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Grazer control of nitrogen fixation: synergisms in the feeding ecology of two freshwater crustaceans

K. D. Hambright[1,]*, N. G. Hairston Jr.[2], W. R. Schaffner[2] and **R. W. Howarth[2]**

With 5 figures, 1 table and 1 appendix

Abstract: Using in situ mesocosm experiments, we compared grazing rates, food-selection patterns and nutrient mineralization rates of a daphniid cladoceran and a diaptomid copepod to determine their influence on phytoplankton composition and the prevalence of N-fixing cyanobacteria. We show that grazing and nutrient mineralization may act synergistically within a grazer taxon. Cladocerans exhibited non-selective grazing and high N:P mineralization; copepods exhibited selective grazing and low N:P mineralization. Through such synergistic effects, freshwater systems dominated by copepods would be expected to be more vulnerable to blooms of N-fixing cyanobacteria than those dominated by cladocerans.

Key words: cladocera, copepod, grazing, cyanobacteria, mesocosms, nitrogen to phosphorus ratio.

Introduction

Cladocerans, especially large taxa like *Daphnia*, are considered non-selective filter feeders that accumulate all encountered particles small enough to fit between the ventral carapace gap, but large enough to be retained by the filtering apparatus – typically 0.5–40 µm diameter (Lampert 1987). Some selection may occur post-capture, such as the rejection of large particles just prior to ingestion (Bern 1990), though it is more typically believed that particle rejection is also non-selective, with an entire bolus of collected food containing objectionable algae rejected en masse (Kirk 1991).

In contrast, copepods, particularly in the Diaptomidae, are often characterized as highly selective grazers, typically processing phytoplankton cells or colonies individually (Burns & Hegarty 1994). As a result, copepods are thought to be capable of avoiding consumption of objectionable particles such as toxic or otherwise poor-quality cells, including many cyanobacteria. Such selectivity may also carry costs: for example, copepod feeding rates per individual have been reported to be relatively low compared with non-selective grazers like *Daphnia* (Muck & Lampert 1984).

The study of zooplankton grazing impacts on phytoplankton has been a central focus in limnology, particularly since Haney (1973) demonstrated that natural zooplankton assemblages were capable of "clearing" immense volumes of water free of phytoplankton daily (often exceeding the volume of a lake's epilimnion) and thereby in principle of consuming > 100 % of the lake's daily net primary production. The different feeding behaviors of cladocerans and copepods are often reflected in planktonic consumer-resource dynamics. During periods in which phytoplankton assemblages are dominated by small forms, such as is typical in north temperate lakes during spring mixis, large populations of *Daphnia* can greatly suppress phytoplankton, producing what is known as a spring clear-water phase (Lampert et al. 1986, Sommer et al. 1986). Such

[1] **Authors' addresses:** Program in Ecology and Evolutionary Biology, Biological Station and Department of Zoology, University of Oklahoma, Norman OK 73019, USA.

[2] Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, New York 14853, USA.

* Corresponding author; E-mail: dhambright@ou.edu

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decimation of phytoplankton by cladocerans is usually not possible in summer, when a broader array of species, including grazing resistant taxa, and larger cell sizes dominate (Sommer et al. 1986). Such assemblages, especially those containing abundant cyanobacteria, are generally considered poor food for generalist grazers like *Daphnia* (but see Epp 1996), and are better tolerated by selective grazers, like calanoid copepods. Likewise, copepods not only can persist under such conditions by consuming large phytoplankton selectively (Burns & Hegarty 1994), but can cause a shift in the phytoplankton assemblage back towards domination by smaller forms (Yoshida et al. 2001, Kagami et al. 2002). Thus, cladocerans and copepods may be considered complementary in their ecological impacts in lakes, each producing food conditions favorable to the other (Sommer et al. 2001, Sommer et al. 2003).

Grazing patterns alone, however, cannot reliably predict seasonal phytoplankton dominance and community composition (Lehman 1980a, Sommer et al. 1986, Kagami et al. 2002). Other factors must be involved. For example, phytoplankton population dynamics reflect a balance between growth potential and grazing mortalities, with phytoplankton growth varying, among other factors, as a function of nutrient availability, which is in turn affected by excretion and egestion of nutrients by grazing zooplankton.

Nutrient recycling by grazers can be a dominant source of nutrients supporting phytoplankton growth, often supplying a majority of the net daily requirements of primary production (Wen & Peters 1994). The importance of nutrient mineralization by zooplankton depends on a variety of environmental and biological factors, including temperature, light availability, and species and size composition of both phytoplankton and zooplankton. Hudson et al. (1999) demonstrated that the P mineralization rates increase linearly with lake total P concentration (a measure of lake trophic status) and P turnover (a measure of regeneration rate relative to the particulate P pool) is relatively constant at about 20 % daily across a range of lakes differing in trophic status. Elser (1999) suggested that even in systems receiving high external nutrient loads, nutrient regeneration by herbivores may determine nutrient availability to phytoplankton, not by increasing absolute availabilities, but by altering the relative availabilities of N and P, and thus altering competitive outcomes between different algal taxa with different nutrient requirements.

Differences in N : P mineralization ratios between copepods and cladocerans are thought to reflect grazer physiology, and in particular homeostatic control of tissue nutrient contents of herbivorous zooplankton (Sterner 1990, Sterner et al. 1992). Animals with relatively high N : P in their body tissue tend to return a lower N : P to the water than those with relatively low body tissue N : P. Support for homeostatic control of N and P in zooplankton has come principally from work showing that N and P are indeed relatively constant within individual zooplankton species, but that the tissue content of cladocerans is relatively enriched in phosphorus compared with copepods, while copepods are relatively enriched in nitrogen (e.g., Andersen & Hessen 1991). Thus, copepods by retaining high N : P should release low N : P to the water, whereas the opposite should be the case for cladocerans. Considerable evidence has accrued in the past decade in support of this "ecological stoichiometry" concept (Sterner & Elser 2002), but the extent to which differential recycling rates of N and P by grazers can impact ecological communities still remains to be elucidated.

