

Empirical analysis of the effect of phosphorus limitation on algal food quality for freshwater zooplankton

Michael T. Brett¹

Department of Civil and Environmental Engineering, Box 352700, 301 More Hall, University of Washington, Seattle, Washington 98195-2700

Dörthe C. Müller-Navarra and Sang-Kyu Park

Department of Environmental Science and Policy, University of California, Davis, California 95616

Abstract

Herbivorous zooplankton production is often constrained by algal food quality, and variation in algal food quality has been attributed to its phosphorus (P) content. To test the hypothesis that mineral P limitation is an important constraint on freshwater herbivorous zooplankton production, we conducted a quantitative analysis of published data on lake seston carbon to phosphorus (C:P) ratios and daphnid growth responses to P-limited algal diets. Our summary of 276 observations of mean lake seston C:P ratios showed 38% exceeded a critical threshold of 300, above which daphnid production is often predicted to be limited by algal P content. Our analysis of 91 observations of daphnid growth responses to nutrient-limited algal diets indicates the true daphnid critical threshold lies within the range of 225–375. Algal food quality is consistently reduced at algal C:P ratios above 300; however, the predicted reduction in algal food quality with P limitation is greater than the observed reduction at critical thresholds <390. Mineral P limitation of *Daphnia* production may be quite strong in some lakes; however, the production of most zooplankton taxa would not be expected to be limited by mineral P in a large proportion of lakes. After correcting for the algal C:P ratio, large differences in food quality were found between the major algal taxa, with diatoms and cryptophytes high food quality, chlorophytes intermediate food quality, and small ingestible cyanophytes very low food quality. A Monte Carlo simulation indicated that shifts in algal community composition similar to those seen during seasonal succession or across lake trophic state gradients explained four times more variability in daphnid growth rates across a range of seston C:P ratios representative of natural lakes. Overall these results tend to support the mineral P limitation hypothesis for daphnids, but they also suggest other factors will be the primary determinants of algal food quality for most zooplankters in most lakes.

“The question of existence of food quality constraints in some places for some zooplankton is no longer the chief question. Attention now turns to the more sophisticated questions about when and where food quality constraints are important.”

Sterner (1997)

It is believed that algal mineral P content limits the production of certain herbivorous zooplankton (Hessen 1992; Urabe and Watanabe 1992; Elser and Hassett 1994; Sterner 1997). This hypothesis was generated from the observation that zooplankton elemental ratios for carbon (C), nitrogen (N), and phosphorus (P) are nearly constant (Andersen and Hessen 1991; Sterner and Hessen 1994), while the nutrient content of lake seston can be quite variable. *Daphnia* are particularly rich in P with molar C:P ratios of ≈ 80 –90 (Sterner and Hessen 1994). Lake seston on the other hand can have C:P elemental ratios of 50–1,000. Given a scenario where the zooplankton's relative P content is much higher

than the seston's, one could expect zooplankton production to be strongly limited by seston P availability (Hessen 1992; Urabe and Watanabe 1992). Several researchers have suggested the most likely critical seston C:P ratio above which daphnid production will be limited by seston P content is ≈ 300 (Urabe et al. 1997; Sterner 1997, 1998). However, critical thresholds ranging from 90 to 385 have also been suggested (Hessen 1992; Elser and George 1993; DeMott et al. 1998; MacKay and Elser 1998).

The zooplankton mineral P limitation hypothesis has generated some debate and much research during the last 5 yr. It is now possible to conduct quantitative tests of the mineral P limitation hypothesis because a substantial number of empirical tests of its predictions have been conducted. Seven large-scale surveys of lake seston C:P ratios have been published and 10 studies have examined the food quality of nutrient-limited algae for *Daphnia*. This amounts to 276 observations of lake seston C:P ratio and 91 observations of daphnid growth response to nutrient-stressed algae. This investigation will quantitatively analyze these studies and draw inferences about the relative importance of mineral P limitation for freshwater zooplankton.

Methods

Data collection—Lake seston C:P ratio: The seven large-scale surveys of mean lake seston C:P ratios were published

¹ Corresponding author.

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Table 1. Mean molar C:P ratios for various lakes.

Lake	C:P elemental ratio	Sample dates (averaged)	Source
Lake Begnas	335	4	Nakanishi et al. (1988)
Lake Biwa	228	5	Urabe et al. (1995)
Blelham Tarn	87	64	Reynolds (1996)
Castle Lake	792	6	Elser and George (1993)
Lake Erken	145	20	Ahlgren et al. (1997)
Funada-ike pond	142	24	Urabe (1995)
Lake Gardsjon	366	63	Broberg (1987)
Lake Huron	230	22	Johengen et al. (1995)
Lake Lacawac	498	8	Kreeger et al. (1997)
Lake Muhazi	243	15	Mukankomeje et al. (1993)
Lake Njupfatet	266	26	Blomqvist et al. (1995)
Lake Norman	590	9	Rodriguez (1985)
Lake Okaro	102	3	Lean et al. (1989)
Lake Phewa	496	3	Nakanishi et al. (1988)
Pyramid Lake	200	12	Reuter et al. (1993)
Rimov Reservoir	196	15	Vrba et al. (1995)
Lake Rupa	232	2	Nakanishi et al. (1988)
Lake Stora Hastevatten	390	100	Broberg (1987)
Lake Superior	190	186	Halfon (1984)
Lake Tjeukemeer	86	130	DeHaan and De Boer (1986)
Tuesday Lake	567	9	Vanni et al. (1997)
Lake Waynewood	90	8	Kreeger et al. (1997)

by Shortreed and Stockner (1986), Fee et al. (1989), Hessen (1992), Hecky et al. (1993), Guildford et al. (1994), Elser and Hassett (1994), and Sterner et al. (1997). We also located an additional 22 observations of natural lake C:P ratios published in a large number of studies (Table 1). The great majority of these data are for epilimnetic seston samples, and a few are averages for the entire water column. Most of these data are averages of multiple observations, but the data from Fee et al. (1989) and Elser and Hassett (1994) are for single sampling dates collected from the epilimnion during the summer. When it was obvious that a lake was listed multiple times by different authors, for example when two studies listed the lake by name, we did not include the older record of the lake's C:P ratio in our analysis. In some cases we suspected that the same lakes were listed multiple times; however, they were not listed in such a way that we could distinguish them. For example, both Elser and Hassett (1994) and Sterner et al. (1997) probably included the same high seston C:P lakes from the Experimental Lakes Area (ELA). In these cases, we included both sets of observations in our analysis. We believe our selection process was conservative and tended to slightly overestimate the incidence of high C:P lakes in the published literature.

