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Grazing on bacteria by flagellates and cladocerans in lakes of contrasting food-web structure

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Abstract. We tested the hypothesis that grazing on bacteria would vary between lakes with differing plankton community structures. Paul and Tuesday lakes (Gogebic County, MI) are respectively dominated by piscivorous and planktivorous fish. Consequently, zooplankton in Paul are primarily large daphnids, while zooplankton in Tuesday are primarily small cladocerans and copepods. We measured flagellate grazing on bacteria using a fluorescent minicell method, while cladoceran grazing was estimated from the relationship between body length and filtering rate. We predicted that cladoceran grazing on bacteria would be higher in Paul, and flagellate grazing would be higher in Tuesday. Cladoceran grazing on bacteria was important in both lakes contrary to our initial expectation. Large populations of the small cladoceran, *Bosmina longirostris*, in Tuesday exerted a grazing pressure ($0.18–35 \times 10^6$ bacteria l^{-1} h^{-1}) approximately equal to that of the large cladoceran, *Daphnia pulex*, in Paul ($0.34–30 \times 10^6$ bacteria l^{-1} h^{-1}). Flagellate grazing was higher in Tuesday as predicted (range: Paul, $0.1–6 \times 10^6$ bacteria l^{-1} h^{-1}; Tuesday, $0.2–20 \times 10^6$ bacteria l^{-1} h^{-1}). However, there was not a simple relationship between total abundance of flagellates and total grazing rates. High community grazing by flagellates occurred when attached choanoflagellates were present. These flagellates had higher ingestion rates than free forms. We find no clear evidence that differences in food-web structure between the two lakes influence the process of grazing on bacteria. Instead, our results emphasize the significance of cladocerans and attached flagellates as consumers of bacteria in freshwater ecosystems.

Introduction

Food-web structure is an important feature regulating the productivity of aquatic ecosystems (Carpenter and Kitchell, 1988). In the plankton of lakes the relative dominance of piscivorous and planktivorous fishes is a significant determinant of zooplankton community structure (Lazzaro, 1987). The effects of changes in fish community structure may cascade to the phytoplankton by changes in planktivory either promoting the development or removal of large species of zooplankton (e.g. Carpenter *et al.*, 1987). In particular, large species of *Daphnia* are effective herbivores which have the capacity to suppress phytoplankton biomass and productivity below levels expected from nutrient concentrations (Shapiro, 1980; Carpenter *et al.*, 1985).

While the response of zooplankton and phytoplankton communities to manipulations of higher trophic levels have been documented, the responses of heterotrophic microbial processes remain largely unstudied. Changes at higher trophic levels may cascade to bacteria and protozoans via a number of pathways. For example, some crustacean zooplankton such as cladocerans consume both bacteria and protozoans in addition to algae, whereas most copepods do not graze on bacteria (Stockner and Porter, 1988). Furthermore, indirect effects of zooplankton on heterotrophic microbes are probably also important. For example, reduction of algal biomass by zooplankton grazing may reduce the quantities of algal-derived substrate available to bacteria. Alternatively if

mixotrophic algae or protozoans are important consumers of bacteria, zooplankton grazing may indirectly favor bacteria by eliminating their predators. The relative magnitude of these interactions will determine how the biomass of different microbial groups and processes such as growth, grazing, and nutrient regeneration within microbial food webs respond to changes at higher trophic levels.

Recent methodological advances allow measurements of grazing on bacteria by protozoans and metazoans (Børsheim, 1984; Güde, 1986; McManus and Fuhrman, 1986; Wikner *et al.*, 1986; Bjørnsen *et al.*, 1986; Sherr *et al.*, 1987). Studies using these techniques have concluded that heterotrophic flagellates are often the major grazers on bacteria in freshwater and marine systems (McManus and Fuhrman, 1988; Bloem *et al.*, 1989; Sanders *et al.*, 1989; Wikner and Hagström, 1988; Pernie *et al.*, 1990). There are exceptions to this generalization. For instance, ciliated protozoans may consume bacteria at rates equal to flagellates in estuarine systems (Sherr *et al.*, 1989a). A number of investigations have also demonstrated that some algal flagellates consume substantial quantities of bacteria (Bird and Kalff, 1989; Sanders *et al.*, 1989). Additionally in freshwater lakes, populations of the cladoceran *Daphnia* may account for more bacterial grazing than heterotrophic flagellates (Pace *et al.*, 1990). These results based on direct measurements suggest that in addition to heterotrophic flagellates other consumers of bacteria may be important.

Here, we contrast rates of grazing on bacteria in two well-studied lakes with differing food webs. We consider the hypothesis that the utilization of bacteria by heterotrophic flagellates and cladoceran zooplankton is dependent on food web structure.

Study sites and hypotheses

This research was conducted in Paul and Tuesday lakes (Gogebic County, MI, USA) during 1989. These lakes are the site of ongoing experiments on the effects of fish manipulations on lower trophic levels and ecosystem processes (Carpenter and Kitchell, 1988). Paul and Tuesday are small (<1.5 ha), oligotrophic–dystrophic lakes whose basic limnological characteristics have been described previously (Elser *et al.*, 1986; Carpenter *et al.*, 1986, 1987; Leavitt *et al.*, 1989). These lakes are similar morphometrically, with Tuesday being deeper (mean depth = 7 m, maximum depth = 19 m) than Paul (mean depth = 4 m, maximum depth = 15 m). Both lakes have anaerobic hypolimnia throughout the summer season and appear to be meromictic.

The main differences between the lakes during 1989 was in fish biomass and composition. Paul was dominated by piscivorous largemouth bass (*Micropterus salmoides*) with virtually no small planktivorous fish, other than young-of-the-year bass. Tuesday supported dense populations of planktivorous minnows, while piscivores were absent. These differences in fish composition were the main cause of contrasting zooplankton communities in these two lakes. During 1989, zooplankton biomass in Tuesday Lake was dominated by an assemblage of small zooplankton, including *Bosmina longirostris*, *Daphnia parvula*, *Ortho-*

cyclops modestus and *Cyclops varicans rubellus*. The large cladoceran *Daphnia pulex* was the most important zooplankter in Paul.

