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The importance of *Daphnia* in determining mortality rates of protozoans and rotifers in lakes

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Abstract

We measured mortality of protozoans and rotifers in three lakes of contrasting zooplankton communities. We also compared protozoan growth in an experiment which controlled *Daphnia* biomass but varied body size. Mortality was determined as the difference between growth rates over 24 h in containers with and without zooplankton. Growth rates of heterotrophic flagellates and ciliates were high in the presence of a small assemblage of zooplankton and near zero or negative when either *Daphnia pulex* or *Daphnia galeata* was the dominant zooplankton species. Growth rates of rotifers were also usually lower in the presence of *Daphnia*. Mortality rates of heterotrophic flagellates, ciliates, and rotifers were positively related to the mean body size of *Daphnia* in comparisons among experiments. In an experiment with equal biomasses but different sizes of *D. pulex*, flagellate growth rates were lower in treatments with large *Daphnia*. High mortality in zooplankton communities dominated by larger species of *Daphnia* appears to be important in determining differences in the abundances of protozoans and rotifers among lakes.

Protozoa are important as consumers, nutrient regenerators, and prey for larger organisms in the plankton (Fenchel 1987). The abundance and biomass of planktonic protozoa tend to be correlated with the availability of resources within and among systems (e.g. Pace 1982; Carlough and Meyer 1989; Sanders et al. 1992), but there is also evidence that larger zooplankton may limit the abundance and biomass of protozoa in lakes. For example,

the ratio of bacteria to heterotrophic flagellates is ~1,000:1 across trophic gradients in marine and freshwater systems (Sanders et al. 1992). This ratio, however, is significantly higher in lakes with abundant populations of the cladoceran *Daphnia* (Gasol and Vaqué 1993).

These comparative observations among ecosystems are supported by laboratory studies which demonstrate that *Daphnia* is an effective predator of heterotrophic flagellates and ciliates (Porter et al. 1979; Sanders and Porter 1990; Jack and Gilbert 1993). Furthermore, reduced abundances of heterotrophic flagellates and ciliates have been observed in field enclosure experiments with *Daphnia* (Gilbert 1989; Pace and Funke 1991; Wickham and Gilbert 1991, 1993) and seasonally in lakes during periods of high grazing by *Daphnia* (Güde 1988; Weisse et al. 1990). Carrick et al. (1991) directly measured the mortality of het-

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erotrophic flagellates and ciliates in relation to zooplankton biomass in Lake Michigan and found significant zooplankton predation at all times of the year. Highest rates were observed when *Daphnia* constituted a major component of the zooplankton biomass (Carrick et al. 1991).

Studies of rotifers have also indicated that cladocerans kill and consume rotifers and are capable of limiting rotifer populations (Gilbert 1988*a,b*). The susceptibility of rotifers to cladoceran-induced mortality is positively related to cladoceran size and to the structural and behavioral characteristics of particular rotifer species (Gilbert 1988*a,b*). Rotifers with hardened lorica, extensive spines, and escape responses suffer less mortality in the presence of large cladocerans. Ciliates also exhibit differential mortality due to cladoceran predation. Larger ciliates appear less susceptible to predation, and some species also have escape responses that reduce capture by cladocerans (Jack and Gilbert 1993).

These considerations suggest protozoan mortality should be related to the abundance and size of cladocerans in lakes. In particular, consumption of protozoans by large-bodied species of *Daphnia* (> 1-mm body length) may reduce protozoan biomass below expected levels based on resources alone. However, there have been few direct measures of protozoan mortality in freshwater systems. In the current study, we measured protozoan mortality in lakes with differing zooplankton communities. We hypothesized that heterotrophic flagellates and ciliates should experience higher mortality in zooplankton communities dominated by large *Daphnia* and lower mortality in communities dominated by smaller cladocerans and copepods. We also considered whether protozoan mortality was primarily related to *Daphnia* biomass or body size. Analyses of size and biomass are easily confounded, because in zooplankton communities there is a positive relationship between mean body size and community biomass (Cyr and Pace 1992). We tested whether protozoan mortality differs in an experiment where we established equal biomasses of different-sized *Daphnia*.

Methods

To test these ideas we designed an experiment which was repeated twice in three lakes

with contrasting zooplankton community structure. Upton and Tyrrel lakes are relatively small (20 ha), eutrophic systems in Dutchess County, New York. Zooplankton biomass in Upton is dominated by two species of *Daphnia*. A medium-sized species, *Daphnia galeata*, occupies the upper portion of the metalimnion by day and migrates into the epilimnion at night. The larger species, *Daphnia pulex*, occurs deeper in the metalimnion during the day and does not migrate into surface waters at night. Tyrrel Lake is dominated by a spectrum of small species including the cladocerans *Daphnia catawba*, *Ceriodaphnia* sp., and *Bosmina* sp. West Long Lake is in Gogebic County, Michigan, and two large cladocerans, *D. pulex* and *Holopedium gibberum*, dominate the zooplankton biomass.

To measure mortality, we compared protozoan and rotifer growth rates in treatments with and without zooplankton. To one set of triplicate containers, we added either the dominant daphnid species (*D. galeata* in Upton, *D. pulex* in West Long) or the entire assemblage of zooplankton in Tyrrel at about the same concentration as found in the lake. The second set of triplicate containers contained no zooplankton. In some experiments we added an additional treatment designed to detect predation on heterotrophic flagellates by microzooplankton. For this treatment three additional containers were filled with <20- μ m-filtered lake water (described below). This treatment removed microzooplankton including copepod nauplii, rotifers, and larger ciliates.

