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## REGULATION OF PLANKTONIC MICROBIAL COMMUNITIES BY NUTRIENTS AND HERBIVORES<sup>1</sup>

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**Abstract.** We conducted a series of single-factor and factorial experiments in two north-temperate lakes (Peter and Paul lakes, Gogebic County, Michigan, USA) to study regulation of heterotrophic microbial communities. In single-factor experiments large *Daphnia* were added to enclosures, and these were compared to enclosures without *Daphnia*. These treatments contrast the major food-web configurations that occur in these lakes as a consequence of cascading trophic interactions. Bacterial abundance and the incorporation of methyl [<sup>3</sup>H]-thymidine into DNA by bacteria were similar in treatments with and without *Daphnia*. Ciliates were significantly reduced while heterotrophic flagellates were only marginally reduced by *Daphnia* additions in the Peter Lake experiments. Protozoans were not affected by *Daphnia* additions in the Paul Lake experiment. Factorial experiments compared the relative significance of large daphnids and nutrients as regulators of bacteria and protozoa. Treatments included controls, *Daphnia* additions, nutrient (N plus P) additions, and a combined *Daphnia*-nutrient treatment. Bacteria responded to nutrient additions but not to *Daphnia*. Protozoa, on the other hand, were strongly affected by *Daphnia* and to a lesser extent by nutrients. These experiments suggest that the trophic cascade is truncated at the level of protozoans. Protozoa appear to be partially regulated by predators, whereas bacteria show no response to changes in either *Daphnia* or protozoa.

**Key words:** *aquatic food webs; bacterial production; bottom-up vs. top-down control; ciliates; heterotrophic flagellates; heterotrophic microbial communities; plankton; protozoa; trophic cascade; zooplankton.*

### INTRODUCTION

Inconspicuous heterotrophic microbes drive many ecosystem processes as a consequence of their metabolism. Environmental regulation of microbial metabolism and hence biogeochemical cycling has been widely considered and summarized in a number of texts (e.g., Fenchel and Blackburn 1979, Bolin and Cook 1983, Brock et al. 1984, Paul and Clark 1989). Ecological constraints on heterotrophic microbial activity, particularly those related to food-web interactions, is a relatively new subject (e.g., Pomeroy 1974, Coleman et al. 1978, Hobbie and Williams 1984, Ingham et al. 1985). In this context, ecological constraints refer to the resource, or bottom-up, and predatory, or top-down, controls on heterotrophic microbial biomass and productivity. Many bacteria and protozoa are considered to live in extremely resource-poor environments (Fenchel 1982, Sieburth 1984, Wright 1984, Morita 1985, Williams 1985); therefore, mechanisms controlling resource supply are likely to be important. Consumers may regulate heterotrophic microbial processes in at least three ways: (1) by direct predation, (2) by indirect effects on microbial resources such as stimulation or, alternatively, inhibition of nutrient recycling, and (3) by changing microbial habitats. The significance of consumer regulation of microbial metabolism, partic-

ularly relative to processes that control microbial resources, is poorly known.

In aquatic ecosystems nutrients and predators are important regulators of plankton biomass and productivity. Nutrient loading explains a substantial amount of the variation among lakes in algal biomass and primary production (Nicholls and Dillon 1978, Schindler 1978, Smith 1979, 1982, McCauley et al. 1989). Furthermore, lake nutrient status predicts zooplankton biomass in comparisons among lakes (Hanson and Peters 1984, Pace 1986). Whole-lake experimental and comparative studies have also demonstrated the profound impact of vertebrate predators on lake food webs (Hrbacek et al. 1961, Brooks and Dodson 1965, Benndorf et al. 1984, Shapiro and Wright 1984, Carpenter et al. 1987). Predatory interactions cascade down the food web from top-level piscivores to the phytoplankton (Carpenter et al. 1985). Changes in the vertebrate planktivory influence the size structure of zooplankton communities, and changes in zooplankton size structure may have an impact on phytoplankton equal to that of changes in nutrient loading (Carpenter et al., *in press*).

These qualitative and quantitative models of plankton dynamics have focused on explaining variability in phytoplankton and zooplankton. Comparatively little is known about how nutrients and consumers may interact to regulate planktonic heterotrophic microbes. Recent studies of these organisms—including bacteria,

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flagellated protozoa, and ciliated protozoa—reveal that they account for a major portion of the biomass, respiration, nutrient recycling, and productivity of the pelagic zone in both marine and freshwater environments (Williams 1984, Porter et al. 1985, Riemann and Sondergaard 1986, Caron and Goldman 1988, Cho and Azam 1988, Cole et al. 1988, Stockner and Porter 1988). Analyses of carbon flow through food webs have documented that more than half the carbon fixed by phytoplankton moves through bacteria (Azam et al. 1983, Ducklow 1983, Pace et al. 1984, Cole et al. 1988, Vézina and Platt 1988). Given the quantitative significance of heterotrophic microbes (in this paper referring collectively to bacteria and protozoa), a major challenge is to identify factors controlling the biomass and productivity of these organisms. As a beginning, we can ask if heterotrophic microbes, like phytoplankton and zooplankton, are regulated by nutrient loading and cascading trophic interactions.