We also compiled the published determinations of daphnid somatic phosphorus and carbon content, as well as molar C:P ratios (Table 2).

Daphnia growth on phosphorus-limited algae: Ten recently published studies have been specifically designed to assess the effect of phytoplankton nutrient limitation on its food quality for *Daphnia* (Sterner et al. 1993; Sterner and Hessen 1994; Sterner and Robinson 1994; Müller-Navarra 1995a; Kilham et al. 1997a; Lürling and Van Donk 1997;

Urabe et al. 1997; Van Donk et al. 1997; Weers and Gulati 1997b; DeMott 1998). We conducted a meta-analysis of these experimental results to compare the magnitude of the observed reduction in algal food quality with increasing algal C:P ratios to the theoretically expected reduction in algal food quality with increasing C:P ratios. This analysis also allowed us to compare the relative magnitude of food quality differences between alga taxa to the depression in algal food quality with increasing C:P ratios.

In accordance with the prediction that *Daphnia* production will be limited by seston P content at C:P ratios >300, we normalized the results within each experiment to all phytoplankton treatments with C:P ratios <300. If a study had three treatments (nutrient-saturated, -N, and -P) with growth rates of 0.40, 0.35, and 0.20, respectively, and algal C:P ratios of 200, 150, and 800, respectively, then the -P treatment would be normalized to the mean of the nutrient-saturated and -N treatments. This process would yield normalized values of 1.07, 0.93, and 0.53, respectively. Because the actual critical threshold is not known, we also normalized the experimental data to hypothetical critical ratios of 200 and 400, according to the procedures outlined above. These normalized values allowed us to directly compare a variety of studies, each of which may have been run at different temperatures, used a different *Daphnia* species, or employed different experimental devices. Each of the studies summarized in this analysis presented their results as the instantaneous rate of change in individual numbers or biomass, which give analogous results (Lampert and Trubetskova 1996), or these parameters could be directly derived from the original data. This resulted in 91 cases where daphnid growth could be compared across a gradient of phytoplankton C:P ratios.

Table 2. Published values for daphnid phosphorus content (P mass/dry weight) and C:P ratio (molar). The average estimate of $45.7 \pm 2.0\%$ carbon (C mass/dry weight) for *Daphnia* spp. was obtained from individual values reported by Andersen and Hessen (1991), Hessen and Lyche (1991), Urabe and Wantanbe (1992), DeMott (1998), and Sterner and Schulz (1998). $n = 8$.

<i>Daphnia</i> taxa	% P of dry wt	C:P ratio	Source
<i>D. cucullata</i>	1.58	72	Hessen and Lyche (1991)
<i>D. galeata</i>	1.11	106*	Langeland et al. (1985)
<i>D. galeata</i>	1.11	109	DeMott (1998)
<i>D. galeata</i>	1.35	87	Urabe and Watanabe (1992)
<i>D. hyalina</i>	1.12	106*	Baudouin and Ravera (1972)†
<i>D. hyalina</i>	1.66	71*	Vijverberg and Frank (1976)†
<i>D. longispina</i>	1.47	85	Andersen and Hessen (1991)
<i>D. longispina</i>	1.55	78	Hessen and Lyche (1991)
<i>D. lumholtzi</i>	1.06	112*	Maine et al. (1997)
<i>D. magna</i>	1.42*	83	Sterner and Schulz (1998)
<i>D. magna</i>	1.01	106	DeMott (1998)
<i>D. magna</i>	1.02	116*	Main et al. (1997)
<i>D. magna</i>	1.56	75	DeMott et al. (1998)‡
<i>D. obtusa</i>	1.24	98*	Sterner et al. (1993)
<i>D. obtusa</i>	1.45	82*	Main et al. (1997)
<i>D. pulex</i>	1.53	77*	Birge and Juday (1922)†
<i>D. pulex</i>	1.25	95*	Lehman (1980)†
<i>D. pulex</i>	1.53	77*	Langeland et al. (1985)†
<i>D. pulicaria</i>	0.81	149	DeMott (1998)
<i>D. rosea</i>	1.80	66*	Peters and Rigler (1973)†
Mean \pm 1 SD	1.33 \pm 0.26	93 \pm 20	

* Calculated assuming a mean carbon content of 45.7% of dry weight.

† Cited in Andersen and Hessen (1991).

‡ Although DeMott et al. (1998) originally reported a C:P ratio of 55, that value was based on a calculation error (W. R. DeMott pers. comm.).

When investigators used multiple *Daphnia* spp. (e.g., DeMott 1998), we normalized the data by individual species. When multiple food concentration levels were used, we used the highest food concentration reported by the authors. We used treatment means for our analyses. For Sterner et al. (1993), the daphnid growth rates were interpolated for the 3 mg L⁻¹ food level from their figure 6, and the algal C:P ratios were obtained from their Table 2. For Sterner and Hessen (1994), the daphnid dry weights and algal C:P ratios were interpolated from their figure 5. The *Daphnia* dry weights at age 5 d were converted to instantaneous growth rates by assuming a neonate weight of 1.9 μ g individual⁻¹ from Sterner (1993). For Sterner and Robinson (1994, p. 1231), the daphnid growth rates were calculated according to the regression formula provided for a food concentration level of 0.25 mg L⁻¹, and the algal C:P ratios were taken from their table 1. For Müller-Navarra (1995a), the daphnid growth rates were obtained by using the regression equations for growth provided in her figure 1 and averaging the growth rates obtained for algal carbon concentrations of 0.2, 0.3, and 0.4 mg L⁻¹; the algal C:P ratios were obtained from her table 1. For Kilham et al. (1997a), the daphnid growth rates were taken as the average of the high ration growth rates for days 8, 10, and 12 from their table 1. The results for the nitrogen and phosphorus limitation experiments were normalized separately. The algal C:P data was taken from Kilham et al. (1997b, table 2). For Lüring and Van Donk (1997), the daphnid growth rates were taken directly from

their table 4, and the algal C:P ratios were taken from their table 1. For Urabe et al. (1997), the daphnid growth rates were interpolated from their figures 3, 4, and 5, and the algal C:P ratios were taken from their table 1. For Van Donk et al. (1997), the daphnid growth rates were taken from their table 4, and the algal C:P ratios were taken from their table 1. For Weers and Gulati (1997b), the daphnid growth rates were taken from their table 4, and the algal C:P ratios were taken from their table 3. For DeMott (1998), the daphnid growth rates and algal C:P ratios were taken from his tables 2, 3, and 4.

