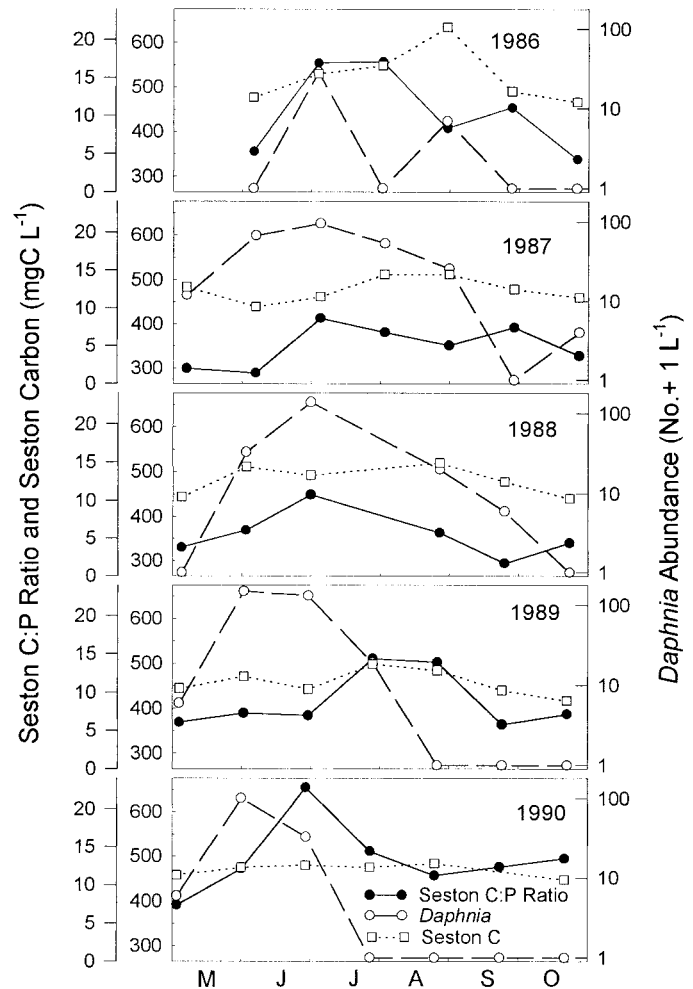


Fig. 3. Dynamics of *Daphnia*, the seston C:P ratio, and seston concentration in Lake Breukeleveen during the last 5 yr of the study. Note the log scale for *Daphnia* abundance.

Daphnia was more abundant at high seston C:P ratios in Lake Breukeleveen than in the other two lakes. Apparent time lags between peaks in seston C:P ratio and declines in *Daphnia* abundance, as shown in Fig. 3, account for some instances of high *Daphnia* abundance and high seston C:P ratios in Lake Breukeleveen. The fish removal project may have also caused a temporary increase in *Daphnia* in Lake Breukeleveen. This lake had higher concentrations of dissolved organic carbon and seston POC and lower Secchi depths than did the other two lakes (Van Tongeren et al. 1992). Thus, reduced water transparency in Lake Breukeleveen may have consistently reduced planktivory in comparison to that in the other two lakes.

Heteroscedasticity and zero values for *Daphnia* preclude a rigorous statistical analysis of the data for individual sampling dates. However, *Daphnia* densities show a gradual decline in the seston C:P ratio when averaged over arbitrary intervals (Fig. 4, bottom left). In contrast to *Daphnia*, the abundance of *Bosmina* does not show a trend with the seston C:P ratio (Fig. 4, right).