In this study, we addressed this question with enclosure experiments performed in two lakes where cascading trophic interactions have been studied in detail. Experimental manipulations of fish populations in several lakes located at the University of Notre Dame Environmental Research Center have demonstrated changes in the structure of aquatic communities as a consequence of the balance between piscivory and planktivory (Carpenter et al. 1987, Carpenter and Kitchell 1988). The plankton in these lakes shift between two configurations. When planktivory is strong, a small zooplankton community, consisting primarily of small species of cladocerans and copepods, dominates (Carpenter et al. 1987, Elser and Carpenter 1988). When piscivory is strong, a large zooplankton community consisting primarily of the large cladoceran *Daphnia pulex* dominates. Differences in the zooplankton community, in turn, affect phytoplankton community composition and primary production (Carpenter et al. 1987, Elser and Carpenter 1988). To test the effect of top-down controls on heterotrophic microbes, we conducted a set of single-factor experiments in Peter and Paul lakes comparing enclosures with and without large *Daphnia*. These experiments mimic at a small scale the major contrast that occurs in these lakes in response to food-web conditions. To investigate the relative importance of nutrient loading and cascading interactions, we conducted a set of factorial experiments in Peter and Paul lakes comparing fertilized and unfertilized enclosures that either contained or excluded large *Daphnia*. These experiments address the relative significance of nutrient loading and cascading trophic interactions on heterotrophic microbes.

## METHODS

### *Study site*

Experiments were carried out during the summers of 1988 and 1989 in Peter and Paul lakes, which have

an extensive history of experimental limnology. An analysis of changes in these lakes over the last 45 yr has recently been presented by Leavitt et al. (1989). Basic limnological characteristics as well as information on phytoplankton and zooplankton communities are presented elsewhere (Elser et al. 1986, Carpenter et al. 1987, Elser and Carpenter 1988).

### *Experimental design*

We carried out paired experiments in each lake using 45-L plastic enclosures anchored to a floating frame described by Elser et al. (1988). Enclosures were filled with surface lake water filtered through a 150- $\mu\text{m}$  net to remove large zooplankton. Experiments were run for 4 d, because previous studies indicated that unmanipulated enclosures are indistinguishable from the lake for a period of 4 d and that treatment effects are apparent within 4 d (Bergquist and Carpenter 1986). This experimental time scale allows for a potential response of several generations of the heterotrophic microbes, depending on growth rates, while limiting the ability of zooplankton to change substantially from initial densities.

The initial set of experiments tested for differences in the effect of *Daphnia* on heterotrophic microbes (Fig. 1a). Experiments were run simultaneously in Peter (1988 June 24–28) and Paul (1988 June 25–29) lakes. *Daphnia* were collected the night prior to initiating the experiment, sorted in batches of 30–50 animals, and added to bottles containing filtered lake water (Whatman glass fiber [GF/F] filters). The *Daphnia* were sampled from a population dominated by *D. pulex* with some *D. rosea*. We added both species to the enclosures and discuss only the aggregate effect of *Daphnia* below, but *D. pulex* was the primary species in the enclosures. Animals were stored overnight in the filtered lake water at in situ temperatures (experimental temperatures ranged from 20° to 25°C). Prior to adding *Daphnia* to the enclosures, we gently rinsed the animals onto a 35- $\mu\text{m}$  mesh net while washing with filtered water. This procedure removed any dissolved and particulate nutrients excreted or egested by the *Daphnia* during the previous 12 h. Each treatment (*Daphnia* addition and controls) was run in triplicate.

To test the joint effects of nutrients and large daphnids, we performed a pair of factorial experiments in Peter and Paul lakes (Fig. 1b). Treatments were controls, nutrient additions, *Daphnia* additions, and combined *Daphnia*-nutrient additions. Each treatment was run in triplicate. Nutrients were added as  $\text{NH}_4\text{Cl}$  and  $\text{KH}_2\text{PO}_4$  to establish initial nutrient concentrations of 10  $\mu\text{mol/L}$  of N and 1  $\mu\text{mol/L}$  of P. Atomic N:P ratios of 10:1 are close to those typically found in Peter and Paul lakes, and previous studies of nutrient limitation in these lakes have suggested that algae respond most strongly to joint additions of N and P (Elser et al. 1988). *Daphnia* additions were carried out in the same manner as described for the single factor experiments. The

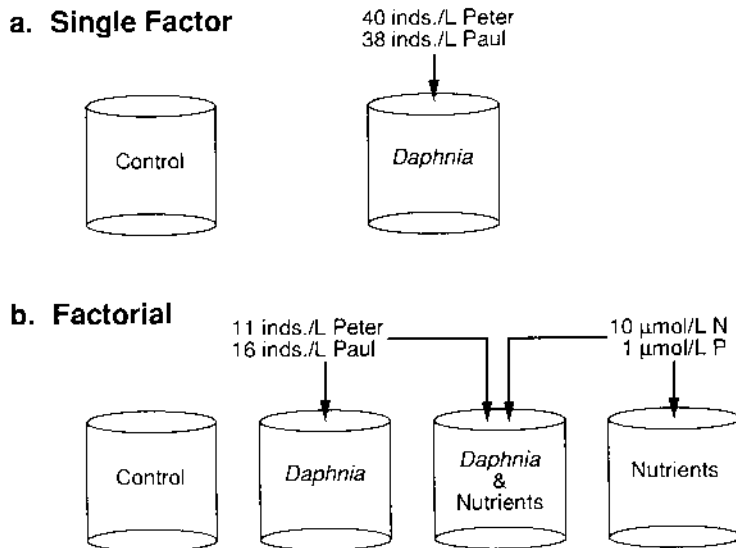


FIG. 1. Schematic representation summarizing enclosure treatments in Peter and Paul lakes in the (a) single-factor and (b) factorial experiments. There were three replicates of each 45-L treatment enclosure.

Peter Lake factorial experiment was carried out 24–28 August 1988, while the Paul Lake experiment was done a year later: 20–24 August 1989.

#### Response parameters—sampling and analysis

Homogeneity of the enclosures was tested initially by taking samples at the beginning of each experiment for chlorophyll *a* (hereafter, "chlorophyll"). At the end of each experiment samples were also taken from each enclosure to measure chlorophyll, bacterial abundance, thymidine incorporation, heterotrophic flagellates, and ciliates. Zooplankton in the enclosures were captured by pouring the contents through a 150- $\mu$ m net and preserving the sample in a sucrose-formalin solution.