Here we report results from a series of experiments in which we compared the feeding rates, food-selection patterns and nutrient mineralization rates of a diaphniid cladoceran with those of a diaptomid copepod. These studies were undertaken simultaneously with a larger replicated pond experiment designed to differentiate between the direct grazing and indirect nutrient-recycling impacts of cladocerans and copepods on pelagic ecosystem dynamics. Results of the pond experiment are presented by Hambright et al. (2007a). Our goal here was to understand better the roles of overall grazing mortality, selective-consumption, and nutrient mineralization (including stoichiometric effects) on phytoplankton taxonomic composition and how zooplankton taxonomic composition can mediate the phytoplankton response. If copepods are relatively selective grazers, then they should feed less on poor food such as N-fixing cyanobacteria and more on other taxa. If they mineralize nutrients at a low N : P, then this too would mean that their presence would lead to N-limiting conditions and so favor N-fixing cyanobacteria. In contrast, less-selective cladocerans should include cyanobacteria in their diets thus imposing mortality on cyanobacteria, and if they mineralize nutrients at a high N : P, taxa other than N-fixing cyanobacteria would be expected to benefit. Thus, through synergistic effects of direct grazing and nutrient mineralization, freshwater systems dominated by copepods would be expected to be more vulnerable to blooms of N-fixing cyanobacteria than those dominated by cladocerans. The goal of the research we report here is to quantify the relative magnitudes of grazer selectivity and differential N and P mineralization by typical calanoid

copepod and anomopod cladoceran species with the objective of understanding their potential impacts on the structure of phytoplankton assemblages.

Methods

Experimental design

We measured grazing and nutrient release rates of diaptomid copepods, mainly *Skistodiaptomus pallidus*, and cladocerans, mainly a hybrid of *D. pulicaria* and *D. pulex* (M.G. Boileau, pers. comm.) by the simultaneous application of two separate methods developed by Lehman (1980b, 1980a) and by Lehman & Sandgren (1985), in which a natural phytoplankton assemblage is exposed to a gradient of grazer densities and the rates of change in either algal abundance or nutrient concentration are measured. This method allows for simultaneous estimation of mass-specific ingestion and nutrient release rates by the grazers and species-specific maximum potential growth and instantaneous mortality rates of the algae (e.g., Hambright et al. 2007b).

All experiments were carried out at the Cornell Experimental Ponds Facility (described by Hambright 1994). We ran feeding trails on ten separate dates during two summers using natural algal assemblages collected from one or more ponds. Pond water was filtered through an 80 µm-mesh net to remove macrozooplankton, and mixed in a 200-L polyethylene barrel with addition of sufficient H_3PO_4 and NH_4NO_3 to bring dissolved nutrient concentrations up to 50 µg P L^{-1} and 500 µg N L^{-1}. The levels of nutrient added were previously determined to be sufficient to saturate phytoplankton nutrient uptake rates and retain the N : P environment from which plankton originated. Nutrients were added so that the only detectable grazing effect would be mortality, mineralization effects on phytoplankton growth having been made negligible. Creating nutrient-sufficient conditions for phytoplankton was essential for obtaining mass-specific mineralization of N and P by daphniid cladocerans and diaptomid copepods (Lehman 1980b).

Twelve liters of the algal-nutrient mixture were added, under continuous stirring, to each of thirteen 12.5-L polycarbonate carboys. Samples were collected to establish initial experimental conditions: duplicate whole water samples for nutrient and chlorophyll analyses, and duplicate 100 mL acid-Lugol's-fixed samples for determination of initial densities of individual phytoplankton taxa. Live *Daphnia* collected from one of the ponds were added to five of the carboys and live *Skistodiaptomus*, also from the ponds, were added to five additional carboys. Three carboys in each experiment contained no added zooplankton. Two carboys of *Skistodiaptomus* and two of *Daphnia* each had a sufficient number of animals added to make up roughly a natural concentration (hereafter: 1× treatment). Three carboys of each species had roughly four times this concentration added. Thus in each experiment, for each grazer species, there was a zooplankton density gradient ranging from none to approximately 4× natural density, each exposed to the same algal assemblage. Actual densities of animals used in each carboy were determined by direct counts of samples preserved at the end of the experiment. All carboys were suspended 0.5 m below the surface of one of the experimental ponds for about 24 h (the exact duration of each experiment was recorded). The carboys were inverted at roughly 6-h intervals to reduce algal settling. At the end of the experiment each carboy was mixed and sampled for phytoplankton (100 mL, acid-Lugol's-fixed sample), zooplankton (1 L, 80 µm-mesh filtered, sugar Formalin-preserved sample), and chemical analysis (2 L whole water for nutrients and chlorophyll). In each experiment, grazer biomass was dominated by either *Daphnia* or *Skistodiaptomus*, but other species were present as well (the combined biomass of other taxa was always < 5 % of the total biomass). In all cases, total zooplankton biomass was included in the independent regression variable.

We did not estimate grazing and nutrient mineralization rates for microzooplankton (heterotrophic protists, rotifers), because we do not believe that they represented a significant confounding factor in our experiment. Microzooplankton have been shown to play important roles in various systems, particularly those in which crustacean zooplankton are dominated by relatively small cladocerans (e.g., *Bosmina*, *Ceriodaphnia*) and cyclopoid copepods (Hambright et al. 2007b), but they were very rare (as revealed in both phytoplankton and zooplankton microscopical analyses) in our experimental plankton assemblages, presumably due to the domination by large crustacean grazers which consume them (Fenchel 1987, Porter 1996).

Phytoplankton samples were settled for at least 24 h and counted at 400× under a Wild M40 inverted microscope (Lund et al. 1958). Additional counts were made at 100× to estimate abundances of larger, rarer forms. Typical cells of each taxon identified were measured using an eye-piece micrometer and cell biomasses were calculated using standard volumetric formulae for the geometric shapes most closely matching those of the cells in question and assuming a specific gravity of 1 (e.g., Hillebrand et al. 1999). Zooplankton samples were subsampled (5 % to 50 % depending on density) and counted in a Bogorov-Litt tray at 25× under a Wild M8 stereomicroscope. Ten to 30 representatives of major taxa were sorted onto nylon mesh, dried at 60 °C and weighed on a Sartorius ultra-microbalance to estimate biomass. Biomasses of less abundant species were estimated by applying measurements of species lengths to standard length-weight regressions (Culver et al. 1985).

Grazing rate estimates

As described by Lehman & Sandgren (1985), grazing rates were determined by linear regression ($\alpha = 0.05$) of the exponential rates of change both of bulk phytoplankton (as chlorophyll) and of each algal taxon against zooplankton biomass (Z) (Fig. 1A). Algal growth rates were estimated as $\ln(A_f\ A_i^{-1})t^{-1}$; where A_i and A_f are initial and final densities of the algal taxon, A, and t is the experiment duration in days. The slopes of the regression lines describe the clearance rate (mL water cleared [mg Z]$^{-1}$ day^{-1}) which we multiplied by mean algal biomass in each experiment to obtain grazing rate (mg chlorophyll or mg A [mg Z]$^{-1}$ d^{-1}). The intercept of the regression line is an estimate of the maximum potential growth rate, μ_{max}, for the algal taxon under nutrient-saturating conditions. Instantaneous mortality rates for each algal taxon were estimated as the product of the taxon-specific clearance rate and mean zooplankton biomass for each experiment.