Given these contrasting food webs, we hypothesized that there would be differences in the rate of grazing on bacteria by cladocerans and flagellates in the two lakes. First, we predicted cladoceran grazing on bacteria would be greater in Paul relative to Tuesday for the following reasons. The biomass of cladocerans is higher in Paul than in Tuesday (Carpenter *et al.*, 1987). In addition, the zooplankton community in Paul is dominated by daphnids and these organisms are known to graze on bacteria (Porter *et al.*, 1983), whereas some of the taxa in Tuesday (e.g. cyclopoid copepods) graze on bacteria with a low efficiency if at all (Pedrós-Alió and Brock, 1983). A second hypothesis concerned the significance of grazing by flagellates in the two lakes. We predicted that flagellate grazing would be higher in Tuesday than in Paul. The higher biomass of zooplankton in Paul would reduce the abundance of heterotrophic flagellates and thereby lower flagellate grazing. To test these ideas grazing on bacteria by flagellates and cladocerans was estimated in monthly experiments conducted in each lake during summer stratification (May–August 1989).

Method

Sampling and enumeration of bacteria, flagellates and cladocerans

Samples were collected weekly with a Van Dorn bottle during summer stratification from the epilimnion (mixed layer) and metalimnion (oxygen maximum) to determine bacterial and heterotrophic flagellate abundance. Zooplankton were collected with duplicate vertical net hauls (80 μm mesh) with the efficiency of the net calibrated by a vertical series of Schindler–Patalas trap samples.

Bacterial abundance was determined by epifluorescence microscopy using the acridine orange direct-count method (Hobbie *et al.*, 1977). Heterotrophic flagellates were stained with proflavin (Haas, 1982), preserved with glutaraldehyde (1% final solution), immediately filtered on 1 μm Nuclepore filters, and counted with an epifluorescent microscope. Zooplankton were counted with a stereomicroscope.

Measurement of grazing

Four experiments to measure grazing by flagellates on bacteria were carried out from May to August 1989. Triplicate 1 l water samples were taken once a month in the epilimnion and in the metalimnion. Samples were immediately placed in sterile 1 l Whirlpak bags and maintained at *in situ* temperature. Feeding by heterotrophic flagellates was measured using a fluorescent minicell technique (Pace *et al.*, 1990), based on a modification of the method of Wikner *et al.* (1986). A minicell-producing strain of *Escherichia coli* was cultured in liquid medium, and minicells separated using sucrose-gradient centrifugation. Minicells are spherical and ~0.5 μm in diameter (volume = 0.065 μm^3). The minicells are slightly larger than the average bacteria in Paul and Tuesday lakes

(n = 652, mean volume = 0.021 μm^3, equivalent spherical diameter = 0.34 μm). Minicells were stained with 5(4,6-dichlorotriazin-2-yl) aminofluorescein (DTAF) following the protocol of Sherr *et al.* (1987). Prior to use in experiments, minicells were sonicated to aid dispersal.

Minicells were added to each replicate 1 l sample at ~10% of bacterial concentrations. Subsamples were taken immediately and at 1 h. Subsamples were preserved with an equal volume of ice-cold 4% glutaraldehyde (Sanders *et al.*, 1989). Minicells and bacteria in the subsamples were counted using epifluorescence microscopy. Flagellates were stained with 4,6-diamindino-2-phenylindole (DAPI), filtered onto 1 μm Nuclepore filters, and frozen. Random strips on each filter were counted at ×1250 until ~100 flagellates were enumerated. The number of minicells within each flagellate was recorded.

We observed that choanoflagellates attached to *Dinobryon* ingested high numbers of minicells. We enumerated these flagellates separately by examining up to 25 *Dinobryon* colonies on each filter, recording the number of choanoflagellates and the number of ingested minicells. Colonies of *Dinobryon* in the epilimnion were enumerated weekly from whole-water samples. We used these data with estimates of the number of choanoflagellates per colony determined in each experiment to estimate the abundance of attached flagellates. For experimental samples taken from the metalimnion, we counted all the *Dinobryon* colonies on each filter prepared for epifluorescence microscopy.

For each replicate Whirlpak bag, we determined the number of minicells, bacteria, free and attached flagellates, and minicells ingested per flagellate. Ingestion rates (bacteria flagellate^{-1} h^{-1}) were calculated by multiplying the number of minicells ingested per flagellate by the ratio of bacteria to added minicells (see Pace *et al.*, 1990). Community grazing rates (bacteria l^{-1} h^{-1}) were calculated by multiplying ingestion rates by flagellate abundance.

Estimation of grazing by cladocerans

We compared our measurements of grazing by flagellates to potential rates of grazing by cladocerans, another major group of bacterial consumers. In Paul Lake, *D.pulex* was the dominant cladoceran. For this species we used the equation of Peterson *et al.* (1978):

$$CR = 0.497 * L^{0.70} \quad (1)$$

This equation relates clearance rate (CR in ml animal^{-1} h^{-1}) on bacteria to body length (L in mm). In Tuesday Lake, *B.longirostris* was the dominant cladoceran. For this species we used the equation of Porter *et al.* (1983):

$$CR = 0.538 * L^{1.545} \quad (2)$$

with the same units as in equation (1). The equation of Porter *et al.* (1983) was developed using *Bosmina* and a number of other cladocerans but did not include *D.pulex*. For this reason, we used two equations to estimate grazing.

In an independent experiment, we tested the utility of this approach by measuring grazing rates of *D.pulex* (average length 2.0 mm) on minicells in the laboratory. The mean of four replicate measurements was 0.55 (95% CI: 0.47–0.63) ml animal^{-1} h^{-1}. This estimate corresponds reasonably well with the value predicted by the equation developed for *D.pulex* by Peterson *et al.* (1978) of 0.76 (95% CI: 0.48–1.23) ml animal^{-1} h^{-1}.