Chlorophyll samples were filtered through GF/F filters, frozen, and subsequently extracted in methanol, and pigments were measured fluorometrically using the acid-correction method (Parsons et al. 1984).

Bacterial abundance was determined using the acridine orange direct-count method of Hobbie et al. (1977). At least 800 bacterial cells were enumerated from every sample. Incorporation of a nucleic acid precursor, methyl  $^3$ H-thymidine, into DNA was measured following the protocol of Findlay et al. (1984). Thymidine incorporation by bacteria is related to DNA synthesis rates and has been used as a measure of bacterial production (Fuhrman and Azam 1980, 1982, Riemann and Sondergaard 1986). From each enclosure, four 10-mL samples were taken, and 7.4 kBq of  $^3$ H-thymidine (specific activity: 3.0 PBq/mol) were added to each sample. Preliminary experiments indicated that 7.4 kBq of thymidine were sufficient to saturate incorporation. One of the samples was immediately killed by adding 2 mL of a 5% formalin solution. This sample controlled for non-biological incorporation of the radiolabel. Samples were incubated in the

dark at in situ temperature for 1 h, filtered on 0.2- $\mu$ m polycarbonate filters, and rinsed several times (including the filter edge) with a 5% trichloroacetic acid solution. Filters were frozen and subsequently extracted using acid-base hydrolysis to isolate DNA (Findlay et al. 1984). Radioactivity associated with the DNA fraction was assayed using a liquid scintillation counter. Counts were corrected for quench. Disintegrations per minute incorporated into DNA were corrected by subtracting counts in the killed control.

The abundance of heterotrophic flagellates (i.e., flagellates without chloroplasts) was determined by epifluorescence microscopy using the stain proflavin at a mass-to-volume concentration of 0.033% (Haas 1982). Duplicate subsamples of 10–30 mL were stained for 2 min with 4  $\mu$ L of stain per millilitre of sample, preserved with addition of 10% glutaraldehyde (final concentration 1%), and filtered on 1.0- $\mu$ m polycarbonate filters under low vacuum (<13 kPa). The filters were air dried, sandwiched between two drops of non-fluorescent immersion oil, and frozen. At least 50 heterotrophic flagellates were enumerated on each filter by accumulating counts of cells in randomly selected fields. This epifluorescent method does not provide sufficient resolution for taxonomic categorization of the flagellates, so we consider here only the community-level effects of the manipulations. We did not consider mixotrophic flagellates, because these species are not easily distinguished from strictly autotrophic forms.

Ciliated protozoans were preserved by adding a 100-mL water sample to 1 mL of Lugol's iodide solution. Ciliates were concentrated from this sample by settling 50–100 mL of it in a graduated cylinder overnight. The overlying solution was then gently aspirated, and the remaining 8–10 mL of concentrated sample resettled in 10-mL plankton-counting chambers. The entire

sample was then enumerated with an inverted microscope at a magnification of 200 $\times$ .

Crustacean zooplankton were enumerated with a stereomicroscope (25 $\times$ ). At least 350 *Daphnia* were counted in subsamples from *Daphnia* enclosures, and an approximately equal volume of the sample was counted from enclosures that did not contain *Daphnia*. Biomass of *Daphnia* was determined by measuring body lengths of 50 individuals per enclosure, converting lengths to body mass using length-mass regressions (McCauley 1984), calculating mean mass (Bird and Prairie 1985), and estimating biomass from density and mean mass.

#### Statistical analysis

Treatment effects were assessed in the single-factor experiments using *t* tests. Reduction in heterotrophic microbes or thymidine incorporation in *Daphnia*-addition treatments relative to controls indicates either a direct effect of grazing by *Daphnia* or possibly an indirect effect through changes in microbial resources (e.g., a parallel reduction in phytoplankton). A two-factor analysis of variance (ANOVA) was used to assess the impact of *Daphnia* and nutrients on the heterotrophic microbial response variables. We tested the hypotheses: (1) no effect of *Daphnia*, (2) no effect of nutrients, and (3) no interaction between *Daphnia* and nutrients. Reductions in heterotrophic microbes or thymidine incorporation in *Daphnia* treatments are interpreted as in the single-factor experiments. Increases in microbes or thymidine incorporation with nutrient addition are interpreted as a response to increases in resources. A significant interaction between the *Daphnia* and nutrient treatments indicates a complex non-additive response to the joint effect of these treatments (for an ecologically oriented discussion see Morin et al. 1988).

## RESULTS

### *Treatment integrity and homogeneity of initial conditions*

Our initial additions of *Daphnia* were designed to add about 10 animals per litre to the enclosures. In order to avoid additional handling of the animals, we did not measure initial *Daphnia* densities. There was recruitment of *Daphnia* in the enclosures in the two single-factor experiments, resulting in final densities that were higher than our target density of 10 animals/L (Fig. 1a). *Daphnia* dry biomass varied from 240 to 520  $\mu\text{g/L}$  in the Paul Lake enclosures and 250 to 360  $\mu\text{g/L}$  in the Peter Lake enclosures. Other crustaceans occurred at low abundances: 5–29 copepod nauplii/L, <5 post-naupliar copepods/L. In the factorial experiments there was relatively little recruitment, and *Daphnia* densities were in the range of 8–18 animals/L (dry biomass 160–180  $\mu\text{g/L}$  in Peter Lake; Paul Lake not measured), which was reasonably close to the target

density. Other crustaceans were present in the enclosures at the end of the experiments at low abundance: 1–10 copepod nauplii/L, <3 post-naupliar copepods/L.