Differences in the net growth rates of protozoans and rotifers in treatments with and without zooplankton reflect mortality due to *Daphnia* or zooplankton (in the case of the Tyrrel Lake experiments) as follows:

$$b_{wz} - d_i = 1/t \times \ln(N_t/N_0) = r_{wz}; \quad (1)$$

$$b_z - (d_i + d_z) = 1/t \times \ln(N_t/N_0) = r_z. \quad (2)$$

b is the growth rate with (z) and without (wz) zooplankton, d_i the incidental mortality rate, d_z the mortality due to zooplankton, t the experimental time, and N_t and N_0 are the abundances at the beginning and end of the experiment. We assumed that b_{wz} and b_z were equal,

and we tested the importance of d_i (e.g. flagellate mortality due to ciliates and rotifers) in several experiments and found it to be negligible (*see below*). Given these assumptions and results, the differences between the treatments with and without zooplankton reduce to

$$r_{wz} - r_z = d_z. \quad (3)$$

For the experiments in Upton and Tyrrel lakes, surface water was collected with a pump and filtered through a 150- μm net into 25-liter carboys. The in situ abundance of zooplankton was also measured before the experiment as described by Pace et al. (1990). Zooplankton were collected in horizontal net tows, returned to the lab, and sorted into filtered lake water, except in the Tyrrel Lake experiments. In the latter case, zooplankton were not sorted; rather, animals from net tows were counted and added directly to the bottles at a final concentration approximating in situ conditions. Four-liter cubitainers were filled with lake water and zooplankton added at about the same concentration as they occurred in the lake at the time of the experiments. An additional set of triplicate cubitainers was filled with lake water gently siphoned through 20- μm Nitex netting. All cubitainers were placed in constant-temperature incubators in the dark at in situ temperature for 24 h.

The West Long Lake experiments were different in three ways. First, the <20- μm treatment was excluded based on prior results in the Upton and Tyrrel experiments. Second, larger enclosures (40 liter) were used, and these enclosures were incubated in situ. Third, we did not measure the in situ concentration of zooplankton immediately before the West Long experiment. Instead, we used extensive prior data on zooplankton abundances (S. Carpenter, unpubl. obs.) to select a target concentration of *D. pulex* approximating in situ conditions.

For all experiments, subsamples from each replicate were taken initially and after 24 h to determine chlorophyll concentration and bacterial, heterotrophic flagellate, ciliate, and rotifer abundances. Chlorophyll in methanol extracts was determined by fluorometry with corrections for pheopigments. Bacterial abundance was determined by epifluorescence microscopy with the acridine orange direct-count

method (Hobbie et al. 1977). Heterotrophic flagellates were stained with proflavin, preserved with glutaraldehyde (1% final solution), immediately filtered on 1- μm Nuclepore filters, and counted with an epifluorescence microscope. A 100-ml sample was preserved in a 1% final concentration of Lugol's solution for enumeration of ciliates. We used an inverted microscope at 200 \times magnification to count ciliates after settling. Rotifers were concentrated by pouring 1 liter of water through a 35- μm sieve. These samples were preserved in a sucrose-Formalin solution and subsequently counted with an inverted microscope at 100 \times magnification.

Macrozooplankton were recovered after 24 h with a 100- μm net. Samples were preserved in sucrose-Formalin and counted with a stereomicroscope at 25 \times magnification. *Daphnia* biomass was estimated by measuring the lengths of 75 randomly chosen individuals from within each replicate and converting these lengths to weights with a generalized length-dry weight regression (Bottrell et al. 1976). Weight estimates were made following the recommendations of Bird and Prairie (1985).

We conducted a single experiment using surface water from Upton Lake to separate the effects of *Daphnia* body size and biomass on protozoan growth. In this experiment we collected *D. pulex* from Upton Lake and separated animals into two size classes. We added sufficient numbers of each size class to achieve equal biomasses in replicate 1-liter polycarbonate culture bottles. A treatment without *Daphnia* was also included. This experiment followed the same sampling and analysis protocols described above. Chlorophyll, bacteria, heterotrophic flagellates, and ciliates were measured in each bottle at the beginning and after 24 h. We also measured the abundance of *Cryptomonas* sp. to see whether responses were similar for a common, edible algal species. At the end of the experiment we collected, enumerated, and sized the *Daphnia*. Because of the smaller container size used in this experiment, we did not determine rotifer abundance.

For all experiments, we used one-tailed *t*-tests to determine whether growth of protozoans and rotifers was greater in treatments without zooplankton relative to treatments with zooplankton.

Table 1. Initial mean concentration ($n = 3 \pm \text{SD}$) of chlorophyll *a* and abundance of bacteria ($\times 10^6 \text{ ml}^{-1}$), heterotrophic nanoflagellates ($\times 10^3 \text{ ml}^{-1}$), ciliates ($\times 10^3 \text{ liter}^{-1}$), and rotifers ($\times 10^3 \text{ liter}^{-1}$) for each experiment. (ND—not determined.)

Lake	Date	Chl	Bacteria	Flagellates	Ciliates	Rotifers
Upton 1	4 Jul 1991	6.1 \pm 0.2	17.7 \pm 2.7	0.5 \pm 0.1	ND	0.4 \pm 0.0
Upton 2	28 Jul 1991	3.0 \pm 0.2	10.8 \pm 1.5	1.1 \pm 0.3	8.5 \pm 1.2	0.3 \pm 0.1
Tyrrel 1	8 Aug 1991	10.9 \pm 0.0	12.2 \pm 3.6	1.1 \pm 0.0	55.8 \pm 16.3	4.1 \pm 0.1
Tyrrel 2	8 Nov 1991	24.6 \pm 0.7	10.9 \pm 3.7	1.1 \pm 0.1	23.6 \pm 3.7	0.8 \pm 0.0
W. Long 1	9 Sep 1991	ND	5.9 \pm 0.8	2.2 \pm 0.2	3.5 \pm 1.1	0.5 \pm 0.0
W. Long 2	23 May 1992	9.3 \pm 0.15	10.4 \pm 1.4	9.2 \pm 1.4	4.9 \pm 0.2	0.1 \pm 0.0

Results

Experimental conditions—The most important differences among the lakes were related to overall trophic conditions and to the structure of the zooplankton communities (Tables 1 and 2). Tyrrel Lake was the most eutrophic of the three systems based on chlorophyll concentrations (Table 1). Chlorophyll was lowest in Upton Lake and representative of midsummer conditions (Pace et al. 1990). Chlorophyll in West Long Lake was also typical of values found throughout the summer season (S. Carpenter unpubl. data).