Zooplankton nutrient mineralization estimates

Nutrient analyses were performed on whole water samples passed through a Whatman GF/C glass fiber filter using stand-

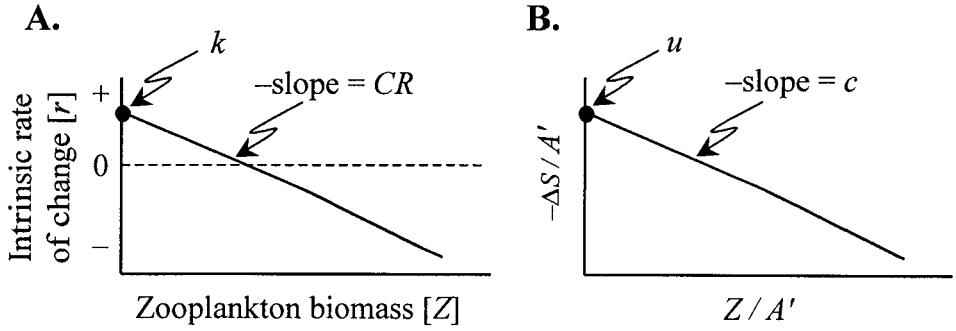

Fig. 1. A. Relationship between phytoplankton rate of change, *r*, and zooplankton biomass, *Z*, along a zooplankton biomass gradient, showing estimation of clearance rate, *CR*, and maximum phytoplankton growth rate, *k*, in the absence of grazing. Grazing rate, *GR*, is calculated as the product of mean phytoplankton biomass, *A'*, and *CR* (see text). **B.** Relationship between changes in nutrient concentration, *S*, and *Z*. Maximum nutrient uptake rate, *u*, for phytoplankton and excretion rate, *c*, of that nutrient by zooplankton are calculated when both *S* and *Z* are standardized to *A'* (Lehman 1980b).

ard methods (American Public Health Association 1985). The material retained on the filter was analyzed for chlorophyll content following homogenization and extraction in 90 % acetone for 24 hr and analyzed spectrophotometrically (American Public Health Association 1985). The filtrate was analyzed in triplicate for NH_4^+ and total soluble phosphorus, TSP, the forms of nitrogen and phosphorus most likely to be released by zooplankton (Lehman 1980b). In the first experiment we also analyzed for total soluble nitrogen, TSN, but found no indication of release above that present as NH_4^+. Details of chemical analyses can be found in Hambright et al. (2007a).

Release rates of nutrients by zooplankton were obtained by regressing the change in nutrient concentration over the 24-hour period against zooplankton biomass (Lehman 1980b) (Fig. 1B). In this method, both nutrient concentration and zooplankton biomass are divided by the mean algal biomass, *A'*, (calculated as $(A_0 - A_t)\ (r_A\, \Delta t)^{-1}$) over the course of the experiment, to correct for algal uptake. The slope of the regression line ($\alpha = 0.05$) gives the zooplankton mass-specific nutrient release rate and the intercept is the saturated rate of nutrient uptake by algae.

Results

Grazing

Phytoplankton density and taxonomic diversity varied substantially among experiments, thus exposing grazers to a broad range of conditions. In the ten experiments, total phytoplankton biomass (wet weight) averaged 3.7 ± 2.0 µg mL^{-1} (± SE) but varied across three orders of magnitude: 0.46 – 19.3 µg mL^{-1}. Cyanobacteria comprised greater than 30 % of the biomass in four experiments (range for all experiments: 0–86 %), Chlorophyta greater than 30 % in four experiments (range: 2–99 %), Chrysophyta greater than 30 % in four experiments (range: 1–79 %) and Cryptophyta greater than 30 % in two experiments (range: 0–65 %). Variation in the density of zooplankton used was much lower: ambient (1× treatments) total zooplankton biomass (dry weight) only varied among experiments by about a single order of magnitude (*Daphnia,* range: 0.21–2.8 mg L^{-1}; *Skistodiaptomus,* range: 0.35–0.84 mg L^{-1}).

We detected significant grazing in all experiments, indicating that a substantial fraction of the phytoplankton biomass (measured as chlorophyll) in the mesocosms was grazeable by both *Daphnia* and *Skistodiaptomus* (Fig. 2). Clearance rates for chlorophyll ranged between 0.09 and 1.02 L [mg *Z*]$^{-1}$ d^{-1} with mean clearance rates by *Skistodiaptomus* (0.654 ± 0.079 L [mg *Z*]$^{-1}$ d^{-1} ± SE) 70 % higher than those by *Daphnia* (0.387 ± 0.093 L [mg *Z*]$^{-1}$$d^{-1}$± SE; $t_9 = 2.180$; $P = 0.057$).

Sixty-four phytoplankton taxa (including size variants) were present in sufficiently high concentration in at least one experiment to be included in our analysis, with an average of 21.2 ± 1.9 (± SE) taxa present in each experiment (Appendix 1). Most (58) phytoplankton taxa present were significantly grazed in at least one experiment. Clearance rates ranged between 0 and 5.5 L [mg *Z*]$^{-1}$ d^{-1} for individual taxa. *Daphnia* consumed on average 64 % (12.7 ± 1.8 taxa) of available taxa in any given experiment, whereas *Skistodiaptomus* consumed fewer, 49 % (9.8 ± 1.6 taxa) of available taxa. Overlap in consumed taxa was moderate; only 33 % (7.1 ± 1.4 taxa) of available taxa were consumed by both grazers. On average, about one quarter of available taxa were not significantly grazed in each experiment.