In both the single-factor and factorial experiments, *Daphnia* densities in the treatments that did not receive additions were 0.0–0.5 animals/L except in two enclosures in the single-factor experiments where densities were 1.7 animals/L. Given the differences in abundance of *Daphnia* between treatments and the low density of other crustacean zooplankton, we judged these manipulations successful.

At the beginning of each experiment we measured chlorophyll in the enclosures prior to establishing treatments. There was no difference among treatments except in the Peter Lake factorial experiment where there was a small initial difference ( $P = .03$ ) between enclosures receiving nutrients ( $\bar{X} = 4.23$ ) and those without nutrients ( $\bar{X} = 3.89$ ). This difference is small relative to the differences we discuss below (see *Daphnia- and nutrient-addition experiments*), which we believe can be attributed to the nutrient treatment. We cannot, however, exclude the possibility that final differences in chlorophyll in this experiment arose from the initial slightly higher levels of chlorophyll in enclosures receiving the nutrient additions.

### *Daphnia-addition experiments*

During late June of 1988, chlorophyll, bacteria, thymidine incorporation, and heterotrophic flagellates were, as is typical, higher in Paul Lake than in Peter Lake (S. Carpenter and M. Pace, unpublished data). The primary contrast between the lakes, however, was that *Daphnia* spp. were relatively abundant in Paul Lake (9 animals/L) and virtually absent in Peter Lake (<0.1 animals/L).

Additions of *Daphnia* had minimal effects in the single-factor experiments (Table 1). Chlorophyll in enclosures with *Daphnia* was significantly lower in Peter Lake, but not in Paul Lake. Means of bacterial abundance and thymidine incorporation were lower in each case in the *Daphnia* treatment, but these differences were small and not significant. Similarly, *Daphnia* had no effect on either the heterotrophic flagellates or the ciliates in the Paul Lake experiment. In contrast in the Peter Lake experiment, mean ciliate and heterotrophic flagellate abundances in the *Daphnia*-addition treatment were 55% and 77% respectively of the control (Table 1). Differences in flagellates were of marginal significance ( $P = .12$ ), while ciliates were clearly different in the two treatments ( $P = .01$ ). These experiments indicate that increases in *Daphnia* have little effect on the bacteria, but may reduce protozoan densities. The stronger effects of *Daphnia* additions on protozoans in Peter Lake may be related to the low natural abundance of *Daphnia* in that lake at this time. The protozoan community present in Peter Lake may have been more susceptible to grazing.

TABLE 1. Results of single-factor experiments comparing control enclosures (C) and enclosures with *Daphnia* (D) in Paul and Peter lakes. Data are means  $\pm$  1 SE,  $n = 3$ . *P* refers to the probability of a difference between treatments based on a *t* test.

Lake	Treatment	Chlorophyll ( $\mu\text{g/L}$ )	Bacteria ( $10^9$ cells/L)	$^3\text{H}$ -Thymidine incorporation ( $\text{pmol}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ )	Heterotrophic flagellates ( $10^6$ inds./L)	Ciliates ( $\times 10^3$ inds./L)
Paul	C	8.16 $\pm$ 0.22	4.80 $\pm$ 0.24	20.1 $\pm$ 1.98	3.46 $\pm$ 0.01	2.22 $\pm$ 0.16
	D	7.62 $\pm$ 0.27	4.67 $\pm$ 0.31	18.6 $\pm$ 2.47	3.47 $\pm$ 0.43	1.99 $\pm$ 0.09
	<i>P</i>	0.19	0.80	0.67	0.98	0.27
Peter	C	7.52 $\pm$ 0.45	4.55 $\pm$ 0.45	12.1 $\pm$ 1.16	1.39 $\pm$ 0.16	1.50 $\pm$ 0.23
	D	5.38 $\pm$ 0.15	4.32 $\pm$ 0.16	11.7 $\pm$ 1.18	1.07 $\pm$ 0.08	0.82 $\pm$ 0.10
	<i>P</i>	0.002	0.65	0.81	0.12	0.01

#### *Daphnia*- and nutrient-addition experiments

Chlorophyll, bacteria, thymidine incorporation, and heterotrophic flagellates were similar in Peter and Paul lakes during late August when the factorial experiments were performed (S. Carpenter and M. Pace, unpublished data). The primary contrast between the lakes was that *Daphnia* spp. were very abundant in Peter Lake (15 animals/L) relative to Paul Lake (2.5 animals/L).

**Phytoplankton.**—In the factorial experiments chlorophyll increased in response to nutrient additions, and in the combined nutrient-*Daphnia* treatment this increase was ameliorated by grazing (Fig. 2). ANOVA revealed that the main effects, nutrients and *Daphnia*, were highly significant in the Peter Lake experiment, and there was a significant interaction (Table 2). In the Paul lake experiment *Daphnia* alone had no effect on chlorophyll concentrations, but nutrient additions caused dramatic increases in chlorophyll. Again in this experiment there was a significant interaction between *Daphnia* and nutrients.

The significant interaction in both experiments reflects the difference in the effect of *Daphnia* at low- and high-nutrient conditions. Under low-nutrient conditions *Daphnia* influence phytoplankton both by nutrient regeneration and by grazing. The net effect of these positive and negative feedbacks from *Daphnia* on phytoplankton biomass is near zero. Under high-nutrient conditions *Daphnia* crops some of the increase in phytoplankton, and nutrient regeneration is unimportant. The net effect of *Daphnia* in this case is negative.

**Bacteria.**—In both the Peter and Paul lakes experiments, bacteria increased in response to nutrient additions, and *Daphnia* had no effect (Fig. 3, Table 2). The abundance of these cells appears, therefore, to be determined in these experiments primarily by bottom-up (=resource) controls. Bacteria may have responded to increases in phytoplankton, which are a major source of organic carbon for bacteria in pelagic systems (Cole 1982), or directly to nutrients if nitrogen or phosphorus were limiting growth.