Results

Seasonal patterns of bacteria, heterotrophic flagellates and cladocerans

Bacteria were more abundant in the epilimnion of Tuesday relative to Paul (*t*-test, $P = 0.019$), but there was no difference in abundance between the metalimnia of the two lakes ($P = 0.573$, Figure 1). Flagellates were on average about twice as abundant in the epilimnion of Tuesday ($P = 0.021$) while differences in abundance between the metalimnia of the two lakes was marginal ($P = 0.136$, Figure 1). Bacteria were relatively constant across the season in each lake. The range of the coefficients of variation (CVs) for the four seasonal patterns was 14–26%. Compared with bacteria, flagellates were more variable (range of CVs 46–62%) showing different seasonal patterns in each lake (Figure 1).

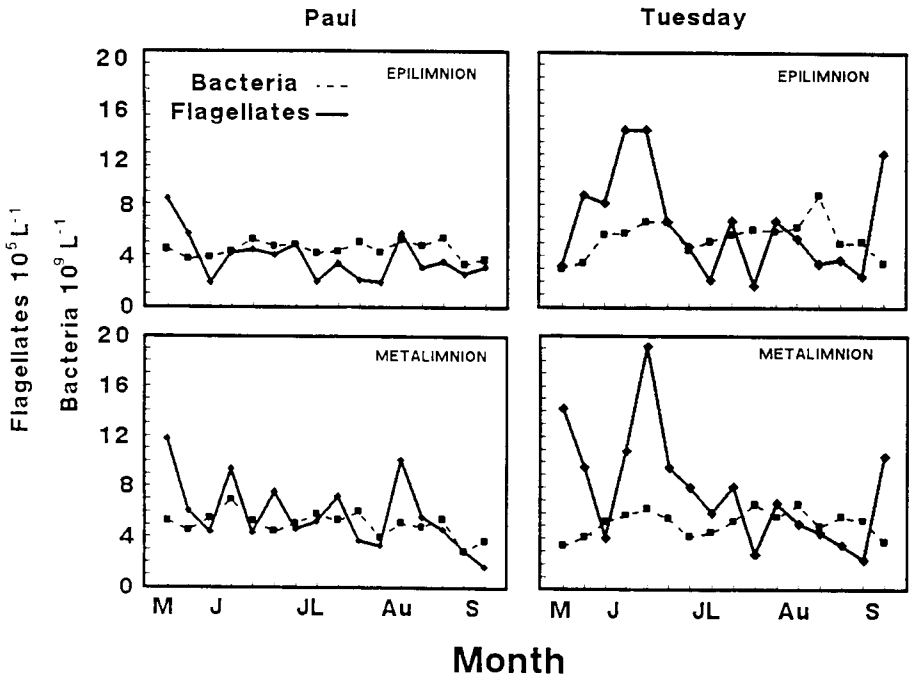

Fig. 1. Bacterial and flagellate abundance in the epilimnion and metalimnion of Paul and Tuesday Lakes during the summer of 1989.

In Paul Lake which was dominated by piscivorous fish, the zooplankton community consisted mainly of *D.pulex* (mean body length = 1.02 mm) with *Daphnia rosea* (mean size = 0.7 mm) being the second most important species (Figure 2). In Tuesday Lake which was dominated by planktivorous fish, the zooplankton community consisted mainly of *B.longirostris*, with a mean body length of 0.27 mm. This population reached a maximum density of 75 animals l^{-1} in June. *Daphnia parvula* was also present (mean body length = 0.61 mm) but always at low abundance (<1 animal l^{-1}). Considering only the four cladocerans noted above, average biomass for the summer period was 25 μg dry wt l^{-1} in Paul and 12 μg dry wt l^{-1} in Tuesday.

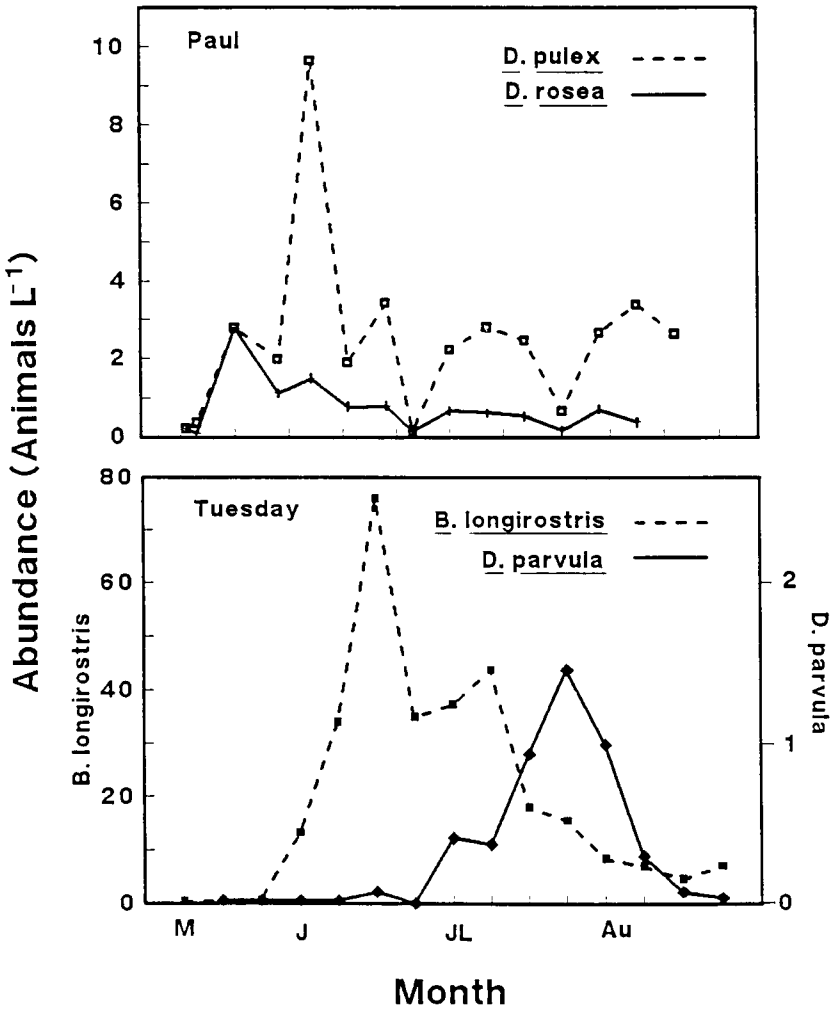

Fig. 2. Abundance of the principal species of cladocerans in Paul and Tuesday Lakes during the summer of 1989.