Heterotrophic microbial communities were similar among the three lakes, with some gradients in abundance and size related to trophic conditions (Table 1). Bacteria were relatively abundant during experiments, particularly in the first Upton Lake experiment. Heterotrophic flagellates were at high abundances only in the second West Long Lake experiment (9,200 ml^{-1}). Otherwise, flagellate abundances were moderate and similar in the three lakes (Table 1). We did note that flagellates were larger in Tyrrel Lake; spherical diameters ranged from 5 to 10 μm compared to 3 to 5 μm in Upton Lake and 2 to 6 μm in West Long Lake. Ciliates were least abundant in West Long

Lake and most abundant in Tyrrel (Table 1). The most common species in all three lakes were oligotrichs of the genera *Halteria*, *Strombidium*, and *Strobilidium*. Other important ciliate taxa included *Mesodinium*, *Gymnostoma*, *Vorticella*, and *Askenasia*. In the second West Long Lake experiment *Paradileptus* sp. and the rotifer-feeding ciliate *Teuthophrys trisulca* (Hutchison 1967) were also present. Aside from these two large species, ciliate community biomasses were dominated by smaller species (15–30 μm in diameter).

Rotifers were more abundant in Tyrrel than in Upton and West Long (Table 1). The most common rotifers in all experiments were species of *Polyarthra* and *Keratella*. Together, these two genera represented >70% of the total community abundance in each experiment, with the exception of the second West Long Lake experiment when the colonial species, *Conochilus unicornis*, was numerically dominant. We excluded *Conochilus* colonies in our analyses of the experimental results because we assumed these colonial forms were too large to be affected by *Daphnia*. Other genera with significant representation in the Upton and Tyrrel experiments were *Trichocerca*, *Ascomorpha*, and *Collotheca*, while in West Long *Asplanchna* and *Pleosoma* accounted for 20%

Table 2. Average ($n = 3 \pm \text{SD}$) zooplankton concentration (animals liter^{-1}) in each lake enclosure experiment. (NP—not present.)

Lake	<i>Daphnia catawba</i>	<i>Daphnia galeata</i>	<i>Daphnia pulex</i>	<i>Bosmina</i> spp.	<i>Ceriodaphnia</i> spp.	<i>Holopedium</i> spp.	Copepods
Upton 1	NP	35.5 \pm 16.7	NP	NP	NP	NP	15.6 \pm 7.3
Upton 2	NP	24.7 \pm 4.7	NP	NP	NP	NP	12.3 \pm 4.7
Tyrrel 1	1.5 \pm 0.9	NP	NP	47.7 \pm 8.8	12.8 \pm 3.4	NP	20.0 \pm 6.2
Tyrrel 2	26.1 \pm 1.4	NP	NP	30.1 \pm 4.1	13.9 \pm 1.0	NP	55.2 \pm 3.5
W. Long 1	NP	NP	9.4 \pm 0.0	NP	NP	2.7 \pm 0.0	3.4 \pm 0.0
W. Long 2	NP	NP	44.3 \pm 10.1	0.1 \pm 0.2	NP	10.2 \pm 1.7	0.8 \pm 0.4

Table 3. Results of one-tailed *t*-tests of the hypothesis that growth rates (d^{-1}) were higher in treatments where zooplankton were removed. (NS—not significant.)

Lake	Exp.	Taxa	<i>t</i>	<i>P</i>
Upton	1	Flagellates	3.77	<0.025
		Rotifers	1.54	NS
	2	Flagellates	3.10	<0.025
		Ciliates	2.85	<0.025
Tyrrel	1	Rotifers	2.53	<0.05
		Flagellates	0.57	NS
		Ciliates	-0.02	NS
	2	Rotifers	0.80	NS
		Flagellates	1.19	NS
		Ciliates	1.79	<0.1
W. Long	1	Rotifers	0.89	NS
		Flagellates	1.75	<0.1
		Ciliates	1.60	<0.1
	2	Rotifers	1.06	NS
		Flagellates	8.20	<0.001
		Ciliates	1.86	<0.1
		Rotifers	0.19	NS

of the total abundance of noncolonial rotifers in the second experiment.

Zooplankton in the Upton experiments were primarily *D. galeata* and in the West Long experiments, primarily *D. pulex* (Table 2). Some copepods and cladocerans were also present, but these taxa were typically small and accounted for a minor component of the community biomass except in the second West Long experiment, where *Holopedium* was prominent (Table 2). In both Tyrrel Lake experiments, the enclosed zooplankton assemblage consisted of small species (*D. catawba*, *Bosmina* sp., *Ceriodaphnia* sp., and copepods). Copepods were 2–20-fold more abundant than *Daphnia* in Tyrrel Lake (Table 2).

Chlorophyll and bacteria—For all experiments bacteria and chlorophyll were similar between treatments with and without zooplankton after 24 h (Fig. 1). These parameters are an index of the resources available to protozoans, rotifers, and macrozooplankton. We conclude that the changes observed in our experiments were not complicated by changes in the overall level of resources.