Bacterial abundance provides an integrated measure of the response of the community over the 4-d experiment. Thymidine incorporation into DNA provides

a measure of the rate of bacterial growth on the final day of the experiment. The most rapid incorporation rates were observed in the nutrient addition and combined treatments (Fig. 4), and nutrients were the most important factor determining the rate of thymidine incorporation (Table 2). The effect of *Daphnia* additions was mixed. In Peter Lake *Daphnia* had a significant positive effect on thymidine incorporation, whereas in Paul Lake there was no significant effect of *Daphnia* (Table 2). Both phytoplankton and zooplankton release dissolved organic matter, and bacterial growth may be a function of the increase in labile substrates in the treatment enclosures.

In the Paul Lake experiment the interaction term for thymidine incorporation was of marginal significance

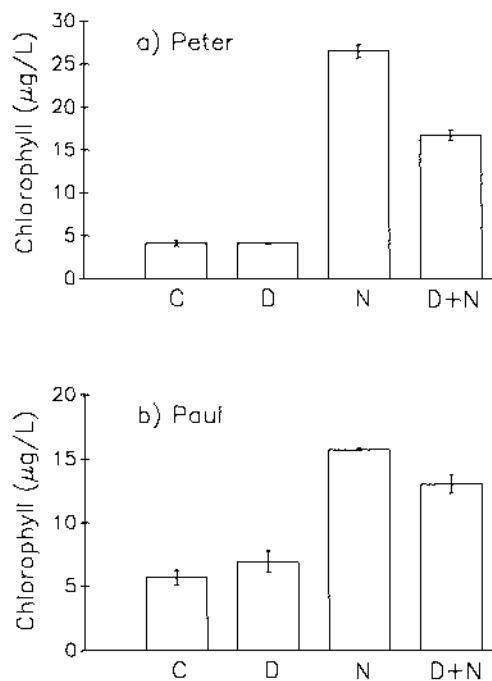


FIG. 2. Chlorophyll concentration in each treatment of the Peter Lake and Paul Lake factorial experiments (means  $\pm$  1 SE). Treatment codes are C = controls, D = *Daphnia* additions, N = nutrient additions, and D + N = *Daphnia* and nutrient additions.

TABLE 2. Summary of probabilities from *F* tests of the analysis of variance for the Peter and Paul lakes factorial experiments. Note: *df* = 1 in all cases, D = *Daphnia*, N = nutrient, D × N = *Daphnia*-nutrient interaction.

Lake	Source	Chlorophyll	Bacteria	<sup>3</sup> H-Thymidine incorporation	Heterotrophic flagellates	Ciliates
Peter	D	0.0001	0.8156	0.0127	0.0011	0.0203
	N	0.0001	0.0005	0.0061	0.2359	0.0287
	D × N	0.0001	0.7327	0.4586	0.0565	0.3747
Paul	D	0.2812	0.1907	0.8266	0.0001	0.0001
	N	0.0001	0.0001	0.0009	0.0001	0.0017
	D × N	0.0120	0.9183	0.0854	0.0023	0.1860

(*P* = .09), because the effect of *Daphnia* was opposite depending on the presence or absence of nutrients. Mean thymidine incorporation rates were higher in the *Daphnia* addition treatment relative to the control, but lower in the combined treatment relative to the nutrient addition treatment (Fig. 4).

Interestingly, the higher thymidine incorporation rates in the *Daphnia* treatments in Peter and Paul lakes did not lead to increases in bacterial abundance. As with the phytoplankton, it appears that *Daphnia* under low-nutrient conditions acts as both a consumer and a regenerator of growth-limiting substrates for bacteria, with the net effect on bacterial abundance being neutral.

In comparing rates of thymidine incorporation we must consider, if possible, whether differences among treatments in the dilution of radioactive thymidine by unlabeled thymidine confound our interpretation

(Moriarty 1986). The strongest result of our experiments is that nutrient additions increase the rate of thymidine incorporation. This result would only be confounded if dilution occurred in a specific pattern of higher dilution (i.e., more unlabeled thymidine) in the treatments without nutrients. Our result is robust if isotope dilution is either zero, constant, or varies positively with nutrient addition. Given these latter possibilities and the saturating levels of <sup>3</sup>H-thymidine we used, it seems unlikely that isotope dilution was a significant problem.

*Protozoa*.—Heterotrophic flagellates responded somewhat differently in the two factorial experiments (Fig. 5). In Peter Lake there was a strong effect of *Daphnia* addition, and nutrients were not important (Table 2). These results suggest direct grazing control of *Daphnia* on heterotrophic flagellates. In the Paul Lake experiment *Daphnia* additions also had a strong effect on heterotrophic flagellates, and, in addition, flagellates responded positively to nutrient additions (Fig. 5, Table 2). The Paul Lake experiment provides evidence for the joint effect of predatory and resource controls on flagellates. Flagellate community composition did not appear to differ among treatments. In all enclosures the communities were dominated by small 1–5 μm free-living forms, which were visually similar to species we observed in the epilimnions of Peter and Paul lakes.