Grazing by flagellates

Grazing on bacteria by flagellates varied in Paul from 0.1 to 6 × 10^6 bacteria l^{-1} h^{-1}. A larger range of grazing rates was observed in Tuesday with values ranging from 0.2 to 20 × 10^6 bacteria l^{-1} h^{-1} (Figure 3). Half of the measurements we made in Tuesday, both in the epi- and metalimnion, were 2–20 times higher than in Paul (Figure 3). A three-way analysis of variance was used to partition variance due to date (May–August), depths (epilimnion versus metalimnion) and lakes (Paul versus Tuesday). Flagellate grazing was significantly related to

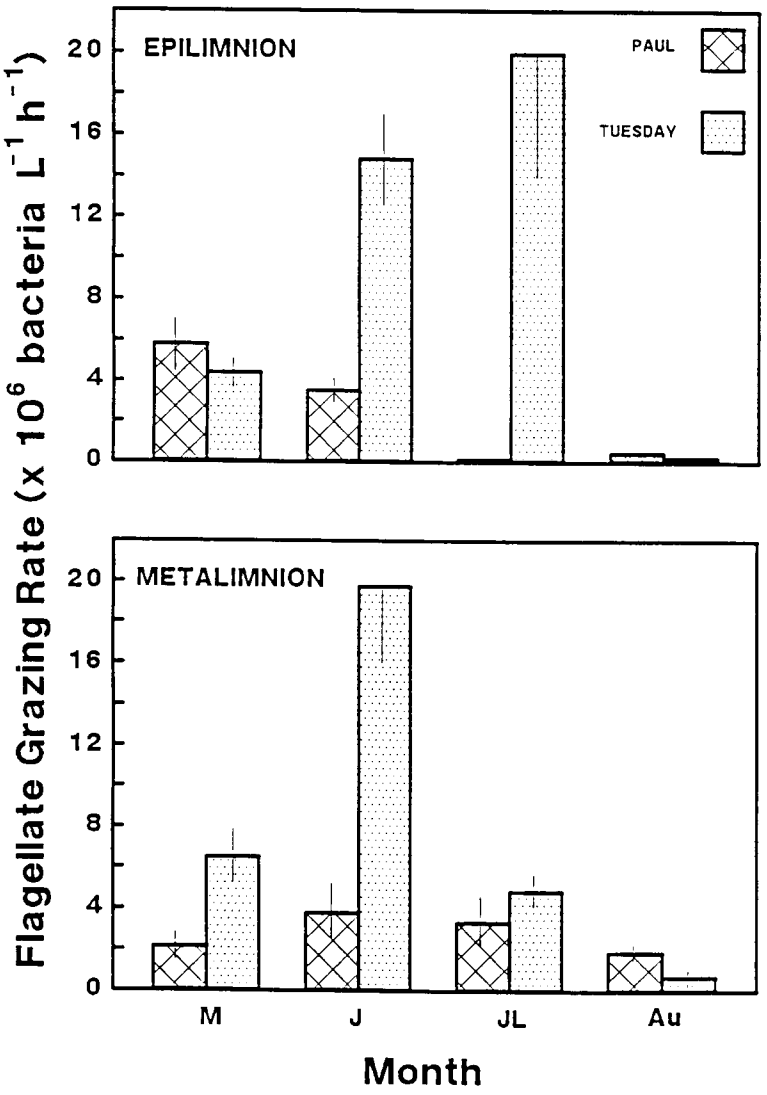

Fig. 3. Grazing on bacteria by heterotrophic flagellates in Paul and Tuesday Lakes. Bars are the standard errors, $n = 3$.

Table I. Density of free (F) and attached (A) flagellates (no. ml^{-1}), during summer in Paul and Tuesday lakes

Month	Depth	Paul		Tuesday	
		F	A	F	A
May	Epi	620 (32)	99 (12)	824 (342)	26 (16)
	Meta	591 (62)	21 (8)	1109 (140)	0
June	Epi	414 (98)	66 (7)	283 (116)	198 (57)
	Meta	655 (41)	11 (0.4)	611 (218)	79 (26)
July	Epi	393 (30)	0.5 (0.3)	291 (71)	279 (63)
	Meta	824 (90)	0.7 (0.1)	596 (47)	77 (28)
August	Epi	316 (38)	9 (3)	293 (39)	0.5 (0.6)
	Meta	722 (75)	2 (2)	460 (21)	0.0

Values are the means of triplicate samples. Standard deviations are in parentheses.

differences among dates ($P < 0.0001$) and weakly related to depths ($P = 0.08$). The difference between lakes was also significant ($P = 0.0032$). We conclude grazing by flagellates was higher in Tuesday as predicted by our hypothesis.

These differences in grazing rates were not simply a function of the total number of flagellates. Rather, we observed differences between the lakes in the number of attached choanoflagellates versus free-swimming (hereafter free) flagellates (Table I). Attached flagellates have higher ingestion rates than free forms as previously documented in culture (Fenchel, 1987; Cynar and Sieburth, 1986) and *in situ* (Pace *et al.*, 1990). We also found higher ingestion rates per flagellate for attached forms. In Paul attached flagellate ingestion rates ranged from 13 to 30 bacteria $flagellate^{-1}$ h^{-1} while in Tuesday the range was 39–73 bacteria $flagellate^{-1}$ h^{-1}. These rates were always substantially higher than ingestion rates for free forms (ranges: Paul, 0.24–5.21; Tuesday, 0.19–23.56 bacteria $flagellate^{-1}$ h^{-1}). Total grazing rates by attached flagellates were especially high in the epilimnion of Tuesday during the June and July experiments (Figure 4). We also observed occasions when ingestion rates per cell and total grazing rates by free flagellates were high, as in the metalimnion of Tuesday during the June experiment (Figure 4).

Attached flagellates in Paul and Tuesday lakes were found almost exclusively on colonies of *Dinobryon sertularia* and *D.divergens*. *Dinobryon* colonies when present varied in abundance from 0.3 to 23×10^3 colonies l^{-1}. Flagellates attached to these colonies varied from 0.5 to 35 flagellates $colony^{-1}$. There was, however, no simple relationship between the number of attached flagellates per colony and the abundance of *Dinobryon* colonies. We observed negligible ingestion of minicells by *Dinobryon*, suggesting these chrysophytes were primarily autotrophic, in these lakes.