Although *Skistodiaptomus* clearance rates based on chlorophyll concentrations were higher than those of *Daphnia*, no difference was seen in the clearance of phytoplankton biomass based on microscopic cell

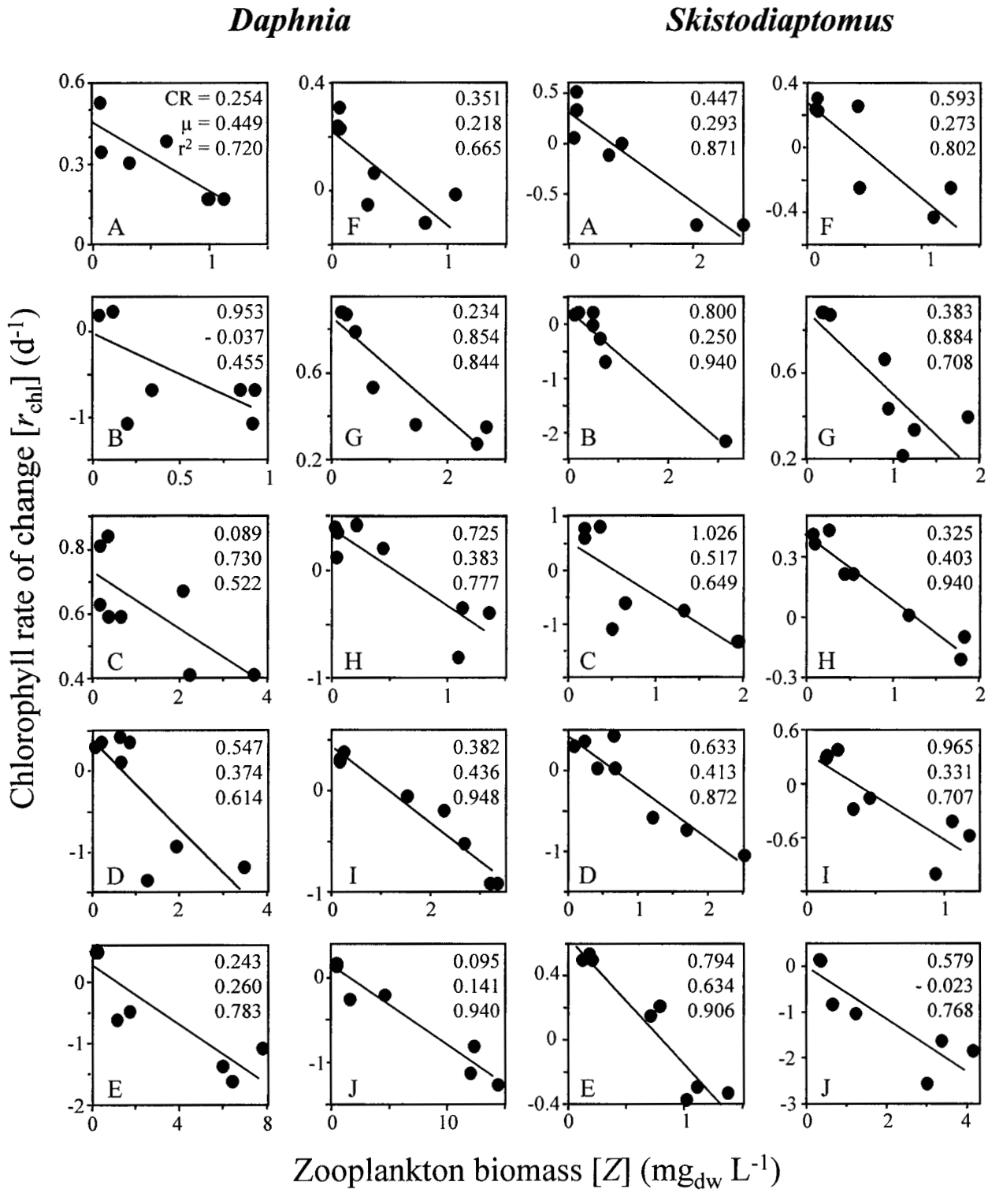

Fig. 2. Estimates of chlorophyll clearance rates by *Daphnia* and *Skistodiaptomus* in 10 paired grazing experiments (panels A – J for each grazer taxon). Data show the calculated rate of change for chlorophyll over the course of ca. 24 hrs in a series of carboys containing a gradient of zooplankton densities. Regressions were fitted by simple linear regression and provide estimates of clearance rate, *CR*, (in units of mL water cleared per [mg *Z*] d^{-1}) as the negative of the slope and of maximum potential growth rates of chlorophyll (in the absence of grazing), μ (in units of d^{-1}), as the y-intercept. All slopes are significantly different from 0 ($P < 0.05$).