A consistent feature of both experiments is the enhanced impact of *Daphnia* on heterotrophic flagellates in the combination treatment (Fig. 5). The magnitude of this effect contributes to the marginally significant (Peter Lake) and highly significant (Paul Lake) interaction terms (Table 2). This result is difficult to explain, since flagellate resources (bacteria and algae) were increasing in the combination treatment (Figs. 2 and 3), and the impact of *Daphnia* predation should have been constant, assuming a constant filtering rate across the gradient of food concentrations in these experiments. A possible explanation is selective feeding by *Daphnia* on heterotrophic flagellates in the combination treatment, which may have occurred as a consequence of the rapid growth of algal nanoplankton observed in the fertilized treatments. These cells were primarily cryptophytes in the same size range as the heterotrophic flagellates. If *Daphnia* were able to focus its grazing on these algal cryptophytes, then it may have had an en-

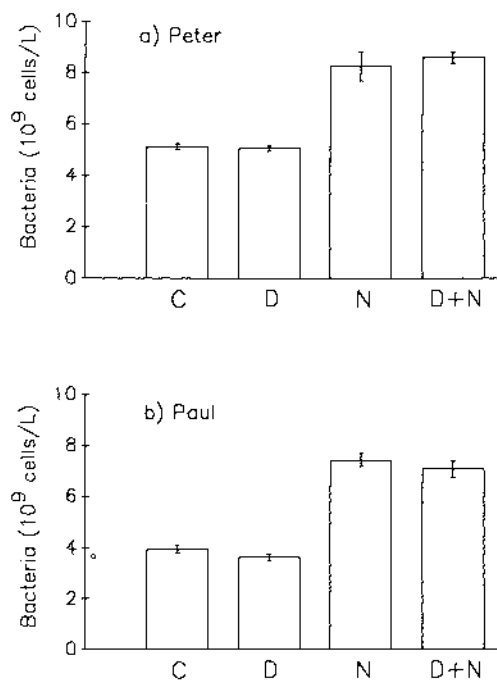


FIG. 3. Bacterial abundance in each treatment of the Peter Lake and Paul Lake factorial experiments (means ± 1 SE). Treatment codes are as in Fig. 2.

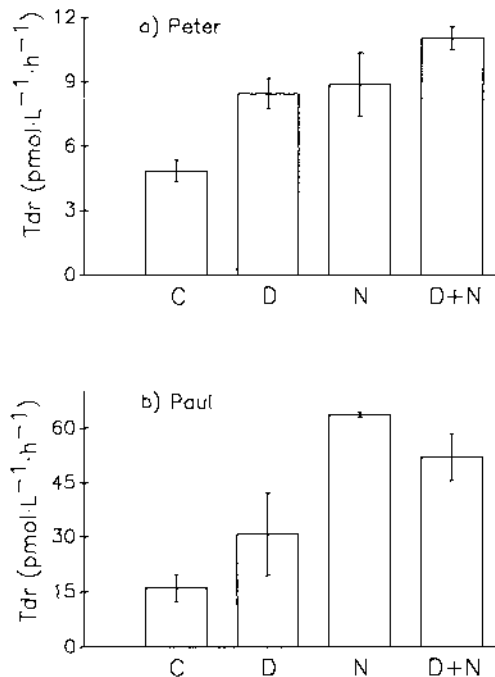


FIG. 4. Thymidine incorporation rate (Tdr) in each treatment of the Peter Lake and Paul Lake factorial experiments (means  $\pm 1$  SE). Treatment codes are as in Fig. 2.

hanced impact on the heterotrophic flagellates. While *Daphnia* is a relatively unselective grazer, several studies have indicated some selectivity, particularly for algal flagellates in the size range of 4 to 10  $\mu\text{m}$  (Lampert 1987).

Ciliates responded most strongly to *Daphnia* additions in both the Peter and Paul lakes experiments (Fig. 6). Ciliates in the *Daphnia* addition treatment were reduced to 65 and 38% of the control density in Peter and Paul lakes, respectively. Nutrient additions lead to modest but significant increases in ciliates in both Peter and Paul (Fig. 6, Table 2). There was no significant interaction in either experiment.

Ciliate communities were always dominated by choanotrichs, particularly a *Strombidium* species and a *Halteria* species. There was little difference in the relative abundance of these species among treatments.

The effect of *Daphnia* on protozoans was greater in Paul Lake relative to Peter Lake (Table 2). Recall that the natural abundance of *Daphnia* was lower in Paul Lake at the time of these experiments. As in the single-factor experiments, initial conditions (high vs. low abundance of *Daphnia* in the lake) appear to be important in determining the strength of the response of protozoans to manipulations of *Daphnia*.

#### DISCUSSION

These experiments clarify the differential role of nutrients and large herbivores in regulating the heterotrophic microbial communities of Peter and Paul lakes.

Fig. 7 illustrates the net responses of major groups (plus, zero, or minus signs) and hypothesized pathways of interaction (arrows) observed in the framework of bottom-up and top-down regulation in the food web. Nutrient additions led to strong increases in the bacteria, flagellates, and ciliates with one exception—flagellates in the Peter Lake experiment. Several pathways for the nutrient effects on bacteria and protozoa are possible, and none of these pathways is excluded from significance by these experiments (Fig. 7). For example, bacteria may respond directly to nutrient additions, indirectly to the effects of nutrients on phytoplankton, or to the combination of nutrient and phytoplankton changes. Similarly, the increases in protozoans may be the result of changes in phytoplankton and/or bacteria, or the joint effect of increases in both groups (Fig. 7). We assume in Fig. 7 that protozoans do not respond directly to nutrients although this may be possible, particularly for ciliates that contain algal symbionts (Stoecker et al. 1987) or for heterotrophic flagellates if dissolved organic matter is a primary resource (Sherr 1989).