Comparison with estimated cladoceran grazing

To test the hypothesis that cladoceran grazing on bacteria was more important in Paul relative to Tuesday, we calculated cladoceran grazing (see Method) from water column averages of cladoceran and bacterial abundance. Flagellate

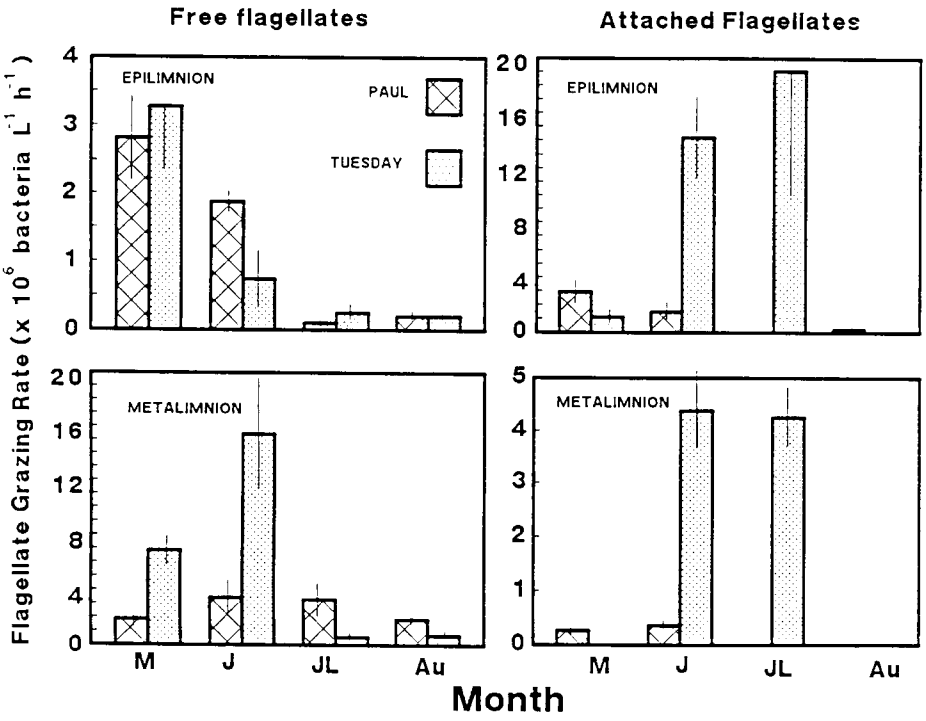

Fig. 4. Grazing on bacteria by free and attached heterotrophic flagellates in Paul and Tuesday Lakes. Bars are the standard errors, $n = 3$. Note different scales.

grazing for the water column was estimated by averaging epilimnion and metalimnion values during each experiment. Weekly estimates of flagellate grazing were based on direct measures of flagellate abundance and estimated clearance rates derived from the monthly experiments (noted by the arrows in Figure 5). Clearance rates for a given week were assumed equal to the value measured during the same month.

The relative magnitude of cladoceran grazing on bacteria in Paul and Tuesday shifted over the season (Figure 5). Clearance rates calculated using the two equations varied from 0.44 to 0.65 ml animal^{-1} h^{-1} for *D.pulex* and 0.055 to 0.097 ml animal^{-1} h^{-1} for *B.longirostris* over the season as a function of minor changes in the average body length of these two populations. Grazing by daphnids was higher in Paul during mid-May to mid-June and again in August. Grazing on bacteria by *Bosmina* in Tuesday was higher than daphnid grazing in Paul generally from mid-June to the end of July. Our weekly estimates support the conclusion that cladoceran grazing was important and roughly equivalent in the two lakes.

Surprisingly, even in Tuesday Lake where *Bosmina longirostris* was dominant, most measurements of cladoceran grazing were either higher or of a similar magnitude to flagellate grazing rates (10 of 14 cases, Figures 5). This comparison does not support our hypothesis that grazing by cladocerans on bacteria would

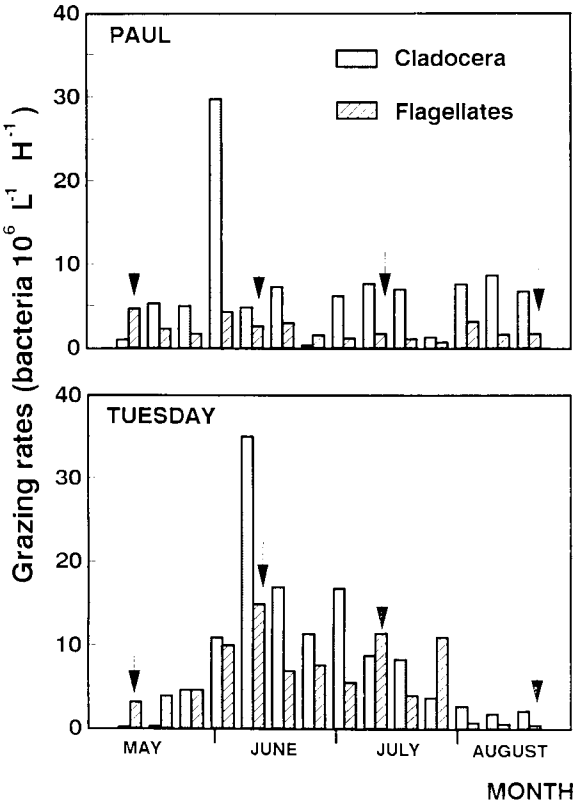

Fig. 5. Grazing on bacteria by cladocerans and flagellates as estimated weekly during the summer of 1989. Rates are based on water column average values. Arrows indicate times when direct measurements of flagellate grazing were made.

be more important in Paul than in Tuesday. Instead, grazing by cladocerans appears to be important even in zooplankton communities dominated by small forms such as *Bosmina*.