Protozoan and rotifer growth rates—There were no differences in the net growth rates of heterotrophic flagellates and ciliates in the containers from which microzooplankton had been removed (<20 μm) compared to containers in which microzooplankton were present but macrozooplankton had been removed (<150

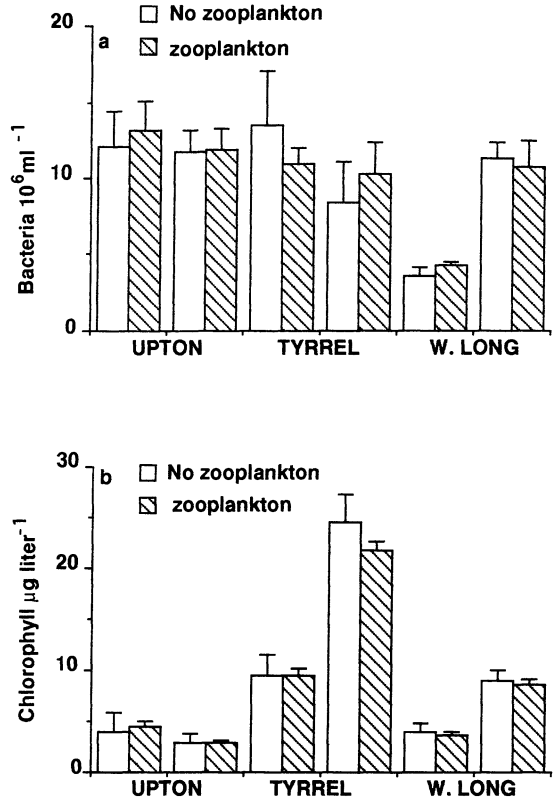


Fig. 1. Comparison of bacterial abundance and chlorophyll concentrations after 24 h in treatments with and without zooplankton. Two pairs of bars are presented for each lake representing the first and second experiments respectively. Values are means \pm SD.

μm). For example, in the second Upton experiment, mean flagellate growth (\pm SD) in the <20- μm treatment was $0.59 \pm 0.18 d^{-1}$ compared to $0.51 \pm 0.14 d^{-1}$ in the <150- μm treatment. Similar results were obtained in the other experiments (all *t*-tests: $P > 0.1$). We conclude that predation on protozoa by microzooplankton (e.g. nauplii, rotifers, and larger ciliates) was not significant in our experiments.

In the Upton and West Long experiments, growth rates of flagellates and ciliates were always positive in the absence of *Daphnia* and near 0 or slightly negative in the presence of *Daphnia* (Fig. 2). As hypothesized, protozoan growth rates were significantly lower in treatments with *Daphnia* (Table 3). In the Tyrrel Lake experiments (where only small-bodied

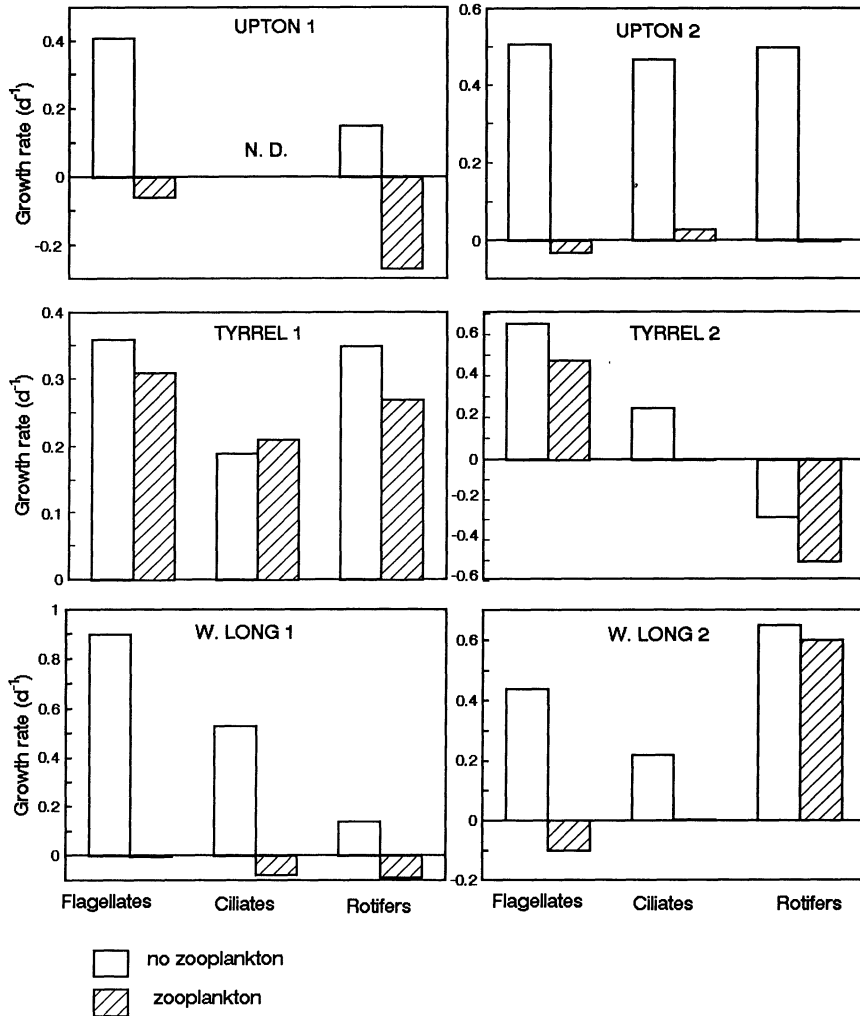


Fig. 2. Net growth rates of heterotrophic flagellates, ciliates, and rotifers in treatments with and without zooplankton. In several cases growth rates were near zero so bars are not visible. (N.D.—not determined.)

zooplankton were present), the growth rates of flagellates and ciliates were positive and similar between treatments with the exception of the second Tyrrel experiment, in which ciliate growth was reduced by zooplankton (Fig. 2, Table 3).