The top-down effects of zooplankton on phytoplankton have been previously described (Carpenter et al. 1987, Carpenter and Kitchell 1988). In our experiments simple additions of *Daphnia* did not change phytoplankton biomass in three out of four cases. Similar results have been observed in other enclosure studies in Peter and Paul lakes (Elser and MacKay 1989). In the context of our experiments, we regard the net

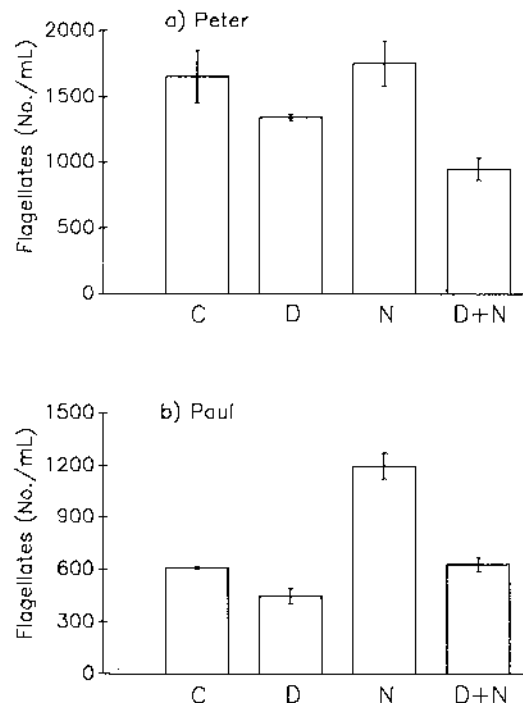


FIG. 5. Abundance of heterotrophic flagellates in each treatment of the Peter Lake and Paul Lake factorial experiments (means  $\pm 1$  SE). Treatment codes are as in Fig. 2.

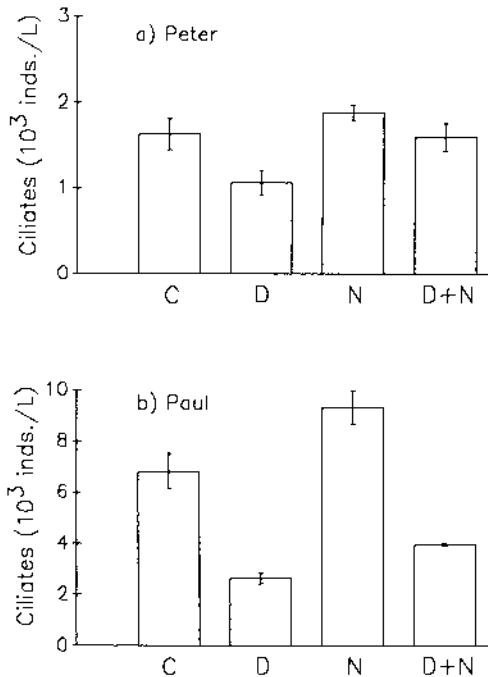


FIG. 6. Abundance of ciliates in each treatment of the Peter Lake and Paul Lake factorial experiments (means  $\pm$  1 SE). Treatment codes are as in Fig. 2.

effect of zooplankton (the result of grazing and nutrient regeneration) on phytoplankton as neutral and the top-down pathway as unimportant (Fig. 7). Top-down effects of zooplankton on both ciliates and flagellates were observed (Fig. 7). In the factorial experiments the top-down effect of zooplankton was usually stronger than nutrient additions in determining final densities of protozoa. Since protozoans generally declined in the absence of any change in their algal and bacterial resources in the *Daphnia* addition treatments, direct grazing rather than exploitative competition is likely the primary mechanism whereby *Daphnia* reduces protozoans.

Bacteria were unaffected by changes in zooplankton, and therefore top-down regulation appears unimportant for these microbes (Fig. 7). There was some evidence of increases in thymidine incorporation in treatments with additions of *Daphnia*, but these changes in the apparent growth rate of bacteria did not lead to changes in abundance. With regard to interactions in the microbial food web, these experiments predict that cascading trophic interactions will be truncated at the level of protozoa. Furthermore, the experiments provide little evidence for a tight linkage between bacteria and protozoans. For example, in cases where *Daphnia* reduced the abundance of flagellates and ciliates, no corresponding increase was observed in bacteria as would be expected if protozoan grazing regulates bacteria (Fig. 7). An alternative explanation is that increased grazing by *Daphnia* compensates for decreased

protozoan grazing so that no net effect on bacteria is observed. This possibility seems unlikely. In experiments where no effect of *Daphnia* was observed on protozoa (e.g., Table 1: Paul Lake), the combined increased grazing pressure by *Daphnia* and protozoans should have reduced bacteria, but no difference was observed. Overall, either bacteria are unaffected by grazers including protozoans and *Daphnia* (grazing mortality is a trivial loss term), or there is rapid compensation for changes in mortality.

One possible limitation to the conclusion that bacteria are not regulated by top-down control is that we observed no effect of zooplankton on phytoplankton. If bacteria are regulated by phytoplankton, changes in phytoplankton in response to zooplankton should eventually be observed in bacteria. In whole-lake manipulations in Peter Lake and an adjacent experimental lake, Tuesday Lake, phytoplankton biomass has been shown to decrease when *Daphnia pulex* becomes abundant in these systems as a consequence of relaxed planktivory (Carpenter et al. 1987, Carpenter and Kitchell 1988). The enclosure experiments may have been of too short a duration to observe this response. Nevertheless, the factorial experiments provide support for the idea that bacteria are not regulated by zooplankton, because bacterial abundance was inde-