Discussion

Cladoceran grazing appears to be a significant mortality factor for bacteria in both lakes. This result is contrary to our initial hypothesis that cladoceran grazing would be most important in Paul (dominated by piscivorous fish) and less important in Tuesday (dominated by planktivorous fish). Large populations of *Bosmina* in Tuesday compensate for the lack of a large cladoceran grazer and have the capacity to exert at least as high a grazing pressure on the bacteria.

Our estimates of these grazing rates are indirect, but our experiment to test the equation of Peterson *et al.* (1978) for *D.pulex* provided support for this approach (see Method). Furthermore, direct measurements of *Daphnia* grazing by Pace *et al.* (1990) fall within the range of values we estimate in this study. In

the case of *Bosmina*, the equation of Porter *et al.* (1983) tends to underestimate the actual values of clearance rates of *Bosmina* they measured in their experiments, because their equation is based on combined results from a number of different species. Our estimated values using the equation of Porter *et al.* (1983), may therefore be conservative, but they fall in the range of *Bosmina* clearance rates 0.015–0.21 ml animal^{-1} h^{-1} measured previously (Pedrós-Alió and Brock, 1983; Porter *et al.*, 1983; Børsheim and Andersen, 1987).

Other researchers have documented that large populations of daphnids can consume a substantial portion of bacterial production during seasonal *Daphnia* maxima (Pedrós-Alió and Brock, 1983; Güde, 1988). Our results suggest cladoceran grazing may be the primary fate of bacterial production during summer stratification in lakes dominated by cladocerans, as has been observed by Kankaala (1988). In contrast, heterotrophic flagellates have been considered the primary consumers of bacteria in marine and many freshwater systems (Coffin and Sharp, 1987; McManus and Fuhrman, 1988; Wikner and Hagström, 1988; Bloem *et al.*, 1989; Sanders *et al.*, 1989; Sherr *et al.*, 1989b; Pernie *et al.*, 1990). We conclude that cladocerans and heterotrophic flagellates are, together, important grazers on bacteria in freshwater with the relative significance of these two groups varying, depending on the time of the year (Figures 3 and 5).

In comparing the relative magnitudes of cladoceran and flagellate grazing we must also consider the limitations of our methods to estimate these rates. The fluorescent minicell method makes several assumptions including: (i) that the added minicells are appropriate analogs of the *in situ* bacteria; and (ii) all consumers ingest the minicells without discrimination. These assumptions are almost certainly not correct. For example, several recent papers document the importance of bacterial size in determining flagellate grazing rates (Andersson *et al.*, 1986; Chrzanowski and Šimek, 1990; Gonzalez *et al.*, 1990; Monger and Landry, 1990). The minicells used in our experiments were reasonably close to the size of the average bacteria in Paul and Tuesday lakes (see Method). Nevertheless, the minicells are uniform particles in terms of size and shape when in reality bacteria in natural communities consist of a diversity of sizes and shapes. Additional tests are required to determine if minicells provide a good estimate of grazing rates, given the potential significance of size-based interactions between bacteria and consumers. There is also uncertainty about how serious discrimination against minicells may be in biasing estimates of grazing rates, but independent experiments with flagellate communities in a temperate eutrophic lake suggest the method yields reasonable clearance rates (Pace *et al.*, 1990).

Grazing on bacteria and substrate supply are considered the main controls of bacterial abundance and growth rates (Coffin and Sharp, 1987). Comparison of grazing rates with bacterial standing stocks and growth rates allows us to assess the percentage of bacteria removed per day and turnover times of bacterial communities. Using our estimates of bacterial ingestion by flagellates, we can calculate the percentage of the bacterial standing stock consumed during each experiment. A small percentage (range 0.2–5.9%) of the bacteria are removed per day (Table II). If we consider grazing rates by flagellates and cladocerans

Table II. Percentage of bacteria removed per day by heterotrophic flagellates (F1) and cladocerans (C1), and the contribution of these two groups (T = F1 + C1)

Month	Paul			Tuesday		
	F1 (%)	C1 (%)	T (%)	F1 (%)	C1 (%)	T(%)
May	2.32	0.50	2.82	2.39	0.55	2.95
June	1.20	2.22	3.41	5.54	13.30	18.8
July	0.85	3.81	4.67	5.89	4.37	10.3
August	0.76	2.99	3.75	0.18	0.96	1.14

this percentage increases (range 1–19%, Table II). Specific bacterial growth rates based on measurements of thymidine incorporation and bacterial abundance for Paul and Tuesday Lakes were in the range of 0.053–0.46 day^{-1} during the summer of 1989 (M.L.Pace, unpublished data). Turnover times calculated from these growth measurements were 2–19 days, within the range of other aquatic systems previously summarized by Pace *et al.* (1990). Grazing rates by cladocerans and flagellates in Paul and Tuesday (range: 1.95–49.9 $\times 10^6$ bacteria l^{-1} h^{-1}; Figure 5) were less than measured growth rates (range: 1.65 $\times 10^7$–7.12 $\times 10^7$ bacteria l^{-1} h^{-1}). Since bacterial densities were relatively constant over the summer (Figure 1), and since grazing by flagellates and cladocerans did not always balance growth, other sources of mortality may be important. For example, we did not consider grazing by ciliates or rotifers. Ciliates in particular may be important consumers during certain portions of the seasonal cycle in lakes (e.g. Sanders *et al.*, 1989; Šimek *et al.*, 1990) and in marine systems (Sherr *et al.*, 1989a,b). Furthermore, parasitic mortality by viruses may be significant (Bergh *et al.*, 1989; Børsheim *et al.*, 1990; Proctor and Fuhrman, 1990), although relatively little is known about lytic bacteriophages in freshwater. It is also important to note that current methodological uncertainties seriously limit attempts to balance measures of grazing and growth for bacterial communities (Sanders *et al.*, 1989; Pace *et al.*, 1990; Wikner *et al.*, 1990). Nevertheless, comparisons of standing stocks, growth rates and grazing rates in our study do indicate that bacterial community turnover times are on the order of days to weeks, as opposed to <2 days, as suggested in some earlier studies (Kirchman *et al.*, 1982; Ducklow and Hill, 1985; Scavia and Laird, 1987).