Expected growth rates: We compared normalized growth rates to growth rates predicted by the most commonly used stoichiometric mass balance model (Hessen 1992; Urabe and Watanabe 1992; Brett 1993). The basic mass balance equation relating zooplankton carbon and phosphorus gross growth efficiency to its own C:P ratio and the C:P ratio of its food is

$$\frac{K_C}{K_P} = \frac{C:P_Z}{C:P_S} \quad (1)$$

K_C is carbon growth efficiency (growth in C/consumption in C), K_P is zooplankton phosphorus growth efficiency, $C:P_Z$ is the zooplankton C to P elemental ratio, and $C:P_S$ is the seston C to P elemental ratio.

The critical seston $C:P_S^*$ ratio above which zooplankton growth will be limited by P availability can be calculated as

$$\text{critical C:P}_s^* = \text{C:P}_z \cdot \frac{K_p}{K_c} \quad (2)$$

At seston C:P ratios greater than the critical threshold potential, zooplankton growth will be linearly related to seston P content. For example, if the true critical threshold is 300 and the seston has a C:P ratio of 600, then the expected growth rate will be 50% of the theoretical maximum for that zooplankter. Similarly, if the seston has a C:P ratio of 1,200, the expected growth rate will be 25% of the theoretical maximum rate. Thus, the relative predicted growth rate at any seston C:P ratio greater than the predicted critical threshold can be calculated accordingly.

$$\text{relative predicted growth} = \frac{\text{critical C:P}_s^*}{\text{seston C:P}_s} \quad (3)$$

According to this approach, the predicted growth at seston C:P ratios below the critical threshold is equal to 1.

Data analyses—Critical thresholds, fit, and bias: Because the actual critical threshold is not known and is the subject of much discussion, we compared predicted to observed growth rates for a range of potential critical thresholds spanning 100–500. This range includes all previously suggested critical thresholds for daphnid zooplankters. We also used observed growth rate values that had been normalized to three potential critical thresholds (i.e., 200, 300, and 400) for these sensitivity analyses. The predicted growth rate is a direct function of the putative critical threshold and the seston or algal C:P ratio (see Eq. 3). Thus, it was not necessary to conduct these sensitivity analyses using the full range of parameters used to actually calculate the critical threshold because we explored the whole range of biologically feasible thresholds. In these sensitivity analyses, the suitability of the various predicted critical thresholds was judged based on two criteria: fit and bias. Fit was calculated as the regression coefficient (r^2) between the predicted and observed growth rates, and bias was calculated as the mean difference between the predicted and observed growth rates.

Because several researchers (Ahlgren et al. 1990; Brett and Müller-Navarra 1997) have pointed out that there are often large differences in algal species food quality, which may be independent of the P content (Müller-Navarra 1995a), we also examined these experimental data to see if there were consistent residual differences in algal taxa food quality once algal P content was accounted for.

Phosphorus limitation of zooplankton communities: Once we empirically derived a range of plausible daphnid critical thresholds, we used these data to generate critical thresholds for other zooplankton taxa. We accomplished this by solving Eq. 2 above for the K_p/K_c ratio using our empirically derived critical thresholds for daphnids and the distribution of published *Daphnia* C:P ratios. We used the observed variability in our estimates of the experimentally derived critical threshold to randomly generate 1,000 observations of the daphnid critical threshold. Similarly, we used the published variability in daphnid somatic C:P ratios to randomly generate 1,000 observations of the daphnid C:P ratio. We then used these two sets of numbers to generate 1,000 estimates of the

K_p/K_c ratio according to Eq. 2, or $K_p/K_c = \text{critical C:P}_s^* / \text{C:P}_z$. Once we established these daphnid K_p/K_c ratios, we applied these ratios, known zooplankter somatic C:P ratios (Sterner and Hessen 1994), and Eq. 2 above to derive critical thresholds for common nondaphnid zooplankters. Once we established these critical thresholds, we calculated theoretical lost zooplankton community production due to mineral P limitation across a range of realistic seston C:P ratios and zooplankton community taxonomic composition. These calculations were repeated 1,000 times (i.e., once for each K_p/K_c ratio). Lost zooplankton production for each zooplankton taxa at each observed lake seston C:P ratio was calculated accordingly.

$$\text{lost production} = 1 - \text{predicted growth} \quad (4)$$

We used the distribution of published lake seston C:P ratios obtained in our compilation of published data to represent natural seston C:P ratios. We used the data of Hessen (1992) and Hessen et al. (1992) for 45 Norwegian lakes to characterize a “typical” temperate crustacean zooplankton assemblage. This assemblage was assumed to be comprised of 20% *Daphnia*, 15% *Diaphanosoma*, 15% *Holopedium*, 15% *Bosmina*, 10% *Cyclops*, and 25% *Diatomus*. To test whether using the Hessen and colleagues data biased our total zooplankton calculations, we also used the zooplankton community biomass data from Hassett et al.’s (1997) survey of 34 North American lakes to characterize a “typical” zooplankton assemblage. Hassett et al. (1987) reported a higher proportion of *Daphnia* (25%) and *Cyclops* (27%) and a lower proportion of *Diaphanosoma* (6%), *Holopedium* (9%), and *Bosmina* (3%) relative to Hessen and colleagues. Total lost community production was calculated as the mean lost production of a matrix of 276 lake seston ratios and 100 zooplankters having the species compositions described above.

Algal P content and taxonomic affiliation effects on food quality for *Daphnia*: Using parameters derived from the previously described analyses of experimental determinations of *Daphnia* growth on nutrient-stressed algae, we conducted a Monte Carlo simulation of the relative importance of algal C:P ratio and taxonomic affiliation for its food quality for *Daphnia*. The objective of this simulation was to answer the question: “Is the taxonomic affiliation or the C:P ratio of algae more important in determining its food quality for *Daphnia*?” Because the experimental data greatly overestimate the true extent of variability in lake seston C:P ratios and are strongly biased in favor of green algae, it was not possible to determine the relative importance of algal P content and taxonomic affiliation from direct analysis of these data (Day and Quinn 1989). This could only be done for a data set with representative numbers of observations for the major algal taxa, as well as a range of algal C:P ratios representative of natural lakes.