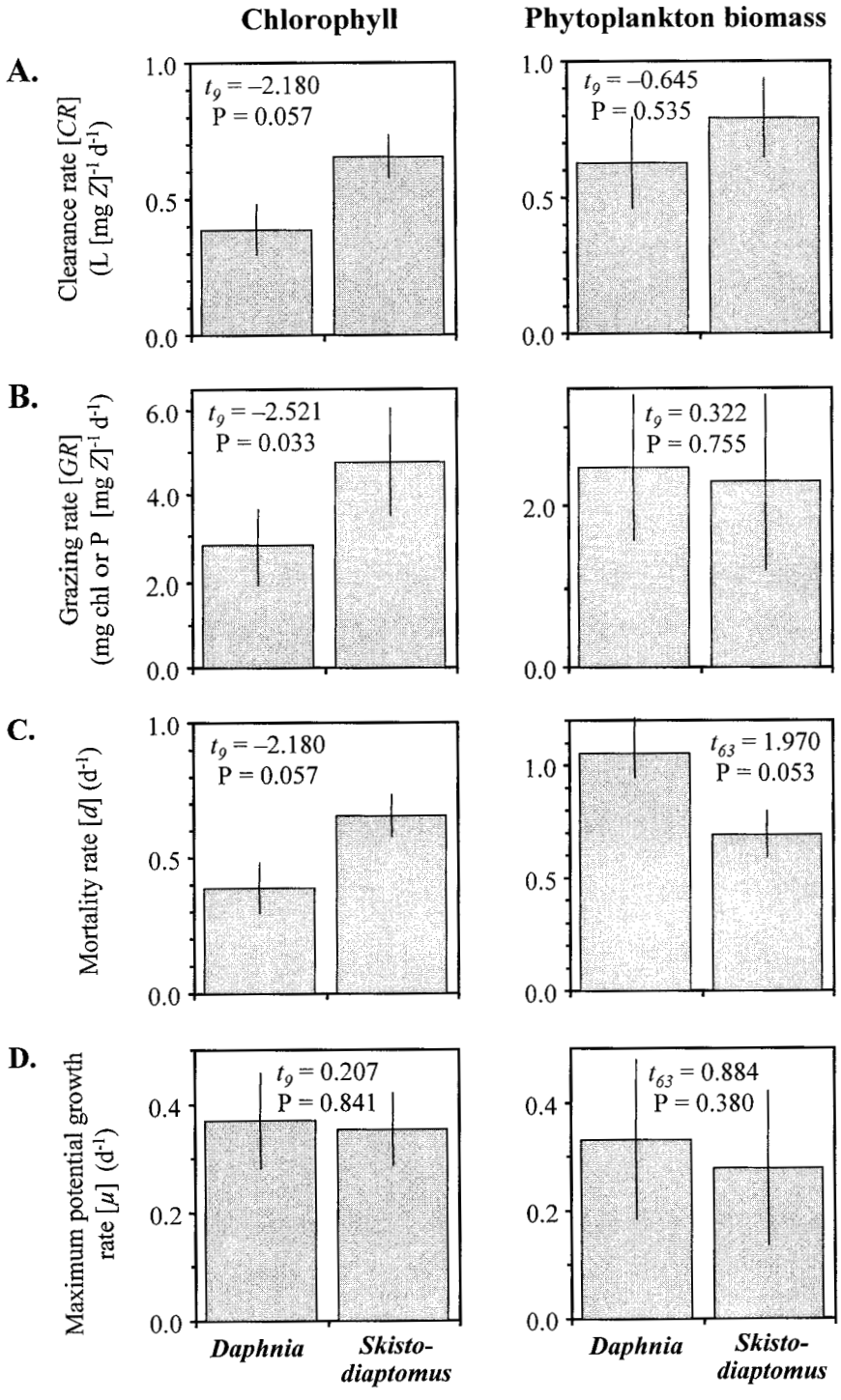

Fig. 3. Comparison of mean (± SE) clearance rates and ingestion rates by *Daphnia* and *Skistodiaptomus*, and phytoplankton mortality and maximum growth rates exposed to grazing by *Daphnia* and *Skistodiaptomus.* Left hand panels are based on chlorophyll; right hand panels are biomass estimates based on phytoplankton cell counts.

counts (Fig. 3). Likewise, grazing (= ingestion) rates for chlorophyll (mg chlorophyll [mg Z]$^{-1}$ d^{-1}) were higher for *Skistodiaptomus* than for *Daphnia*, but were similar based on phytoplankton biomass. Chlorophyll-based mortality rates, d, were consistently high and similar in magnitude to measured maximum potential growth rates, μ_{max}, but there were no differences across experiments in chlorophyll mortality rates inflicted by either grazer. Phytoplankton mortality rates, based on phytoplankton biomass, reveal, however, marginally significantly greater mortalities inflicted by *Daphnia* compared with *Skistodiaptomus*. Hence, from the phytoplankton perspective, mass-specific grazing by *Daphnia* was more costly to individual phytoplankton

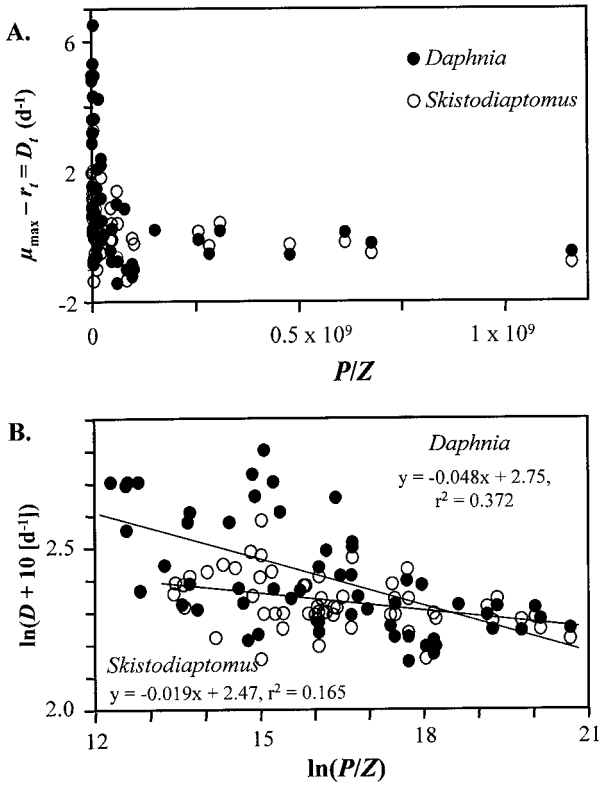

Fig. 4. A. Plot of standardized mortality rates, *D* (calculated by subtracting the measured growth rate of the algal taxon in question, r_t, from its maximum potential growth rate, μ_{max} in individual carboys from all experiments combined) plotted as a function of the ratio of phytoplankton biomass to zooplankton biomass (*P/Z*) using *Ankistrodesmus* sp. as an example typical of many of the taxa in our study. Plots of D_t versus *P/Z* have a negative hyperbolic shape, suggesting that mortality rate imposed by grazing is relatively low as long as *P/Z* is high, but that there is a threshold *P/Z* below which grazer induced mortality rises precipitously. **B.** Natural log-transformed plot of the data in A illustrating estimation of D_{max} (the y-intercept of a simple linear regression). Values of D_t may be zero, or even negative when at very high *P/Z* (i.e., r_t estimates exceed μ_{max} estimates). For this reason, 10 was added to all D_t values before taking logarithms. A linear regression using these transformed data provides a means of comparing grazer impact on different alga taxa. The y-intercept is used for comparison rather than the slope of the regression, as it provides a maximal estimate of grazing mortality, D_{max}, at *P/Z* = 0.

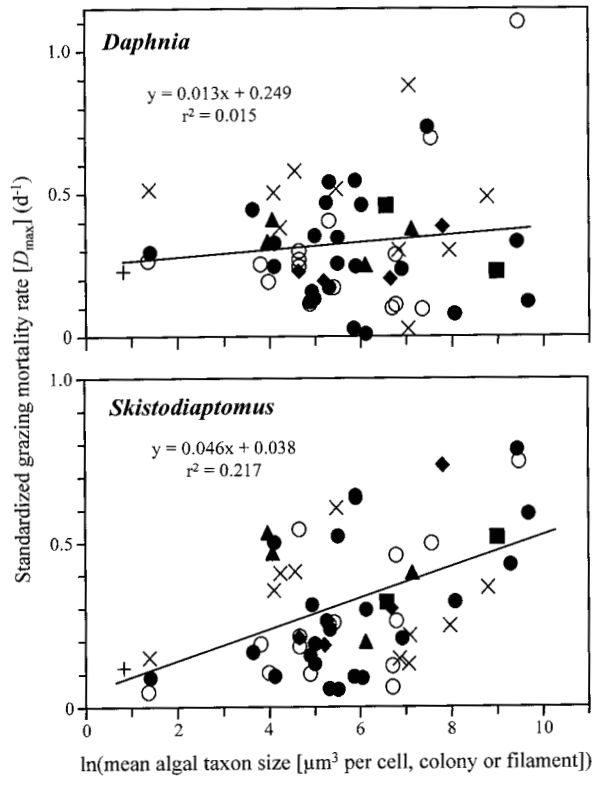

Fig. 5. Plots of D_{max} versus algal cell or colony size (as the natural log of µm^3 per cell, colony or filament). Symbols: solid circle - Chlorophyta; open circle - Chrysophyta; solid square – Dinoflagellata; triangle – Bacillariophyta; diamond – Cryptophyta; × – Cyanobacteria; + – Bacteria.