The long-term data from the Loosdrecht lakes reveal sev-

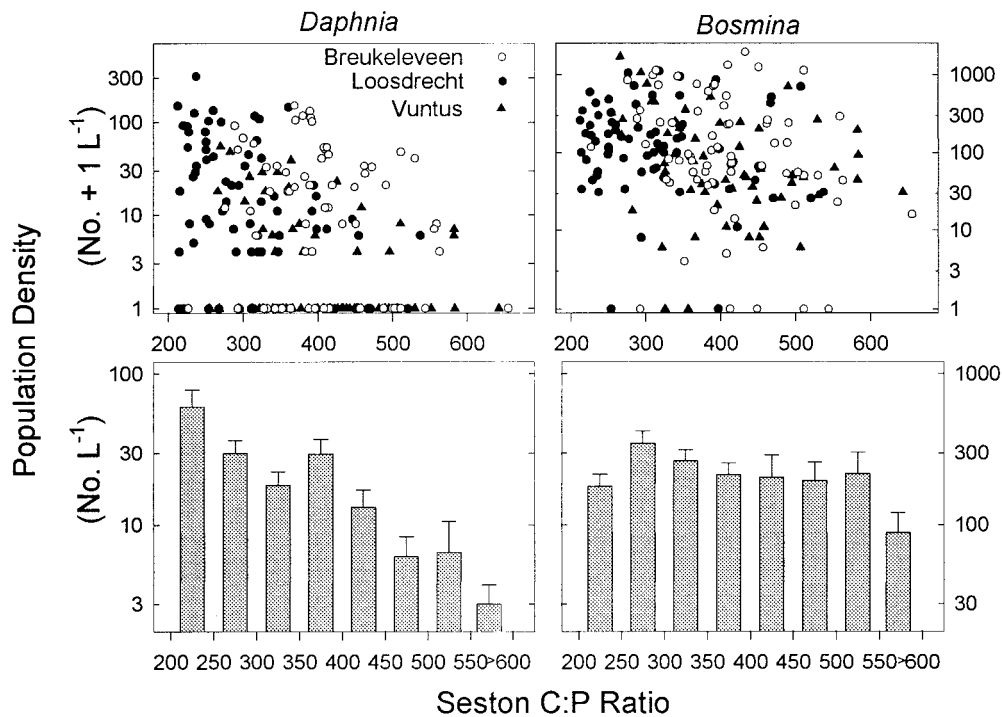


Fig. 4. Scatter plots of abundance and the seston C:P ratio in the same sample in each of three lakes (upper panels). Bars (lower panels) show mean abundance \pm 1 SE for intervals of 50 units in the seston C:P ratio. Bars are centered between the lower and upper limits of each 50-unit interval. Data from all three lakes are combined.

eral patterns consistent with stoichiometric theory, especially the notion that *Daphnia* abundance was limited by the P content of food resources. First, *Daphnia* abundance shows strong inverse relationships with seston C:P ratios. This constraint on the abundance of daphnids appeared strong for analysis based on both annual means and individual sampling dates. Second, declines in *Daphnia* abundance occurred over the range in seston C:P ratios where P limitation of *Daphnia* is expected. Clearly, P limitation is predicted only when seston C:P ratios exceed threshold values. Calculations based on stoichiometric theory suggest a threshold C:P ratio of about 90 to 300 for *Daphnia* spp. (Sterner and Hessen 1994; DeMott et al. 1998), whereas Sterner (1993) noted a detectable decline in the growth of *Daphnia obtusa* at a C:P ratio of about 200 in a laboratory experiment. In the Loosdrecht lakes, *Daphnia* showed marked declines in abundance over mean annual C:P ratios ranging from about 250 to 500. Analysis of data for individual dates suggests that C:P ratios constrained *Daphnia* abundance over the same range in C:P ratios, with the lowest daily C:P ratios exceeding 200. Thus, *Daphnia* in these lakes may have experienced weak P limitation even when the seston C:P ratio was at its lowest values. The decline in *Daphnia* abundance with increasing seston C:P was especially strong and consistent in Loosdrecht and Vuntus Lakes. In these lakes, an increase in the annual mean seston C:P ratio from 250 to 500 was associated with a decline in mean *Daphnia* abundance of nearly two orders of magnitude (Fig. 2). In agreement with stoichiometric theory, *Bosmina* and cyclopoid copepods, taxa that are predicted to have lower P requirements

than *Daphnia* (Sterner and Hessen 1994), showed almost no response to changes in the seston C:P ratio. *Chydorus* also did not seem to respond to the seston C:P ratio, but no data are available on its stoichiometry and P requirements.

Correlations must be interpreted with caution, especially under complex field conditions. However, two lines of evidence further increase confidence in the P limitation hypothesis for *Daphnia* in the Loosdrecht lakes. First, the data and analysis from this long-term intensive project provide no evidence for alternative explanations for the marked changes in *Daphnia* abundance. The extensive data on phytoplankton provide no evidence for significant changes in food composition or quantity apart from the changes in the seston C:P ratio. Filamentous prokaryotes dominated the phytoplankton of all three lakes, and total seston concentrations remained extremely high throughout the study period (Van Tongeren et al. 1992). Second, differences between the lakes in temporal fluctuations in seston C:P ratios also increase confidence in the correlation analysis. For example, during 1983–1985, seston C:P ratios were low in Loosdrecht Lake but high and declining in the two smaller lakes. *Daphnia* showed corresponding changes in abundance between the lakes (Fig. 1), suggesting that *Daphnia* abundance was not being controlled by some gradual change in an unknown factor common to the interconnected lakes.