For the Monte Carlo simulations we started with the observed frequency distribution of lake seston C:P ratios obtained in our summary of published data. We then generated separate data sets for each of the major algal taxonomic categories at each lake seston C:P ratio and assigned each of these algal taxonomic categories mean values equal to their

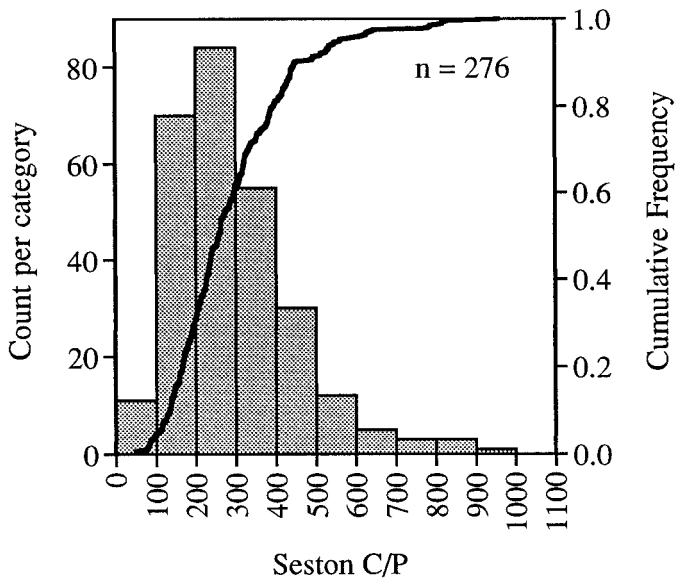


Fig. 1. A frequency histogram and cumulative frequency curve of all published lake seston C:P ratio data, $n = 276$. The mirror image of the cumulative frequency curve gives the proportion of lakes with C:P ratios above a specified critical threshold. In a few cases, data may have been listed more than once in this figure because some authors compiled lists of lake seston C:P ratios without identifying the lakes by name. These data are presented as elemental molar ratios.

mean residual food quality. We then applied a phosphorus limitation correction to the expected daphnid relative growth rate by applying the overall observed slope between algal C:P and normalized experimental daphnid growth rates for the whole distribution of observed lake seston C:P ratios. The stochastic term in these simulations was the mean square error between the predicted and observed algal food quality after accounting for residual differences in algal food quality between the main algal taxa. All data were generated using the random number generator routine of Microsoft Excel®. The results of these simulations were analyzed using analysis of covariance (ANCOVA) with the algal taxonomic groupings as categorical variables and the expected depression in algal food quality with its C:P ratio as a covariate. The relative strength of algal C:P ratio and taxonomic affiliation as determinants of food quality for *Daphnia* was determined by variance partitioning within the ANCOVA.

We conducted these Monte Carlo simulations for three different scenarios. The first scenario compared the importance of algal C:P ratio and taxonomic affiliation with the observed range of natural lake seston C:P ratios and an algal species composition that varied between 100% diatoms, to 100% cryptophytes, to 100% chlorophytes, to 100% cyanophytes. The second scenario only considered cases where the natural lake seston C:P ratio exceeded the putative critical threshold, but with the phytoplankton composition still varying from 100% dominance by one taxonomic grouping to 100% dominance by another algal group. The third scenario relaxed the unrealistic assumption that phytoplankton communities shift from complete dominance by one group to complete dominance by another group. This scenario used

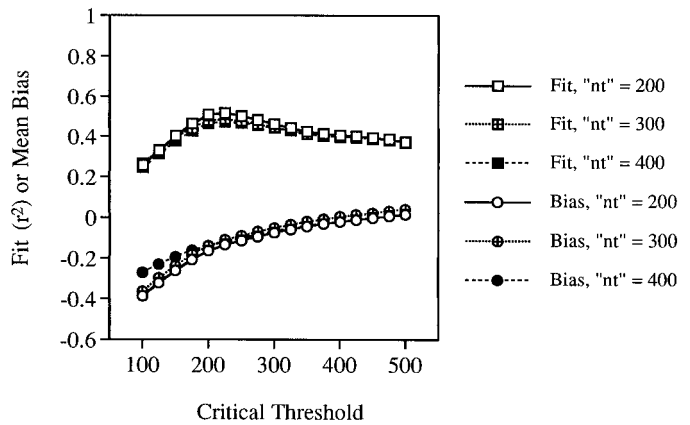


Fig. 2. The fit and bias for comparisons of predicted versus observed declines in algal food quality due to mineral P limitation, $n = 91$. Fit and bias have been calculated for a range of potential critical thresholds according to standard mass balance models. Fit was calculated as the regression coefficient between the predicted and observed food quality. Mean bias is calculated as the average difference between the predicted and observed normalized food quality accordingly: bias = predicted - observed food quality. Fit and bias were both calculated using three different critical thresholds (200, 300, and 400) when initially normalizing the experimentally determined daphnid growth rates. NT, normalized threshold.

the observed range of lake seston C:P ratios, but varied the phytoplankton species composition from 80% diatoms and cryptophytes and 20% other phytoplankton, to a mixed algal assemblage, and finally to 80% cyanobacteria and 20% other phytoplankton. This third scenario is more representative of typical algal seasonal succession or of the type of shifts in species dominance typically seen across lake trophic state gradients. All of these Monte Carlo simulations were for zooplankton communities comprised exclusively of *Daphnia*.

Results

The natural lake seston and daphnid C:P ratios—A frequency distribution of published lake seston C:P ratios shows the average is 292 (median = 261, Fig. 1). According to 20 published values (Table 2) *Daphnia* spp. had an average phosphorus content of $1.33 \pm 0.26\%$ (as P mass/dry weight), an average carbon content of $45.7 \pm 2.0\%$ (as C mass/dry weight) and an average molar C:P ratio of 93 ± 20 .

Predicted versus observed food quality—We tested the fit and bias of predicted versus observed algal food quality across a wide range of hypothetical critical thresholds. This sensitivity analysis showed that the optimal fit was obtained at a critical threshold of 225, whereas bias was equal to zero at a critical threshold of 390 (see Fig. 2). Figure 3 shows a plot of observed versus predicted algal food quality at a critical threshold of 300, the intermediate point between optimal fit and minimal bias. We also conducted these sensitivity analyses using three different critical thresholds when normalizing the data; however, this had a very small effect