taxa than was that by *Skistodiaptomus*. However, from the perspective of the grazers, *Daphnia* and *Skistodiaptomus* ingested similar amounts of phytoplankton biomass, though *Skistodiaptomus* apparently selectively ingested cells of higher chlorophyll content.

The nature of this selection can be explored further by examining mortality rates for individual taxa. As clearance rates suggest, phytoplankton mortality varied greatly both within algal taxa among experiments, and among taxa within experiments, making comparison across taxa and experiments difficult. Much of the variation lies in the fact that phytoplankton abundance relative to zooplankton abundance varied greatly between experiments. When total phytoplankton biomass was high relative to zooplankton biomass, little algal *per capita* mortality was imposed simply because of low grazing capacity (Fig. 4). In contrast, when total phytoplankton biomass was low, even a moderate biomass of zooplankton had a significant impact. This effect of relative densities of phytoplankton and grazers can be explored, one algal taxon at a time, using the data for all experiments in which that taxon was present and looking at maximum per capita mortality rates, D_{max}, imposed by the grazers as a function of algal size. For the grazers in our study, these plots (Fig. 5) reveal size-selective feeding by *Skistodiaptomus* ($P < 0.001$), but not by *Daphnia* ($P = 0.385$). For neither grazer taxon is there any discernible selective feeding according to major taxonomic group (Fig. 5).

Table 1. Mass (dry weight) specific excretion of nitrogen, as ammonia-N (μg N [mg Z]$^{-1}$ d^{-1}) and phosphorus, as total soluble P (μg P [mg Z]$^{-1}$ d^{-1}) by *Daphnia* and *Skistodiaptomus*. Rates and ratios of excretion were compared using paired *t*-tests.

Grazer	Mean (± SE) N excretion (μg N [mg Z]$^{-1}$ d^{-1})	Mean (± SE) P excretion (μg P [mg Z]$^{-1}$ d^{-1})	N : P molar
Daphnia	25.7 ± 7.2	1.9 ± 0.9	27.6 ± 3.3
Skistodiaptomus	20.6 ± 4.6	4.0 ± 0.6	15.7 ± 1.3
	t_7 = 0.652	t_4 = –3.934	t_4 = 4.286
	P = 0.535	P = 0.017	P = 0.013

Nutrient mineralization

We measured significant nitrogen regeneration by grazers in eight of the ten experiments each for *Daphnia* and *Skistodiaptomus* and significant phosphorus regeneration in eight (of ten) experiments for *Daphnia* and five (of ten) experiments for *Skistodiaptomus*. Maximum potential nutrient uptake rates varied considerably among experiments and phytoplankton composition, ranging between 0.8 and 44.3 μg N [μg chl]$^{-1}$ d^{-1} and between 0.8 and 7.5 μg P [μg chl]$^{-1}$ d^{-1}. Mineralization rates of N by *Daphnia* and *Skistodiaptomus* were similar, averaging ca. 23 μg N [mg Z]$^{-1}$ d^{-1} (Table 1). In contrast, P mineralization rates by *Skistodiaptomus* were greater than those of *Daphnia* by 111 %, based on data from the five experiments in which P mineralization rates were obtained for both grazers. Consequently, consistent with other studies of zooplankton excretion stoichiometry (Sterner & Elser 2002), molar N : P of nutrient mineralization by *Daphnia* was significantly greater than that of *Skistodiaptomus*.

Discussion

The results of our mesocosm experiments indicate that consumption and nutrient mineralization by grazing zooplankton may interact synergistically within a taxon, though divergently between the two major crustacean zooplankton taxa in fresh water. Selective grazing and low N : P mineralization by copepods should be conducive to the development of N-fixing cyanobacteria; non-selective grazing and high N : P mineralization by cladocerans would be inhibitory. Both *Skistodiaptomus* and *Daphnia* consumed phytoplankton over a broad taxonomic range, including cyanobacteria. Copepods selectively consumed larger, higher chlorophyll-content cells than *Daphnia*, whereas the latter showed no selectivity. Both grazers consumed similar amounts of total phytoplankton daily on a mass-specific basis and grazed cyanobacteria at similar rates. However, the two grazers mineralized nutrients at markedly different N : P, suggesting that altering relative availabilities via mineralization may be a primary mechanism by which these grazers influence N-fixing cyanobacteria.

Cladocerans and copepods have been shown in several studies to consume phytoplankton, bacteria, and detritus selectively, with cladocerans efficiently consuming bacteria, small algae and detrital particles (e.g., below 30–35 μm in size) whereas copepods tend to selectively ingest larger algal sizes and avoid incidental ingestion of detritus and other low quality food items (Lampert 1987, Bern 1994, Hansen et al. 1994, DeMott 1995). In contrast, using grazer gradient experiments like ours, Cyr & Curtis (1999) found that both cladocerans and copepods tended to consume small algae ranging between 10 and 50 μm in diameter, that copepod grazing was not biased toward larger algae, and that cladoceran and copepod selectivity was affected by factors other than size. Thus there appears to be context-dependence with respect to grazing selectivity, species identity, and experiment location.