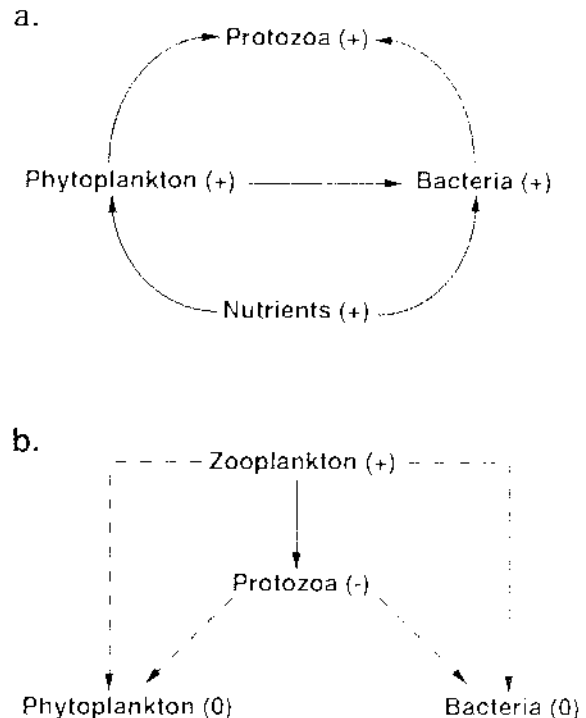


FIG. 7. Schematic representation summarizing net responses (+, -, 0) and control on bacteria and protozoa from (a) nutrients (bottom-up), and (b) zooplankton (top-down), as revealed by the Peter Lake and Paul Lake experiments. Solid arrows indicate important or potentially important pathways of control. Dashed arrows indicate weak or unimportant pathways.

pendent of *Daphnia* in the combined *Daphnia*-nutrient treatment whereas phytoplankton were reduced in the combination treatment relative to the nutrient treatment. The effect of *Daphnia* on phytoplankton was evident in the combined treatment, but there was no evidence of either a direct effect of *Daphnia* on bacteria or an indirect effect of *Daphnia* on bacteria via reductions in phytoplankton.

The limitation of bacterial abundance by nutrients in Peter and Paul lakes contrasts with results of enclosure experiments in two Danish lakes by Riemann and Sondergaard (1986) and Geertz-Hansen et al. (1987). In these Danish systems bacterial biomass and production appeared to be unaffected by nutrient additions and instead were elevated in enclosures containing planktivorous fish. The Danish lakes are quite eutrophic, with chlorophyll concentrations in the range of 10–100  $\mu\text{g/L}$  and phosphate concentrations in excess of 10  $\mu\text{g/L}$  (Riemann and Sondergaard 1986). Under these circumstances, predatory control on bacteria appears to be significant, particularly when dense populations of *Daphnia* develop (Riemann 1985). We have also observed in a eutrophic lake where *Daphnia* populations can be 10-fold higher than in Peter and Paul lakes that the rate of grazing on bacteria by *Daphnia* is often greater than bacterial growth rates (Pace et al. 1990). Hobbie and Cole (1984) found that bacterial biomass was differentially regulated across a gradient of nutrient loading in large seawater enclosures. Bacteria and flagellated protozoans increased in abundance proportionally, except at the highest levels of nutrient loading where there was evidence of top-down control on these two groups. Initial conditions, therefore, are probably quite important in determining the results of enclosure studies. Regulation of bacteria may vary across gradients of system productivity in aquatic environments, with resource control being more important in systems with low productivity like Peter and Paul lakes.

The experiments described in this paper suffer from a common malady of ecological experiments in being of small scale and short duration (Tilman 1989). In one sense the experimental scale is relatively large, since each enclosure contained  $>10^{10}$  bacteria and  $>10^7$  phytoplankton and protozoans. Furthermore, the experimental time allowed the potential for several turnovers of the microbial communities in the enclosures while limiting numerical responses by the zooplankton community. Nevertheless, the most serious potential problems with these types of experiments are that the responses may only reflect transient dynamics of microbial communities in response to perturbation, and indirect effects may not be manifested in the time frame of the experiment (Tilman 1989). In other words, the temporal scale of the experiments may have been too limited to allow useful extrapolation.

Our experiments are representative of the summer stratified period and of the effect of one component of

the zooplankton community (large *Daphnia*). Phytoplankton, zooplankton, and heterotrophic microbes vary seasonally in terms of their dynamics and interactions. Additional experiments are required to determine if the results of our experiments can be generalized across seasons. In addition, the small zooplankton communities that develop in these lakes upon the elimination of large *Daphnia* might have distinct impacts on the heterotrophic microbial community. Additional experiments are required to compare the effects of zooplankton communities of different taxonomic composition and size structure (e.g., Elser et al. 1988).

Our results are consistent with several expectations based on earlier work. First, large-scale comparative studies suggest that bacterial abundance and productivity are regulated by algal biomass, primary production, and system nutrient status (Bird and Kalff 1984, Cole et al. 1988). The strong experimental responses of bacteria to nutrient additions are consistent with results of the comparative studies. Second, earlier studies of the interaction between *Daphnia* and bacteria have suggested the trophic linkage between these two organisms is relatively weak (Peterson et al. 1978, Pace et al. 1983). Again, the current experiments support this notion, but with the caveat that in eutrophic lakes the *Daphnia*-bacteria linkage may be stronger (see discussion above). Third, the strong top-down effects of *Daphnia* on protozoa are also consistent with the known grazing ability of this cladoceran, particularly given that heterotrophic flagellates and ciliates are highly edible by *Daphnia* (Porter et al. 1979; Sanders and Porter 1990). These consistencies give us some confidence in the generality of our results, but we emphasize that predictions derived from these experiments require testing at longer temporal scales. We are currently performing such experiments at the whole-lake level in Peter and Paul lakes.

In summary, heterotrophic microbial communities can be considered within the framework of theory on the regulation of productivity in aquatic ecosystems. Nutrients appear to regulate bacteria in the oligotrophic lakes studied here. Protozoan regulation is more complex, but, in a manner similar to phytoplankton (Carpenter et al. 1987), variation in protozoans is likely to be related to variability in nutrients and food-web structure. Comparisons of the results of these experiments with those in eutrophic lakes suggest regulatory factors may vary across gradients of productivity in lake ecosystems.

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