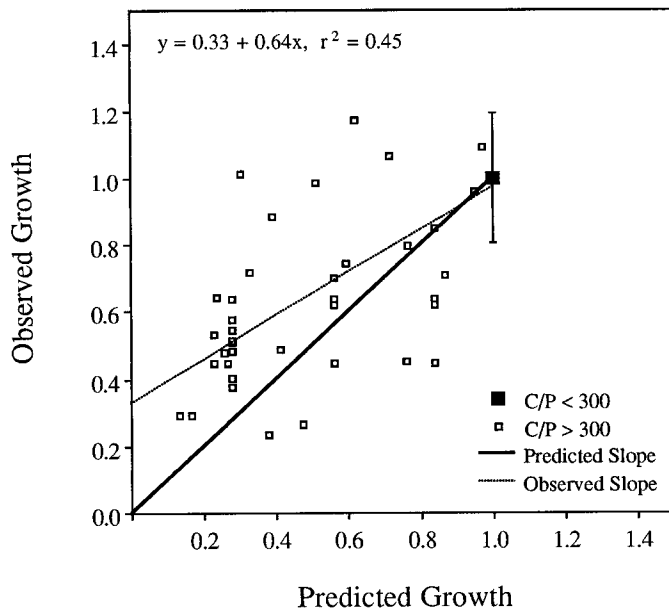


Fig. 3. Comparisons of predicted versus observed food quality for a critical threshold of 300, which is approximately at the midpoint of range of critical thresholds spanning optimal fit and minimal bias. The cases where the phosphorus content of the algae were nonlimiting algae (e.g., C:P < 300) have been summarized as one point with the confidence interval representing ± 1 SD. The 1:1 slope (in bold) represents the theoretically predicted slope for these data.

on the normalized growth rate values as well as fit or bias (Fig. 2). When a low critical threshold of 200 is used, the mass balance model strongly overestimates the actual decline in algal food quality with declining P content. In 81% of the 52 cases where the algal C:P ratio was greater than the hypothetical threshold of 200, the mass balance model overestimated the observed decline in algal food quality. Conversely, there was very little bias when using a critical threshold of 400, but there was a substantial decline in fit. Furthermore, the pattern to the fit was poor: the observed food quality was clearly somewhat depressed below the critical threshold of 400. For all critical thresholds, the observed decline in algal food quality at very high algal C:P ratios (>1,000) was substantially less than predicted for algae with these extreme carbon to phosphorus ratios.

Algal taxa food quality—The database available for these analyses is strongly biased toward experiments examining daphnid growth in nutrient-limited green algae (70 of the 91 summarized treatments used green algae), and especially the green alga *Scenedesmus acutus* (54 of 91 cases). Fortunately, there are 21 cases within this database where experiments were run with cyanophytes, cryptophytes, and diatoms (i.e., seven cases each). To test whether there were consistent food quality differences between these major algal taxa, we examined the residuals for the observed regression in Fig. 3 using a critical threshold of 300. The growth of daphnids feeding on diatoms and cryptophytes was about $25 \pm 13\%$ (± 1 SD) higher than for daphnids consuming green algae. The growth of daphnids feeding on cyanophytes was about

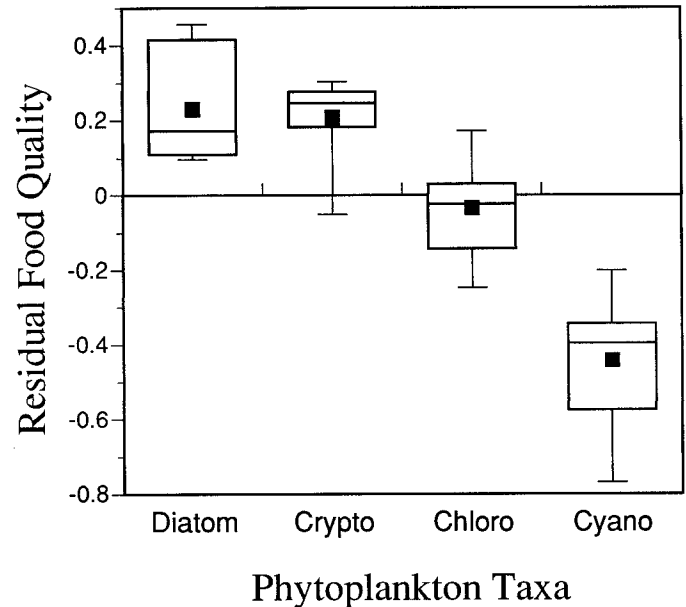


Fig. 4. The normalized food quality for the diatom ($n = 7$), cryptophyte ($n = 7$), cyanophyte ($n = 7$), and green ($n = 70$) algae. The normalized food quality is calculated as the residuals of the “observed slope” from Fig. 3 for experiments using *Daphnia*. Diatom, cryptophyte, and cyanophyte growth responses were all significantly different from the green algae growth responses ($P < 0.001$). The margins of the boxes enclose the 25th and the 75th percentiles, and the whiskers enclose the 10th and 90th percentiles.

$41 \pm 18\%$ lower than that for green algae (Fig. 4). These differences in diatom, cryptophyte, and cyanophyte residual food quality were all statistically significant compared to chlorophyte food quality.

To test whether our data treatment procedures influenced these residuals, we examined their magnitude while varying both the critical threshold assumed when normalizing the data and while calculating the predicted algal food quality. For this analysis, we varied the critical threshold from 200, 300, and 400. This sensitivity analysis showed that these transformations had virtually no effect on the residuals (M. T. Brett unpubl. data).

Because the apparent differences in algal taxa food quality were large, we also conducted our previously discussed sensitivity analyses of P limitation effects on algal food quality (see Fig. 2) after accounting for these differences. This was done by subtracting the mean residual difference in algal taxa food quality (relative to chlorophytes) from each of the normalized observed growth rates. This showed that between algal taxa, differences in food quality were an important source of scatter in our prior analysis; fit was improved by approximately 0.20 across the range of critical thresholds tested. Because these algal taxa residuals had an overall mean of zero, this correction had no effect on bias.

Phosphorus limitation constraints on zooplankton production—Because our sensitivity analysis indicates the most plausible daphnid critical threshold lies within the range of 225–375, when randomly generating critical threshold values for calculations of lost production, we assumed 300 ± 75