Rotifer growth rates were lower in containers with *Daphnia* in the Upton Lake experiments but not in the two West Long Lake experiments (Table 3). In the first West Long experiment, mean rotifer growth was lower in the *Daphnia* treatment (Fig. 2). There was, however, considerable variability among the replicates, and the mean difference was not

sufficiently large to distinguish an effect of *Daphnia*. In the second West Long experiment, rotifers other than *C. unicornus* were at low abundances and were not affected by *Daphnia*. Rotifer growth rates in the Tyrrel experiments were lower in the presence of zooplankton (Fig. 2), but these differences were not significant (Table 3).

Mortality rates of protozoa and rotifers in relation to Daphnia biomass and size—We initially predicted that protozoan mortality would be proportional to *Daphnia* size and biomass. We compared mortality rates (d_z , see Eq. 3) for all experiments as a function of *Daphnia*

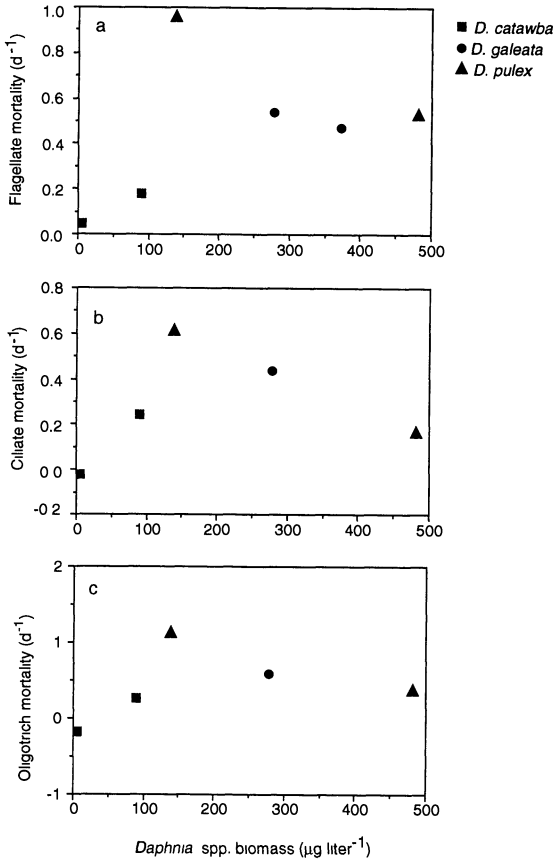


Fig. 3. Protozoan mortality in relation to *Daphnia* biomass for all experiments. *Daphnia catawba* was the dominant daphnid in the Tyrrel Lake experiments. *Daphnia galeata* was used in the Upton Lake experiments and *Daphnia pulex* in the West Long Lake experiments.

biomass and mean body size. In these comparisons, we assumed that *Daphnia* was the dominant consumer of protozoans in the Upton and West Long experiments, as suggested by relative abundances of zooplankton (Table 2). In the Tyrrel experiments, there were several other species present that probably consumed protozoans (e.g. *Ceriodaphnia*, *Bosmina*, and some of the copepods). In this case, we assumed that the low biomass of *Daphnia* was related to the low rates of mortality, but that the small species of *Daphnia* present in these experiments were not the only consumers of protozoans.

Mortality of heterotrophic flagellates and ciliates increased with *Daphnia* biomass (Fig.

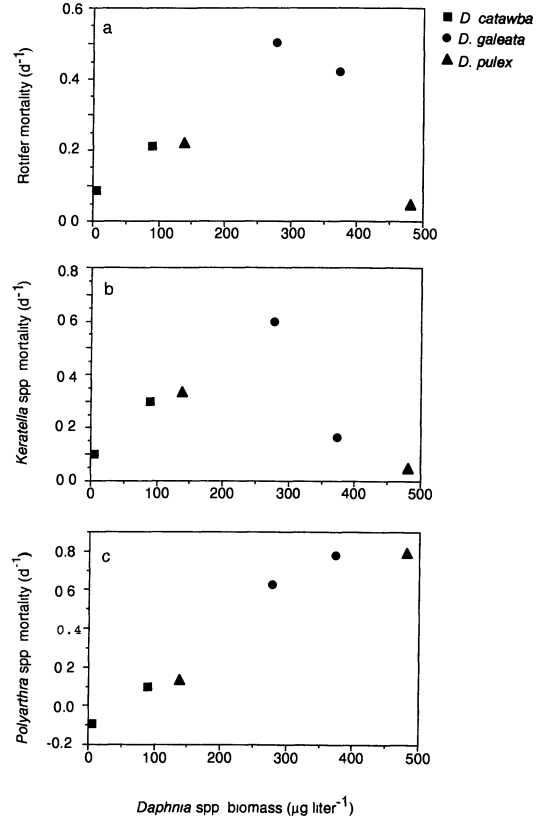


Fig. 4. As Fig. 3, but of rotifer mortality.

3). The highest loss rates of both flagellates and ciliates were observed in the first West Long experiment, where *D. pulex* was the dominant consumer. Mortalities in this case were 0.96 d^{-1} for flagellates and 0.62 d^{-1} for ciliates. Mortality rates for flagellates and ciliates were lower in the second West Long experiment despite relatively high standing stocks of these two groups (Table 1). Oligotrichs suffered the highest overall mortality among the ciliates in the presence of *Daphnia*. For example, in the first West Long experiment, mortality of this group exceeded 1 d^{-1} .