The inverse relationship between the seston C:P ratio and *Daphnia* abundance in these hypereutrophic lakes was strong and consistent. Fish predation combined with the alternative reasons for the poor food quality of filamentous cyanobacteria already seemed sufficient to explain periods of low

Daphnia abundance. Stocks of planktivorous fish were very high. Moreover, the proportion of small (<25 cm), slow-growing bream was much higher in Loosdrecht Lake than in Tjeukemeer, another shallow, hypereutrophic Dutch lake (Lammens et al. 1992), suggesting that fish growth in the Loosdrecht lakes was strongly limited by the small size of planktonic prey and the low abundance of alternative resources. *Daphnia cucullata* typically occupies the extreme end of a gradient of increasing productivity and fish predation in lakes in northern Europe (Hessen et al. 1995). The nearly complete absence of the larger *D. galeata*, a species quite common in many other eutrophic Dutch lakes, is further evidence for very strong fish predation in the Loosdrecht lakes.

Predation and food limitation are often considered alternative mechanisms of population limitation. This reasoning applies where high prey abundance causes declines in the abundance and/or quality of their food resources (e.g., Kerfoot et al. 1988). However, seston C:P ratios are largely determined by the combined effects of nutrient loading, mixing depth, and water transparency (Sterner et al. 1997). In this situation, poor food quality and high predation can occur simultaneously. High predation makes populations especially vulnerable to poor food quality, because high reproductive rates are needed to balance high predatory mortality. Poor food quality can prevent trophic cascades from zooplankton to phytoplankton even when predation rates are low (e.g., Elser et al. 1998; MacKay and Elser 1998). Van Donk et al. (1990) speculated that the failure of their food chain manipulation to improve water clarity in Lake Breukeleveen was due to poor food quality for the zooplankton grazers. Drawing on laboratory experiments, Davidowicz et al. (1988) cited the high abundance of filamentous cyanobacteria as the probable cause of poor *Daphnia* growth in seston from Lake Loosdrecht. Our analysis, however, points to changes in dietary P as the primary cause for fluctuations in *Daphnia* abundance in the Loosdrecht lakes.

The possibility that high seston C:P ratios constrained *Daphnia* abundance does not preclude the possibility that other factors also decreased food quality in the Loosdrecht lakes. Laboratory experiments suggest that *Oscillatoria limnetica*, which dominates the phytoplankton of the Loosdrecht lakes, is a suboptimal resource for *Daphnia*. For example, laboratory experiments with *D. galeata* have shown that the green alga *Scenedesmus acutus* is a better quality food than the filamentous cyanobacterium *O. limnetica* (Repka 1997). However, *O. limnetica*, which has a fatty acid profile similar to that of green algae, is sufficiently nutritious to support growth and reproduction when it is the sole food source (Repka 1997). This situation is in contrast to that of the cyanobacterium *Synechococcus elongatus*, which is highly deficient in unsaturated fatty acids and does not sustain growth and reproduction in *Daphnia* when used as a sole food source (DeMott and Müller-Navarra 1997). Laboratory experiments also suggest that detritus derived from *Oscillatoria* is an even better food resource for *Daphnia* than the living filaments (Repka et al. 1998).

Our correlations suggest that *Daphnia* abundance in the Loosdrecht lakes was strongly constrained by the seston C:P ratio. Moreover, as predicted by stoichiometric theory,

other coexisting zooplankton species were less sensitive to P limitation. Correlations, however, do not provide direct evidence for the underlying mechanisms. Therefore, our results do not directly rule out the possibility that fatty acids, digestion resistance, or another factor influencing food quality covaried with the seston C:P ratio. However, several recent laboratory studies provide support for the elemental limitation hypothesis and/or evidence against alternative hypotheses for mechanisms underlying the poor food quality of P-deficient algae for *Daphnia* (e.g., Sundbom and Vrede 1997; Urabe et al. 1997; Weers and Gulati 1997; DeMott 1998; DeMott et al. 1998). These studies also point to methods for manipulating the quality of natural seston to provide direct experimental tests of the mechanism(s) by which food quality may constrain *Daphnia* growth and abundance.

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