Grazing by mixotrophs did not appear to be important in the epilimnion and metalimnion of the two lakes studied. Bird and Kalff (1989) noted high rates of ingestion by mixotrophic chrysomonads at low light intensities where carbon fixation was reduced. Above these depths, chrysomonad phagotrophy was relatively low. Pace *et al.* (1990) also observed relatively low grazing by *Dinobryon* in epilimnetic samples. Perhaps, the degree of phagotrophy by this genus is related to light and nutrient conditions, although for a related species, *Poterioochromonas malhamensis*, Caron *et al.* (1990) observed that phagotrophy was relatively constant under varying nutrient and light conditions.

Total community grazing rates by flagellates were linearly related to the abundance of attached flagellates ($r^2 = 0.48$; $P < 0.01$). This relationship is a function of the high ingestion rates of bacteria per attached flagellate. Free

flagellates had low ingestion rates and community grazing rates were independent of their abundance ($r^2 = 0.07$; $P > 0.1$). Attached flagellates are potentially important bacterial consumers in lakes where choanoflagellates have been noted to colonize diatoms, blue greens as well as the chrysophyte, *Dinobryon* (Fenchel, 1987; Pace *et al.*, 1990). It is not clear what factors control the abundance of attached flagellates, but the abundance and dynamics of suitable host algae are probably important.

Our initial hypotheses concerning the effect of contrasting zooplankton in the two lakes on bacteria and flagellates were not supported by our experiments. Flagellate grazing was higher in Tuesday than in Paul as predicted; however, the higher grazing was primarily a function of differences in the number of attached flagellates. Furthermore, cladocerans were important grazers of bacteria in Tuesday relative to Paul (Figure 5) contrary to our initial expectation. Grazing by both large and small cladocerans on bacteria and flagellates complicate simple predictions about the effect of different zooplankton community composition on microbial food webs. The results of our study indicate the importance of cladocerans and attached flagellates in the consumption of bacteria in freshwater.

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References

Andersson,A., Larsson,U. and Hagström,Å. (1986) Size-selective grazing by a microflagellate on pelagic bacteria. *Mar. Ecol. Prog. Ser.*, **33**, 51–57.

Bergh,Ø., Børsheim,K.Y., Bratbak,G. and Heldal,M. (1989) High abundance of viruses found in aquatic environments. *Nature*, **340**, 467–468.

Bird,D.F. and Kalff,J. (1989) Phagotrophic sustenance of a metalimnetic phytoplankton peak. *Limnol. Oceanogr.*, **34**, 155–162.

Bloem,J., Ellenbroek,F.M., Bär-Gilissen,M.-J.B. and Cappenberg,T.E. (1989) Protozoan grazing and bacterial production in stratified Lake Vechten estimated with fluorescently labeled bacteria and by thymidine incorporation. *Appl. Environ. Microbiol.*, **55**, 1787–1795.

Bjørnsen,P.K., Larsen,J.B., Geertz-Hansen,O. and Olesen,M. (1986) A field technique for the determination of zooplankton grazing on natural bacterioplankton. *Freshwater Biol.*, **16**, 245–253.

Børsheim,K.Y. (1984) Clearance rates of bacteria-sized particles by freshwater ciliates measured with monodisperse fluorescent latex beads. *Oecologia*, **63**, 286–288.

Børsheim,K.Y. and Andersen,S. (1987) Grazing and food size selection by crustacean zooplankton compared to production of bacteria and phytoplankton in a shallow Norwegian mountain lake. *J. Plankton Res.*, **9**, 367–379.

Børsheim,K.Y., Bratbak,G. and Heldal,M. (1990) Enumeration and biomass estimation of planktonic bacteria and viruses by transmission electron microscopy. *Appl. Environ. Microbiol.*, **56**, 352–356.

Caron,D.A., Porter,K.G. and Sanders,R.W. (1990) Carbon, nitrogen, and phosphorus budgets for