Rotifer mortality rates were also generally greater in treatments with higher *Daphnia* biomass (Fig. 4a), but the impact of *Daphnia* varied among genera and was dependent on rotifer community composition. For *Polyarthra*, mortality was linearly related to *Daphnia* biomass (Fig. 4b), while for *Keratella*, the effect of *Daphnia* was variable (Fig. 4c). We ob-

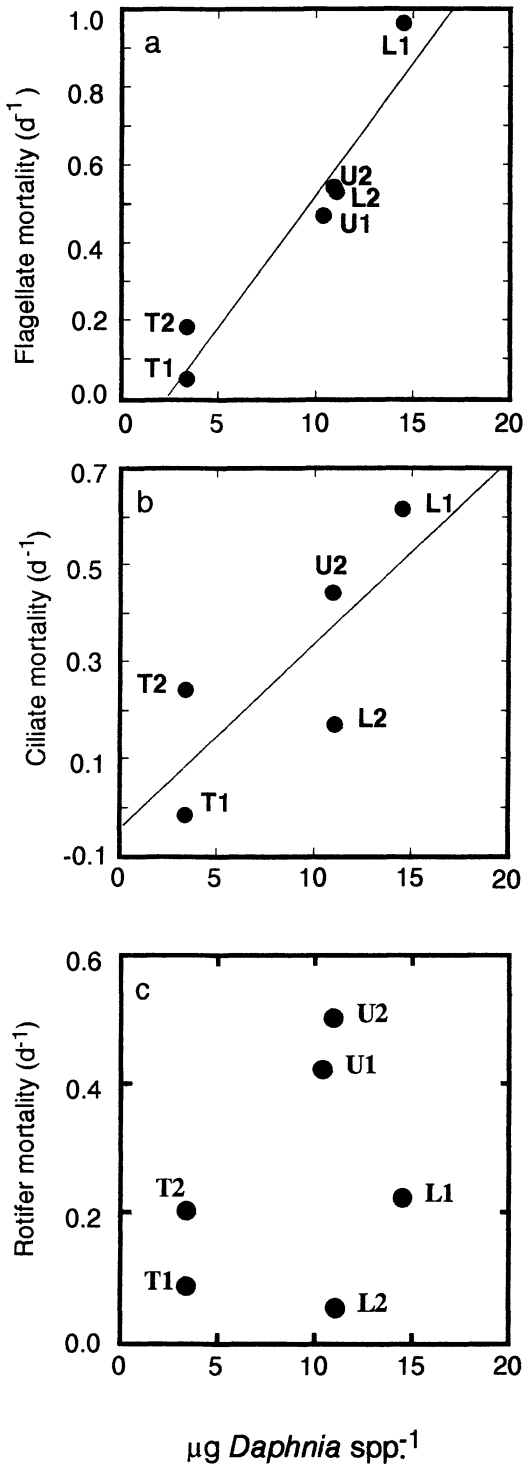


Fig. 5. Relationship between the mean body size of *Daphnia* used in the experiments and flagellate, ciliate, and rotifer mortality. Experimental lakes and experiment

served many apparently damaged rotifers, especially *Polyarthra*, in *Daphnia* treatments, suggesting interference mortality was important during our experiments (Gilbert 1988a,b).

The mean body size of *Daphnia* was more closely related to flagellate and ciliate mortality than to *Daphnia* biomass (Fig. 5a and b compared to Fig. 3a and b). Regressions of *Daphnia* biomass with flagellate, ciliate, and rotifer mortality explained little variation (all $r^2 < 0.16$, $P > 0.44$). Alternatively, the mean body size of *Daphnia* accounted for most of the variation in flagellate mortality among experiments ($r^2 = 0.92$, $P = 0.003$). The relationship between *Daphnia* size and ciliate loss rates was more variable ($r^2 = 0.61$, $P = 0.12$); however, if only the dominant oligotrichs are considered, mortality was more strongly related to *Daphnia* size ($r^2 = 0.78$, $P = 0.048$). Rotifer mortality was not well related to *Daphnia* size (Fig. 5c) even when major taxa (e.g. *Polyarthra*, *Keratella*) were considered separately (data not shown).

Experiment with different-sized Daphnia at equal biomass—In the experiment to test the effect of *Daphnia* size, we were able to achieve similar biomasses in the two treatments. In the bottles with large *D. pulex*, mean biomass (\pm SD) was $340 \pm 61.3 \mu\text{g dry wt}$ compared to $291 \pm 20.5 \mu\text{g dry wt}$ for bottles containing small *D. pulex*. There was a considerable difference in body size between the large and small *D. pulex* treatments. Large animals were typically 1.5 to >2 mm in total length; small animals were 0.7–1.4 mm long. These length differences equate to very large differences in body mass. In bottles with the large animals, average mass was $35.1 \pm 2.03 \mu\text{g dry wt ind.}^{-1}$ —almost 6 times greater than the small animals, which averaged $6.3 \pm 0.58 \mu\text{g dry wt ind.}^{-1}$. As in the other experiments (Fig. 1), bacterial abundances were similar among treatments at the end of the 24-h experimental period (data not shown), but unlike prior experiments, chlorophyll was lower in the two treatments with *Daphnia* than in the treatment without *Daphnia*. The decline in chlorophyll, however, was

←

numbers noted adjacent to symbols: T—Tyrrel; U—Up-ton; L—West Long.

the same (net change = -0.22 d^{-1}) in the two treatments with *Daphnia*, so the general effects of *Daphnia* size described below were not confounded by large differences in resource levels between these two treatments.