In our experimental system, both cladocerans and copepods consumed phytoplankton across all taxonomic groups and sizes. There was no apparent size selection by *Daphnia*, whereas *Skistodiaptomus* consumed larger cells at higher rates. The high non-size-related variability in grazing (i.e., $0 \leq D_{max} \leq 1.1$ d^{-1}) by *Daphnia* from our extensive series of experiments over a phytoplankton size range from 2 to 32 μm suggests that attempts to define "functional food groups" for phytoplankton based on size alone (e.g., < 30 μm, Cottingham et al. 2004) are likely to include many taxa that are selected against by *Daphnia* for reasons other than size. Across grazers, however, *Daphnia* consumed small algae and bacteria at higher rates than *Skistodiaptomus,* while *Skistodiaptomus* consumed larger taxa at higher rates than *Daphnia*. One interesting outcome of our analysis is the fact that although total phytoplankton biomass ingestion was similar between the two grazers, rates of change of total chlorophyll across grazer densities indicate that *Skistodiaptomus* consumed higher chlorophyll-content algae, as total chlorophyll-based clearance rates were nearly 50 % higher than those of *Daphnia*. Presumably, such selection is based on chemical or other cell characteristics associated with high chlorophyll content (e.g., taste, toxicity, or morphology, Burns and Hegarty 1994, Cyr & Curtis 1999). Toxicity was apparently not generally

important in diet selection because both *Daphnia* and *Skistodiaptomus* consumed most cyanobacteria taxa present including single-celled (*Chroococcus* sp. and coccoids), colonial (*Aphanocapsa*), and filamentous (*Oscillatoria* and *Anabaena*) species. One striking exception is *Microcystis* which neither grazer was able to consume as large colonies, but which *Daphnia* ingested as small colonies. Using data from these same mesocosm experiments, Schaffner et al. (1994) noted for filamentous cyanobacteria that *Daphnia* consumed whole, intact trichomes, while *Skistodiaptomus* only partially ingested filaments, clipping them into small segments and consuming some while fumbling others away. Thus the differences that did exist between the grazer taxa in their consumption of cyanobacteria appear to have had more to do with the phytoplankton and zooplankton morphologies present in our study than with chemical characteristics of the cyanobacterial cells.

Selective grazing mortality is only one mechanism linking regulation of phytoplankton composition to grazer assemblages. Elser (1999) proposed a stoichiometric basis for *Daphnia* control of cyanobacteria. He suggested that in lakes receiving high nutrient loading at low N : P (and with proper hydrodynamic and light conditions), *Daphnia* abundance could be a critical determinant of whether cyanobacteria blooms occur. Rather than direct grazing effects, he proposed that high N : P recycling by *Daphnia* could suppress N-fixing cyanobacteria. Based upon a mesocosm experiment conducted in artificially eutrophied Lake 227 (Ontario, Canada), MacKay & Elser (1998) concluded that differential recycling of NH_4^+ relative to P by *Daphnia* was responsible for a 50 % reduction in cyanobacteria abundance and N fixation rates. However, in a subsequent study of the same lake, Paterson et al. (2002), while also showing that *Daphnia* strongly reduced cyanobacteria abundances, concluded that because NH_4^+ additions produced few effects on phytoplankton composition, the influence of N-biased nutrient mineralization by *Daphnia* was small relative to other mechanisms in reduction of cyanobacteria.

Our experimental design required that nutrients be held at concentrations that saturated uptake rates by the phytoplankton (Lehman 1980b). Thus, our goal was simply to determine the potential mineralization effects of both grazers in low N : P environments (i.e., those fostering N-fixing cyanobacteria; in this case, initial molar N : P = 22). As a result, our measured rates of nutrient mineralization do not provide direct estimates of how differential nutrient release by cladocerans and copepods impacted nutrient availabilities in P deficient algal assemblages. Nevertheless, our nutrient mineralization estimates allow us to evaluate the relative importance of the different mechanisms proposed to explain the distinct N : P released to the water by cladocerans and copepods. The pattern of similar N mineralization rates by *Daphnia* and *Skistodiaptomus*, but lower P mineralization by *Daphnia*, while both grazers consumed algae and bacteria in the same low N : P (P replete) environment, is consistent with predictions of the growth rate hypothesis (GRH) of ecological stoichiometry (Elser et al. 2003). According to GRH, N release by the two taxa is expected to be similar, but because faster-growing cladocerans have a higher P requirement, they should release lower amounts of P back to the environment compared with slower-growing copepods.

An intriguing result of our mesocosm studies is that in addition to the selection by grazers for phytoplankton size and chlorophyll content, the high variability in phytoplankton mortalities, D_{max}, suggests that other complexities in food selection exist. Although we have not been able to identify a general pattern to this selection based on taxon or morphology, grazing mortalities induced by both *Daphnia* and *Skistodiaptomus* varied by more than one order of magnitude among very similar (in taxon, size and shape) phytoplankton groups, suggesting some form of either passive or active selection. Such differentiation could be based on other physical attributes (e.g., presence or absence of spines or gelatinous coats) or on cell surface charge and chemistry (DeMott 1986, Burns & Hegarty 1994, Epp 1996).

It may be that there is an interaction between the selective consumption of phytoplankton and the relatively high P mineralization by *Skistodiaptomus* that we observed. In our experiments the cells consumed by the two taxa were different: copepods ingested higher chlorophyll-content cells than did *Daphnia*. If high chlorophyll-content cells were also higher in P content, then the diet of *Skistodiaptomus* would have been relatively P-sufficient compared with that of *Daphnia.* We have no evidence that cell chlorophyll and P covaried with phytoplankton taxonomic identity or cell size in our experiments, but evidence in the literature indicates that such variability is prominent in natural phytoplankton assemblages (Sterner et al. 1998, Urabe et al. 2002). The possibility of such relationships warrants further exploration.

Both differences in diet and in N : P release rates between *Daphnia* and *Skistodiaptomus* influenced phytoplankton community structure. The relative importance of these two distinct processes over the pe-

riod of a seasonal cycle must depend upon the other conditions in a water body such as the particular assemblage of phytoplankton (especially which cyanobacteria species) present and water column N : P. How these influences play out in structuring phytoplankton in the experimental ponds from which our study organisms were taken, is the subject of two replicated longer-term whole-pond experiments (Hambright et al. 2007a). In this study, we found that under conditions of high external nutrient loading at both high and low N : P, grazing-induced mortality dominated phytoplankton impacts because nutrient mineralization by grazers was always small compared with the combined sources of external and internal (sediment) loading. In *Daphnia*-dominated ponds, zooplankton P excretion amounted to ca. 23 % of total nutrient loading (sum of external and internal loading plus excretion), while in copepod-dominated ponds, zooplankton P excretion was only ca. 8 % of the total P supply; N excretion was even less important (Hambright et al. 2007a). Thus it seems likely that the stoichiometry of zooplankton recycling will be much more important in regulating nutrient availabilities to phytoplankton in oligotrophic systems, in which external and internal nutrient loads are small.