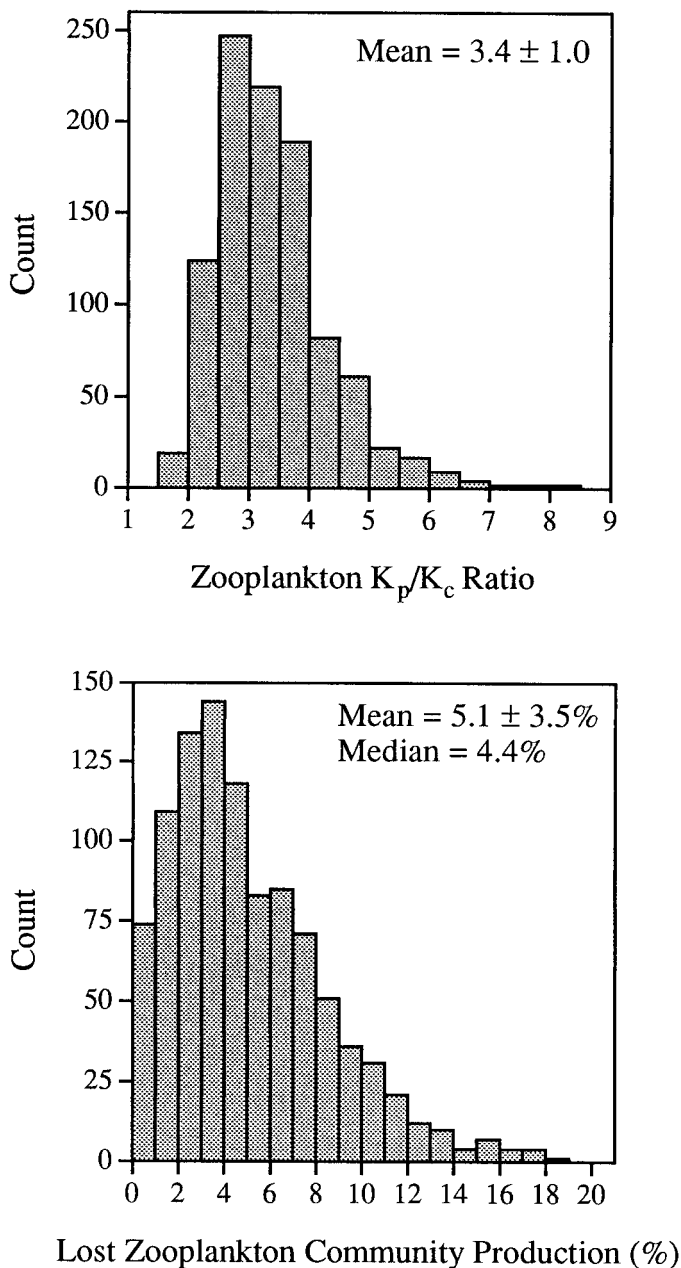


Fig. 5. Frequency histograms for our calculation of the average lost zooplankton community production due to mineral P limitation. The upper panel is our calculated range of likely daphnid K_p/K_c ratios. The lower panel is our estimate of lost zooplankton production derived using critical thresholds specific to zooplankton taxa, the observed distribution of natural lake C:P ratios in our Fig. 1, and "typical" zooplankton community composition. The sample size for these simulations was 1,000.

equaled the 95% confidence interval for these data. As reported in Table 2, we found the 95% confidence interval for daphnid somatic C:P ratios was 93 ± 39 . When we used randomly generated numbers with these distributions to calculate daphnid K_p/K_c ratios, we obtained a mean ± 1 SD of 3.4 ± 1.0 (see Fig. 5).

Figure 5 also presents the lost zooplankton community

production results for this range of K_p/K_c ratios. It is important to emphasize that variation in these results is driven solely by variation in our estimates of the K_p/K_c ratio used when generating individual zooplankton taxa critical thresholds. Variation in the K_p/K_c ratio was, in turn, a function of the daphnid critical thresholds and the daphnid somatic C:P ratios assumed in our calculations. Because zooplankters with much higher P gross growth efficiencies than carbon gross growth efficiencies will be less susceptible to P limitation, the K_p/K_c ratio was inversely related to our estimate of lost zooplankton community production. It should, however, be emphasized that for these calculations, we have assumed that daphnid-derived K_p/K_c ratios are representative of K_p/K_c ratios other zooplankton taxa might have. Our estimate of overall lost community production due to mineral P limitation was moderately skewed with a mean of $5.1 \pm 3.5\%$ (± 1 SD) and a median of 4.4%. We obtained very similar results when we instead used the data of Hassett et al. (1998) to characterize the crustacean zooplankton community structure (i.e., mean = $4.7 \pm 3.1\%$; median = 4.1%).

As previously mentioned, these lost zooplankton community production values were calculated as the average lost production for a matrix of 276 lake seston ratios and 100 zooplankters having the species compositions described by Hessen et al. (1992) or Hassett et al. (1997). The generality of mineral P limitation for zooplankton communities can be further examined by considering the proportion of this matrix where no limitation was predicted to occur. At a K_p/K_c ratio of 3.4, fully 85% of the 276 lake seston values by 100 zooplankton taxa matrix had zero lost production. These results suggest mineral P limitation of typical zooplankton production should be more the exception than the rule.

Although there have been few direct determinations of K_p , DeMott et al. (1988) is the only researcher to have done this recently, we can use our estimates of daphnid K_p/K_c ratios to estimate likely *Daphnia* K_c values for the growth rate experiments summarized in this study. If we assume the maximum K_p is 0.8, as DeMott et al. (1998) found, then K_p/K_c ratios of 2.4 and 4.4 would correspond to K_c values of 0.33 and 0.18, respectively. Thus, if we assume an average K_p/K_c ratio of 3.4 ± 1.0 and a $K_p \leq 0.8$, then we must conclude that the daphnids used in these experiments had rather low carbon efficiency.

Algal P content, taxonomic affiliation, and food quality—For each of the 276 lake seston C:P observations depicted in Fig. 1, four observations (for diatoms, cryptophytes, chlorophytes, and cyanophytes) were randomly generated. For the diatom, cryptophyte, chlorophyte and cyanophyte generated data sets, means of 1.23, 1.21, 0.97, and 0.56, respectively, were used when generating the data. These mean values are equal to one plus the residuals depicted in Fig. 4. In all cases, SD = ± 0.15 ; that is, the mean square error for these data was specified when generating data. Once these data were generated, each of the four observations at each of the 276 lake seston C:P ratios was corrected for the observed reduction in food quality with increasing C:P ratio, as depicted in Fig. 3 for a critical threshold of 300 and described by the appropriate regression formula. This gave a P limitation food quality correction equivalent to the empir-

Table 3. An analysis of covariance (ANCOVA) of the effect of algal taxonomic affiliation and C:P ratio on its food quality for *Daphnia*. The overall r^2 values for these ANCOVAs were 0.783, 0.789, and 0.699, respectively. These data were generated with a Monte Carlo simulation. SD between simulations refers to standard deviation in variance explained between simulations.*

Source	SS	df	MS	Variance explained (%)	SD between simulations (%)
<i>Daphnia</i> all seston					
Algal taxa	71.4	3	23.82	66.5	0.9
C:P ratio	12.7	1	12.70	11.8	0.8
Error	23.4	1100	0.021	21.7	0.8
<i>Daphnia</i> seston C:P > 300					
Algal taxa	20.2	3	6.73	63.2	1.6
C:P ratio	5.0	1	5.01	15.7	1.1
Error	6.7	416	0.016	21.1	1.4
Phytoplankton succession					
Algal taxa	35.2	2	17.62	56.3	1.2
C:P ratio	8.5	1	8.51	13.6	1.3
Error	18.8	825	0.023	30.1	1.7

* SS, sums of squares; df, degrees of freedom; ms, mean square.

ically observed decline in algal food quality with declining algal P content. For example, according to the empirically observed decline in algal food quality with its C:P ratio, an algal monoculture with a C:P ratio of 600 would be expected to have a food quality 32% lower (or =0.68) than an algal monoculture with a C:P ratio of 300. This final data set was balanced in terms of algal species composition, representative of natural lake seston C:P ratios, and based on the trends observed in our earlier analysis.