the mixotrophic phytoflagellate *Poterioochromonas malhamensis* (Chrysophyceae) during bacterial ingestion. *Limnol. Oceanogr.*, **35**, 433–443.
Carpenter,S.R. and Kitchell,J.F. (1988) Consumer control of lake productivity. Large-scale experimental manipulations reveal complex interactions among lake organisms. *BioScience*, **38**, 764–769.
Carpenter,S.R., Kitchell,J.F. and Hodgson,J.R. (1985) Cascading trophic interactions and lake productivity. *BioScience*, **35**, 634–639.
Carpenter,S.R., Elser,M.M. and Elser,J.J. (1986) Chloropyll production, degradation, and sedimentation: implications for paleolimnology. *Limnol. Oceanogr.*, **31**, 112–124.
Carpenter,S.R., Kitchell,J.F., Hodgson,J.R., Cochran,P.A., Elser,J.J., Elser,M.M., Lodge,D.M., Kretchmer,D., He,X. and von Ende,C.N. (1987) Regulation of lake primary productivity by food web structure. *Ecology*, **68**, 1863–1876.
Chrzanowski,T.H. and Šimek,K. (1990) Prey-size selection by freshwater flagellated protozoa. *Limnol. Oceanogr.*, **35**, 1429–1436.
Coffin,R.B. and Sharp,J.H. (1987) Microbial trophodynamics in the Delaware estuary. *Mar. Ecol. Prog. Ser.*, **41**, 253–266.
Cynar,F.J. and Sieburth,J.McN. (1986) Unambiguous detection and improved quantification of phagotrophy in apochlorotic nanoflagellates using fluorescent microspheres and concomitant phase contrast and epifluorescence microscopy. *Mar. Ecol. Prog. Ser.*, **32**, 61–70.
Ducklow,H.W. and Hill,S.M. (1985) Tritiated thymidine incorporation and the growth of heterotrophic bacteria in warm core-rings. *Limnol. Oceanogr.*, **30**, 260–272.
Elser,M.M., Elser,J.J. and Carpenter,S.R. (1986) Paul and Peter lakes: a liming experiment revisited. *Am. Midl. Nat.*, **116**, 262–295.
Fenchel,T. (1987) *Ecology of Protozoa*. Brock/Springer.
Gonzalez,J.M., Sherr,E.B. and Sherr,B.F. (1990) Size-selective grazing on bacteria by natural assemblages of estuarine flagellates and ciliates. *Appl. Environ. Microbiol.*, **56**, 583–589.
Güde,H. (1986) Loss processes influencing the growth of planktonic bacterial populations in Lake Constance. *J. Plankton Res.*, **8**, 795–810.
Güde,H. (1988) Direct and indirect influences of crustacean zooplankton on bacterioplankton in lake Constance. *Hydrobiologia*, **159**, 63–73.
Haas,L. (1982) Improved epifluorescence microscopy for observing planktonic micro-organisms. *Ann. Inst. Oceanogr., Paris*, **58**, 261–266.
Hobbie,J.E., Daley,R.J. and Jasper,S. (1977) Use of Nuclepore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.*, **33**, 1225–1228.
Kankaala,P. (1988) The relative importance of algae and bacteria as food for *Daphnia longispina* (Cladocera) in a polyhumic lake. *Freshwater Biol.*, **19**, 285–296.
Kirchman,D.L., Ducklow,H.W. and Mitchell,R. (1982) Estimates of bacterial growth from changes in uptake rates and biomass. *Appl. Environ. Microbiol.*, **44**, 1296–1307.
Lazzaro,X. (1987) A review of planktivorous fishes: their evolution, feeding behaviours, selectivities and impacts. *Hydrobiologia*, **146**, 97–167.
Leavitt,P.R., Carpenter,S.R. and Kitchell,J.F. (1989) Whole-lake experiments: the annual record fossil pigments and zooplankton. *Limnol. Oceanogr.*, **34**, 700–717.
McManus,G.B. and Fuhrman,J.A. (1986) Bacterivory in seawater studied with the use of inert fluorescent particles. *Limnol. Oceanogr.*, **31**, 420–426.
McManus,G.B. and Fuhrman,J.A. (1988) Clearance of bacteria-sized particles by natural populations of nanoplankton in the Chesapeake Bay outflow plume. *Mar. Ecol. Prog. Ser.*, **42**, 199–206.
Monger,B.C. and Landry,M.R. (1990) Direct-interception by marine zooflagellates: the importance of surface and hydrodynamic forces. *Mar. Ecol. Prog. Ser.*, **65**, 123–140.
Pace,M.L., McManus,G.B. and Findlay,S.E.G. (1990) Planktonic community structure determines the fate of bacterial production in a temperate lake. *Limnol. Oceanogr.*, **35**, 795–808.
Pedrós-Alió,C. and Brock,T.D. (1983) The impact of zooplankton feeding on the epilimnetic bacteria of a eutrophic lake. *Freshwater Biol.*, **13**, 227–239.
Pernie,G.L., Scavia,D., Pace,M.L. and Carrick,H.J. (1990) Micrograzer impact and substrate limitation of bacterioplankton in Lake Michigan. *Can. J. Fish. Aquat. Sci.*, **47**, 1836–1841.
Peterson,B.J., Hobbie,J.E. and Haney,J.F. (1978) *Daphnia* grazing on natural bacteria. *Limnol. Oceanogr.*, **23**, 1039–1044.
Porter,K.G., Feig,Y.S. and Vetter,E.F. (1983) Morphology, flow regimes, and filtering rates of *Daphnia*, *Ceriodaphnia*, and *Bosmina* fed natural bacteria. *Oecologia*, **58**, 156–163.
Proctor,L.M. and Fuhrman,J.A. (1990) Viral mortality of marine bacteria and cyanobacteria. *Nature*, **343**, 60–62.

Sanders,R.W., Porter,K.G., Bennett,S.J. and DeBiase,A.E. (1989) Seasonal patterns of bacterivory by flagellates, ciliates, rotifers, and cladocerans in a freshwater planktonic community. *Limnol. Oceanogr.*, **34**, 673–687.

Scavia,D. and Laird,G.A. (1987) Bacterioplankton in Lake Michigan: dynamics, controls, and significance to carbon flux. *Limnol. Oceanogr.*, **32**, 1017–1033.

Shapiro,J. (1980) The importance of trophic-level interactions to the abundance and species composition of algae. In Barica,J. and Mur,L.R. (eds), *Hypereutrophic Ecosystems*. Dr W.Junk Publishing Co., The Hague, Netherlands, pp. 105–115.

Sherr,B.F., Sherr,E.B. and Fallon,R.D. (1987) Use of monodispersed fluorescently labeled bacteria to estimate *in situ* protozoa bacterivory. *Appl. Environ. Microbiol.*, **53**, 958–965.

Sherr,E.B., Rassoulzadegan,F. and Sherr,B.F. (1989a) Bacterivory by pelagic choreotrichous ciliates in coastal waters of the NW Mediterranean Sea. *Mar. Ecol. Prog. Ser.*, **55**, 235–240.

Sherr,B.F., Sherr,E.B. and Pedrós-Alió,C. (1989b) Simultaneous measurement of bacterioplankton production and protozoan bacteriovory in estuarine water. *Mar. Ecol. Prog. Ser.*, **54**, 209–219.

Šimek,K., Macek,M. and Vynálek,V. (1990) Uptake of bacteria-sized fluorescent particles by natural protozoan assemblage in a reservoir. *Arch. Hydrobiol. Beih. Ergebn. Limnol.*, **34**, 275–281.

Stockner,J.G. and Porter,K.G. (1988) Microbial food webs in freshwater planktonic ecosystems. In Carpenter,S.R. (ed.), *Complex Interactions in Lake Communities*. Springer-Verlag, New York, pp. 69–83.

Wikner,J. and Hagström,Å. (1988) Evidence for a tightly coupled nanoplanktonic predator–prey link regulating bacterivores in the marine environment. *Mar. Ecol. Prog. Ser.*, **50**, 137–145.

Wikner,J., Andersson,A., Normark,S. and Hagström,Å. (1986) Use of genetically marked minicells as a probe in measurements of predation on bacteria in aquatic environments. *Appl. Environ. Microbiol.*, **49**, 19–23.

Wikner,J., Rassoulzadegan,F. and Hagström,Å. (1990) Periodic bacterivore activity balances bacterial growth in the marine environment. *Limnol. Oceanogr.*, **35**, 313–325.

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