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Appendix 1. Phytoplankton taxa, mean biovolume per cell, number of experiments (max = 10) in which individual phytoplankton taxa occurred, number of experiments in which significant grazing by *Daphnia* and *Skistodiaptomus* was detected (i.e., slope < 0, $P \leq 0.05$) for each taxon, and mean (± SE) clearance rates for phytoplankton taxa across experiments. Mean clearance rates for each taxon include all experiments in which a taxon was present, even if not significantly grazed. Numbers following taxon names indicate size classes (μm) that were individually identified and recorded.

Phytoplankton taxa	Bio volume (μm^3)	No. expts. taxon present	No. of experiments taxon grazed by		Clearance Rate (L $[mgZ]^{-1}$ d^{-1})			
			Daphnia	*Skistodiap.*	*Daphnia*		*Skistodiap.*	
					mean	SE	mean	SE
Chlorophyta								
Ankistrodesmus sm.	40	7	5	3	1.40	0.78	0.33	0.19
Chlamydomonas 4–9	144	10	2	2	0.11	0.08	0.17	0.13
Coccoid greens 1–3	4	9	8	3	0.93	0.43	0.18	0.10
Coccoid greens 4–6	65	7	6	4	0.82	0.25	0.66	0.35
Coccoid greens 6–9	221	2	0	0	0		0	
Coelastrum	14476	1	0	1	0		3.68	
Colonial green 2–6	34	1	0	1	0		1.33	
Conochaete	65	1	0	0	0		0	
Gloeocystis	382	2	0	1	0		0.10	0.14
Golenkinia	270	3	2	3	1.48	1.02	2.10	0.59
Kirchneriella obesa	460	2	2	0	3.41	2.21	0	
Kirchneriella spp.	400	1	1	1	1.25		3.85	
Largerheimia	402	2	2	2	0.48	0.13	2.47	2.57
Oocystis pusilla/parva	268	3	2	1	1.91	2.09	0.21	0.26
Oocystis sp.	500	2	0	2	0		1.32	1.06
Pandorina	18416	2	1	1	0.32	0.45	0.38	0.54
Pediastrum	12416	1	0	0	0		0	
Scenedesmus bijuga	224	4	3	1	1.37	0.86	0.26	0.30
Scenedesmus spp.	1120	8	6	4	0.91	0.45	0.58	0.34
Schroederia	159	6	3	3	0.35	0.24	0.48	0.28
Selenastrum 6	160	4	3	1	1.92	1.12	0.11	0.13
Selenastrum minutum	208	2	2	0	0.73	0.41	0	
Staurastrum	2000	1	1	0	4.13		0	
Tetraedron	150	2	0	1	0		0.92	1.30
Westella	3619	1	0	0	0		0	
All chlorophytes					**0.78**	**0.34**	**0.52**	**0.20**
Chyrsophyta								
Chromulina 3–6	48	9	7	6	1.45	0.91	0.54	0.17
Chromulina 12–15	905	3	1	0	0.61	0.75	0	
Chrysamoeba 6–9	221	3	1	2	0.97	1.18	1.57	1.32
Chrysochromulina	57	5	4	3	0.49	0.29	0.58	0.35
Chrysococcus 4–9	144	1	0	0	0		0	
Chrysococcus 10–20	1767	2	0	2	0		0.53	0.53
Dinobryon	2160	2	2	2	4.51	2.12	1.56	0.07
Mallomonas akrokomos	245	3	0	2	0		0.87	0.54
Mallomonas sp2	980	3	2	2	1.84	1.46	1.69	1.52
Mallomonas sp3	980	1	0	1	0		0.66	
Ochromonas 6	113	6	4	1	1.24	0.59	0.27	0.30
Ochromonas 12	905	2	1	1	0.19	0.27	0.49	0.69
Uroglenopsis americanum	15000	1	1	1	5.50		1.37	
μ flagellates	113	9	8	4	1.41	0.67	0.57	0.30
μμ flagellates	4	10	7	3	0.53	0.19	0.15	0.08
colonial μ flagellates	113	1	1	1	0.96		2.01	
All chysophytes					**0.77**	**0.28**	**0.60**	**0.16**
Bacillariophyta								
Centric diatoms 6	57	3	1	2	1.48	1.81	0.95	0.60
Pennate diatoms 11–30	63	2	1	1	2.06	2.92	0.33	0.47
Pennate diatoms 31–50	503	1	1	0	0.02		0	
Pennate diatoms > 50	1414	1	1	1	2.20		1.94	
All bacillariophytes					**1.35**	**1.17**	**0.68**	**0.50**

Appendix 1 (continued).

Phytoplankton taxa	Bio volume (μm^3)	No. expts. taxon present	No. of experiments taxon grazed by		Clearance Rate (L [mgZ]$^{-1}$ d^{-1})			
			Daphnia	*Skistodiap.*	*Daphnia*		*Skistodiap.*	
					mean	SE	mean	SE
Cryptophyta								
Chroomonas	113	10	6	5	0.83	0.48	0.54	0.26
Cryptomonas sm.	200	7	5	5	0.30	0.11	0.71	0.24
Cryptomonas med.	880	4	2	2	0.14	0.10	0.74	0.51
Cryptomonas lg.	2800	3	3	2	1.72	1.33	0.78	0.50
All cryptophytes					**0.57**	**0.29**	**0.56**	**0.15**
Pyrrophyta								
Gymnodinians 9–12	796	1	1	1	1.85		0.91	
Peridinians 12–40	9203	1	1	0	0.37		0	
All pyrrophytes					**1.11**	**1.04**	**0.46**	**0.65**
Cyanobacteria								
Anabaena spiroides 4–6	1309	1	0	1	0		0.40	
Anabaena felisii	1350	1	1	1	1.14		0.64	
Anabaena 9	7634	2	2	2	1.84	0.81	1.39	0.62
Aphanocapsa	105	2	1	0	2.01	2.85	0	
Chroococcus	1072	1	1	1	1.51		0.37	
Coccoid cyanobacteria 1–3	4	1	1	0	5.04		0	
Coccoid cyanobacteria 4–6	65	5	3	1	0.50	0.35	0.08	0.09
Microcystis (sm. colony)	3272	1	1	0	1.29		0	
Microcysis (lg. colony)	> 25000	1	0	0	0		0	
Oscillatoria 1–2	266	5	5	5	1.05	0.28	2.41	1.20
Stichococcus subtilis 6	75	1	1	0	0.24		0	
All cyanobacteria					**1.43**	**0.55**	**0.79**	**0.37**
Bacteria								
Rods 1×3	2	2	1	2	0.74	1.05	0.50	0.26
All bacteria					**0.74**	**1.05**	**0.50**	**0.26**