The results of these simulations (Table 3) show that algal taxonomic affiliation explained 5.6 times more variability in algal food quality than did the C:P ratio (i.e., 66% versus 12% for affiliation and C:P ratio, respectively). This conclusion is only modestly influenced when considering only cases where the seston C:P ratio exceeds 300 (e.g., cases where mineral P limitation is most likely). When we repeated this simulation five times using only the C:P ratios for the 38% of lakes where the C:P ratio exceeded 300, taxonomic affiliation explained four times more variability than did algal C:P ratio in the ANCOVA. When we relaxed the unrealistic assumption that phytoplankton communities shift from 100% dominance by one major taxa to another, we still found algal taxonomic composition had a substantially larger effect on algal food quality. The food quality index of the spring bloom was calculated as the average food quality of diatoms and cryptophytes $\times 0.8$ + the food quality of "other" phytoplankton (i.e., 1) $\times 0.2$, which equals 1.18 ± 0.15 . The food quality of the early summer period was assumed equal to that for green algae, or 1.00 ± 0.15 . The food quality of the cyanobacteria-dominated phase was calculated as the food quality of cyanobacteria $\times 0.8$ + the food quality of "other" phytoplankton $\times 0.2$, which equals 0.65 ± 0.15 . This new simulation showed that phytoplankton "species succession" explained 4.1 times more vari-

ability in algal food quality for *Daphnia* than did the algal C:P ratio for *Daphnia*.

Discussion

Our summary of published lake seston C:P values shows approximately 38% of the lakes exceed a critical threshold of 300, above which Urabe et al. (1997) and Sterner (1997, 1998) have predicted daphnid production will be constrained by mineral P availability. If a critical threshold of 300 is reasonable, these results suggest fully 62% of all published lake seston C:P ratios were below a threshold where any zooplankton taxa would be predicted to be P-limited.

The results of our analyses, comparing predicted to observed changes in algal food quality with declining P content, clearly show phytoplankton food quality decreases as the C:P ratio of the algae increases above 300. These analyses also suggested a critical threshold of 300 is reasonable. This value was in the middle of a range of critical thresholds, which included values having optimal fit and minimal bias. Unfortunately, the results of our analyses of mass balance model fit and bias did not converge. The optimal fit, at a critical threshold of 225, was obtained for predictions that strongly overestimated the true extent of algal declines in food quality with mineral P limitation. Minimal bias at a critical threshold of 375 was obtained for predictions with poor fit.

Our results show mass balance calculations always overestimated the true reduction in the food quality of the most severely P-limited algae (C:P > 1,000). This result could be explained by generally low daphnid carbon gross growth efficiency or some degree of compensation (Brett 1993). Because all of the experiments summarized in our analysis used high algal concentrations relative to natural seston concen-

trations, it is possible that *Daphnia* simply consumed more low-quality food to compensate for the limited supply of P. This result could be an artifact of the very high algal concentrations typically used in these experiments.

Based on our sensitivity analyses of likely daphnid critical thresholds, observed daphnid somatic C:P ratios, and the distribution of published lake seston C:P ratios, we conclude that most lakes will not have P-limited seston for most zooplankton taxa. In fact at a K_p/K_c ratio of 3.4, we predict that only 15% of the zooplankton taxa by lake seston combinations will be subject to any mineral P limitation. These conclusions are diametrically opposed to those of Elser and Hassett (1994, p. 212), who used similar analyses to conclude "C:P ratios exceeded even the most conservative thresholds in the majority of our study lakes. Thus, P-limitation of zooplankton growth may be ubiquitous in lakes." Our analysis, although conceptually identical, differed from that presented by Elser and Hassett in a few critical details. First, we used a more comprehensive sample set of temperate lake seston C:P ratios (276 versus 36 lakes for Elser and Hassett). Second, we derived quantitative predictions from the model instead of purely qualitative tests. Third, we tested the mineral P limitation hypothesis for a variety of common zooplankton taxa and not just the most P-rich cladocerans.

One of the main studies included in this analysis (DeMott 1998) showed that direct P limitation did not account for about 33% of the observed decline in the food quality of P-stressed *Scenedesmus acutus*. DeMott (1998) cultured five daphnid species on P-saturated (C:P ratio = 225), P-stressed (C:P = 1,070) and P-amended *S. acutus* (C:P = 180). The P-amended treatment consisted of P-stressed algae with a short-term phosphate enrichment that restored cell P content but probably had a minimal effect on other cell physiological characteristics. This short-term P amendment only ameliorated $67\% \pm 7\%$ (± 1 SD) of the overall food quality difference between P-saturated and P-stressed *S. acutus*. This result suggests that reduced food quality of severely P-stressed *S. acutus* cannot be solely attributed to its P content. The cell wall indigestibility hypothesis (Van Donk and Hessen 1993; Van Donk et al. 1997) is one potential cause of this unexplained decline in algal food quality.

All of these calculations are for an elemental mass balance model that assumes food quality constraints are independent of food quantity (Hessen 1992; Urabe and Wantanabe 1992; Brett 1993). Sterner has shown theoretically (Sterner 1997) and experimentally (Sterner and Robinson 1994) that elemental food quality constraints are reduced at lower food concentrations. This occurs because at lower food concentrations an increasing proportion of the food consumed is utilized to support basal metabolism and is therefore not used for growth. We used the model presented in Sterner (1997) to test whether food quantity limitation might theoretically lessen P limitation in the 276 lakes depicted in our Fig. 1. We were, however, only able to obtain food quantity data for 227 of the 276 lakes depicted in this figure. We carried out our calculations for the case of a zooplankton community with a K_p/K_c ratio of 3.4 and the zooplankton community composition described by Hessen and colleagues (1992). We calculated that after adjusting for food quantity constraints, food quality constraints were on average 61% as

strong as calculated using the more traditional food quantity independent models; that is, food concentrations were sufficiently low in many of the lakes depicted in Fig. 1 to lessen food quality limitation by 39%.