Growth rates of heterotrophic flagellates (3–4 μm), oligotrichs (16–18 μm), and a large species of *Cryptomonas* sp. (21–24 μm) were lowest in the treatments with large *D. pulex*, intermediate with small *D. pulex*, and highest when *D. pulex* was not present (Fig. 6). This pattern is consistent with our hypothesis that protozoa are disproportionately affected by larger *Daphnia*. Variability among replicates, however, was high in this experiment (Fig. 6). Only heterotrophic flagellate growth was significantly (*t*-test: $P < 0.05$) lower in the large *D. pulex* treatment than in the treatment with small individuals.

There were no differences in the sizes of heterotrophic flagellates, oligotrichs, or *Cryptomonas* in the three treatments after 24 h (data not shown), indicating that *Daphnia* did not alter the size distribution of the three prey groups we analyzed. Oligotrichs, although smaller than *Cryptomonas*, appeared less susceptible to predation by *Daphnia* (Fig. 6). Lower predation on oligotrichs may be related to escape responses exhibited by some of these species (Jack and Gilbert 1993).

Discussion

Large species of *Daphnia* can have a strong influence on the abundance and composition of phytoplankton communities, primary productivity, water transparency, sedimentation, and various other ecosystem processes (e.g. Vanni et al. 1990; Carpenter et al. 1991; Carpenter and Kitchell 1993). Large *Daphnia* may also be important in determining the abundance and pathways of energy and material flow through microbial food webs (Stockner and Porter 1988; Pace et al. 1990; Wylie and Currie 1991). Our experiments demonstrate that high mortality rates are imposed on both heterotrophic flagellates and ciliates by large *Daphnia*. The overall ability of *Daphnia* to regulate protozoan abundance in lakes depends on the balance between growth, *Daphnia*-induced mortality, and other sources of protozoan mortality. In all of our experiments, growth rates were positive and high in treat-

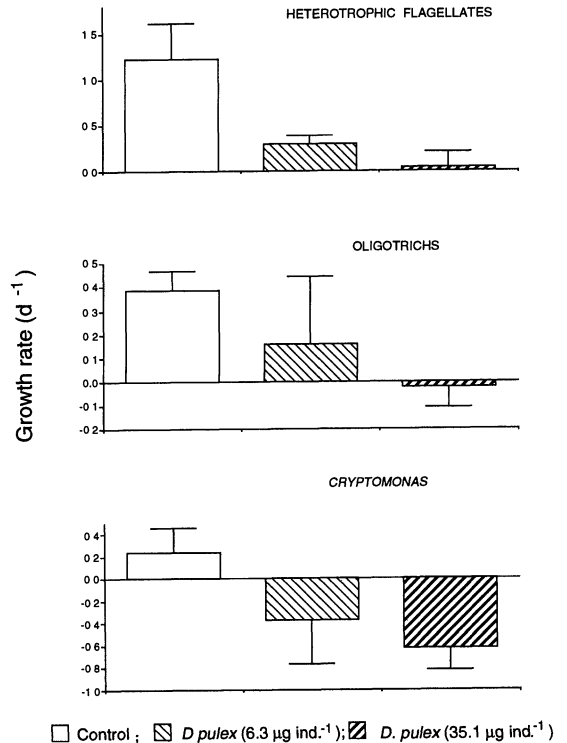


Fig. 6. Mean growth rates (\pm SD) of heterotrophic flagellates, oligotrichs, and *Cryptomonas* sp. in treatments with no (=control) *Daphnia*, with small *Daphnia*, and with large *Daphnia*.

ments without zooplankton (Figs. 2 and 6). Net growth was near 0 in containers with *Daphnia* in Upton and West Long but not in Tyrrel. Resources, therefore, appeared to be adequate to support high protozoan growth rates in all lakes, but this productivity was rapidly harvested by *Daphnia* in Upton and West Long.

Our results are consistent with other field measurements of zooplankton predation on protozoans. In Lake Michigan, nano- and microprotozoa mortality rates were 0.1–0.5 and 0.02–0.17 d^{-1} , respectively (estimated from data of Carrick et al. 1991). Mortality rates were generally higher in the three lakes we studied (Fig. 3), as would be expected since Lake Michigan is more oligotrophic than the systems we studied. Also as in our experiments, zooplankton consumption in Lake Michigan accounted for a major fraction of the estimated growth rates of protozoa (Carrick et

al. 1991). Both copepods and cladocerans appeared to be important consumers of protozoa in Lake Michigan, with highest loss rates measured during periods when *D. galeata* was a significant component of the zooplankton biomass (Carrick et al. 1991). Longer term enclosure studies in a variety of lakes have also documented that zooplankton, especially *Daphnia*, reduce the abundance and net growth rates of protozoa (e.g. Pace and Funke 1991; Wickham and Gilbert 1991, 1993).

We were primarily interested in the community responses of protozoans and rotifers, and so we did not focus on differences among species. In the case of heterotrophic flagellates, it is generally impossible to identify species with current epifluorescent methods (Lim et al. 1993). The relatively uniform pattern of mortality as a function of *Daphnia* body size, however, suggests that most flagellate species must be strongly impacted by predation from *Daphnia*. For ciliates, some taxonomic resolution is possible, although a full analysis of species composition requires a more difficult staining and analysis procedure (Montagnes and Lynn 1993).

Ciliate species vary in their susceptibility to predation by *Daphnia* (Jack and Gilbert 1993), but we found consistent strong differences in ciliate mortality between treatments with and without *Daphnia*, especially for the dominant oligotrichs. Our results lead us to predict a general pattern similar to the one Gasol and Vaqué (1993) found for flagellates: ciliate abundances will be lower in communities dominated by *Daphnia* and in lakes during times of high *Daphnia* abundance.

The individual responses of rotifer species to *Daphnia* have also been well documented (Gilbert 1988a,b). In keeping with these observations, rotifer communities were the most variable in their responses to our experimental manipulations. *Daphnia* may act more strongly in determining community composition than the overall abundance and biomass of rotifers in lakes.