Our observation that, after accounting for algal P content, diatoms and cryptophytes are higher food quality than green algae, which are in turn are higher food quality than cyanophytes (see Fig. 4), is qualitatively similar to the predictions of the highly unsaturated fatty acid (HUFA) hypothesis (Ahlgren et al. 1990; Müller-Navarra 1995b; Brett and Müller-Navarra 1997; Müller-Navarra et al. 2000). This hypothesis predicts algal taxonomic differences in food quality due to differences in their essential fatty acid content. When Weers and Gulati (1997a) added HUFA emulsions to cultures of *Scenedesmus acutus*, they obtained *Daphnia* growth rates similar to those observed for high food quality and HUFA-rich cryptophytes. Similarly, DeMott and Müller-Navarra (1997) found that adding HUFA emulsions to the HUFA-poor cyanophyte *Synechococcus* improved the food quality of this mixture to the level observed for *Scenedesmus acutus*. Thus, it is plausible that the large differences in food quality noted between the major algal taxa are due to differences in their HUFA content. However, very few of the studies summarized in this analysis actually presented fatty acid composition data, so direct comparisons of algal fatty acid content and food quality are not possible for this data set.

The results of our Monte Carlo simulations indicated that the observed differences in algal taxa food quality are substantially larger than one would expect to observe due to daphnid mineral P limitation across a range of natural lake seston C:P ratios. This result is also true when we consider only cases with seston C:P ratios > 300 and when we consider a scenario similar to typical algal community seasonal succession. Furthermore, the difference in average food quality between a diatom and/or cryptophyte versus a cyanobacteria-dominated algal community is greater than the observed decline in algal food quality with P limitation across the entire range of variability in seston C:P depicted in Fig. 1. These results are noteworthy because one of the most predictable responses of algal assemblages to nutrient stress is shifts in phytoplankton community species composition (Tilman 1982; Harris 1986; Sommer 1989; Lampert and Sommer 1997). It is also well known that the phytoplankton communities of meso- to hypereutrophic temperate lakes typically undergo a seasonal succession from spring dominance by cryptophytes and diatoms to an early summer dominance by chlorophytes, a late summer dominance by cyanophytes, which is in turn often followed by a fall diatom bloom (Wetzel 1983; Harris 1986; Sommer et al. 1986; Lampert and Sommer 1997). The differences in alga taxa food quality noted in this study suggest zooplankton will be limited by the food quality of phytoplankton communities whenever these communities are not strongly dominated by diatoms or cryptophytes, or other highly nutritious phytoplankters.

Although most of the studies included in our analysis were specifically designed to test the effect of algal P limitation on food quality, many also included N limitation treatments. These treatments allowed us to test the protein limitation hypothesis described by Kilham et al. (1997a) using the re-

sults of eleven experiments in which the authors included nutrient-saturated and N-limited treatments. This comparison showed that N limitation typically reduced algal food quality (\pm SD) by $23 \pm 13\%$ (paired t -test = -5.59 , $P = 0.0001$). Relative to other trends noted in this study, this is a strong response.

Biases in the database—Sixty-eight percent of the published natural lake C:P ratios presented in Fig. 1 were obtained from lakes located in Canada. In large part, this reflects the fact that Canada has one of the most active limnological research programs and that the Canadian Shield contains many of the world's lakes. To generalize about "typical" natural lake seston C:P ratios, one is forced for now to assume these lakes are representative of lakes in other regions of the world. This may not be true, but it is difficult to say whether this would upwardly or downwardly bias our estimate of typical lake seston C:P ratios.

The lake seston C:P ratios depicted in Fig. 1 may be positively biased because most values reported are for summer epilimnetic seston. Phytoplankton nutrient limitation is generally most pronounced in the epilimnion during the summer, and high seston C:P ratios are considered an index of phytoplankton nutrient limitation. Thus, it stands to reason that the seston C:P ratios are probably somewhat lower during the nonstratified conditions of fall, winter, spring, and especially below the epilimnion during the summer when nutrients are not limiting. It should also be noted that even when summer epilimnetic seston C:P ratios are high, most zooplankton have access to seston with lower C:P ratios below the epilimnion and can balance shortages in surface seston phosphorus content by consuming a mixture of seston of varying phosphorus content from different depths in the lake.

As we previously mentioned, another major bias in the available published data is that 59% of all the experimental observations of *Daphnia* growth on P-limited algae were obtained from experiments using *Scenedesmus acutus*. *S. acutus* is the proverbial "white rat" of zooplankton production studies because it is easy to culture and it is moderately high food quality for daphnids. However, there is no a priori reason to assume that this relatively rare alga in the wild (in terms of percent community biomass) is generally representative of all other algae that zooplankton are likely to feed on in natural lakes. In addition to this obvious problem of pseudoreplication, the observations for nongreen algae phytoplankters are also for only a limited number of taxa. This exacerbates the pseudoreplication problem and may bias this study's conclusion that there are strong differences in food quality between algae taxa.

Perhaps the most obvious bias in the currently existing literature on this topic is the nearly exclusive use of *Daphnia* as the model zooplankter. This is probably the case because *Daphnia* are very easy to culture compared to other common zooplankters, and theoretically speaking, they are the taxa most likely to be limited by the mineral P content of their diets. Few studies have examined this hypothesis for non-daphnid zooplankters (however, see Rothhaupt's (1995) study of the rotifer *Brachionus*, and DeMott's (1998) experiments with the cladoceran *Simocephalus*). Before the min-

eral P limitation hypothesis is proclaimed to be a general hypothesis for all freshwater zooplankton, as some authors have claimed (Elser and Hassett 1995), research on zooplankton taxa other than *Daphnia* will be needed.

The results of the Monte Carlo simulation hinge on certain assumptions. If these assumptions are not valid, then the predictions provided by this analysis may be false. These critical assumptions are: (1) the seston C:P ratio distribution shown in Fig. 1 is more or less representative of temperate lakes, (2) the observed decline in algal food quality with declining P content is generally true for all algae taxa, (3) the food quality differences between the algal taxonomic categories are generally true, and (4) $\pm 15\%$ is representative of the expected uncertainty in this type of analysis. All of these assumptions seem plausible because they were extracted directly from this database, but only future research will show whether they are true.

In addition, our conclusion that nondaphnid zooplankters will only be occasionally limited by the mineral P content of their diets hinges in large part on our assumption that the K_p/K_C ratios we derived from the daphnid experiments are representative of K_p/K_C ratios for nondaphnid zooplankters. At present, we are unaware of any data that support or refute this assumption.

Our study presents several seemingly strong results; however, all of the trends reported in this study may be partial artifacts of biases in the underlying data as well as of the assumptions used in our calculations. Only future research will show whether the trends discussed in this analysis are generally true.

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