It is well known from marine studies that copepods eat ciliates (Stoecker and Capuzzo 1990; Gifford 1991), but little work has been done on the consumption of protozoa by copepods in freshwater. Freshwater copepods also eat ciliates (Gifford 1991), but many heterotrophic flagellates may be too small to be ef-

fectively consumed by copepods. For our study, we intentionally chose lakes with zooplankton communities dominated either by large cladocerans or mixed assemblages of small cladocerans and copepods. Further work on the regulation of protozoa by crustacean zooplankton needs to consider communities dominated by various types of copepods.

Others have demonstrated that microzooplankton, including ciliates and rotifers, can be important predators of heterotrophic flagellates (McManus and Fuhrman 1990; Weisse et al. 1990; Dolan and Gallegos 1991). Over 24 h, we did not observe significant differences in flagellate growth rates between treatments in which we used water filtered through 20- μm nets and those in which we used 150- μm nets. This result implies that in Upton, Tyrrel, and perhaps other lakes, the microzooplankton are not significant consumers of heterotrophic flagellates.

Bacterial abundance did not change in the containers over the experimental period, and chlorophyll concentrations were lowered in only one case. Consumption of protozoans in zooplankton treatments might have been expected to result in increases in bacteria; however, we have previously demonstrated that cladocerans can be the major consumers of bacteria in lakes and that changes in protozoan abundance are not necessarily coupled to changes in bacteria (Pace et al. 1990; Pace and Funke 1991; Vaqué and Pace 1992). We did not expect large changes in chlorophyll over 24 h based on prior experiments in which we observed either little reduction in chlorophyll (Pace and Funke 1991) or low chlorophyll-specific grazing rates (0.03–0.17 d⁻¹) (Cyr and Pace 1992) in these systems. The reduced concentration of chlorophyll observed in the Upton experiment with two size classes of *D. pulex* was reasonable given the higher abundances of *Daphnia* used in this experiment than in in situ conditions (Table 2).

We initially predicted that mortality rates of protozoa and rotifers would be proportional to *Daphnia* biomass. There was such a trend, but the large differences between the two West Long experiments resulted in considerable scatter in the pattern (Figs. 3 and 4). Flagellate mortality was very high in the first experiment, whereas ciliate and rotifer mortalities were relatively low in the second experiment. Initial

conditions, including differences in *Daphnia* size and differences in flagellate, ciliate, and rotifer community composition, probably account for the differences between the two West Long experiments. Mean body size of *Daphnia* was $14.5 \mu\text{g dry wt ind.}^{-1}$ in the first experiment and $11.1 \mu\text{g dry wt ind.}^{-1}$ in the second experiment. The overall trend of steep increases in the mortality rate of flagellates as a function of *Daphnia* size (Fig. 5) explains the large difference in flagellate mortality between the two experiments.

In the case of ciliates, more large species were present in the second West Long experiment (e.g. *T. trisulca*), and these taxa were not greatly affected by *Daphnia*. Further, ciliates vary in their susceptibility to predation by *Daphnia* as a function of their size and avoidance abilities (Jack and Gilbert 1993), so differences among ciliate species will introduce variability in community response. Similarly, the rotifer community varied and was dominated by a colonial species in the second West Long experiment; other rotifers were at low abundance.

Daphnia size appears to be an important factor determining the impact of zooplankton on protozoa and other components of lake ecosystems. In the experiment with large and small *Daphnia*, we established equal biomasses of the two size classes. From the average length and number of animals in each replicate, we can estimate the expected grazing rate by using the allometric equation of Knoechel and Holtby (1986). Ignoring prediction error for the filtering rate equation, we estimated average grazing rates (\pm SD) of 631 ± 44 and 535 ± 84 ($\text{ml liter}^{-1} \text{d}^{-1}$) in the small and large *D. pulex* treatments. Based on these estimates, we would have expected mortality rates in the two treatments to be similar if large and small animals were equally efficient at feeding on the three different food types we analyzed. Instead, we observed differential consumption of the smallest (i.e. heterotrophic flagellates)—not the largest—particles (i.e. *Cryptomonas*).

The presumed higher grazing on small particles may be related primarily to experimental conditions because variability was high and replication low within treatments, so that we were only able to detect one statistically significant result. There was at least a trend in the data that suggests large *D. pulex* grazed more

heavily on all three food particles (Fig. 6). Our experimental design was probably insufficient to detect this effect. These results suggest that large cladocerans such as *D. pulex* may have higher grazing rates within the size spectra of particles consumed by most zooplankton, but this suggestion requires further experimental analysis. The disproportionate effect of grazing by large *Daphnia* may be a function of the higher biomass attained by these animals, their ability to feed on larger particles, and the possibility that they also achieve higher mass-specific grazing rates in situ (Cyr and Pace 1993).

The most important finding of our study is that the mortality rates of protozoans and rotifers in association with *Daphnia* were substantial and roughly equal to their in situ growth rates. Larger species of *Daphnia* appear to exert considerably greater mortality on protozoans and rotifers than on zooplankton communities dominated by a mixture of small copepods and cladocerans. Our results provide a mechanism that explains the observations from a comparative study (Gasol and Vaqué 1993) that the abundance of heterotrophic flagellates in lakes is negatively related to the abundance of cladocerans, especially *Daphnia*. Our results emphasize that large *Daphnia* can consume a large size spectrum of resources, from the smallest bacteria to many of the larger phytoplankton, ciliates, and rotifers. As a consequence, large *Daphnia* can exploit the production of both autotrophic and heterotrophic components of the food web and hence play a key role in regulating lake ecosystem processes.

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