Micrograzer Impact and Substrate Limitation of Bacterioplankton in Lake Michigan

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We estimated Lake Michigan epilimnetic heterotrophic bacterial loss rates, predator size, and substrate limitation in 1986 and 1987. The bacterial growth rates were always enhanced by organic substrate additions indicating that bacterial growth is limited, to some degree, by substrate availability. In this study we obtained loss rates and intrinsic growth rates each between 0.32 and 1.45 d^{-1}. The grazers were predominantly picoplankton-size organisms, presumably heterotrophic flagellates. Using radiolabeled bacteria, only a small percentage (2–3%) of bacterial cells were incorporated into larger size fractions after 24 h. These results indicate that during our experiments heterotrophic bacteria were not a direct, significant, carbon source for the upper trophic levels.

Nous avons évalué le taux de déperdition des bactéries épilimnétiques hétérotrophes, la taille de leurs prédateurs et les contraintes imposées à leur croissance par les ressources nutritives auxquelles elles ont accès dans le lac Michigan en 1986 et 1987. L'addition de matière organique avait toujours pour effet d'accroître les taux de croissance des bactéries, ce qui laisse entendre que leur croissance dépend, dans une certaine mesure, de la quantité de substrat à laquelle elles ont accès. Les taux de déperdition et de croissance intrinsèque que nous avons obtenus se situaient chacun entre 0,32 et 1,43 $jour^{-1}$. Les organismes de taille picoplanctonique, probablement des flagellés hétérotrophes, étaient les principaux brouteurs. Au moyen de bactéries radiomarquées, nous avons observé que seulement un faible pourcentage de cellules bactériennes (2–3%) était incorporé dans des organismes de plus grande taille 24 heures après le début de l'expérience. Ces résultats montrent que, durant nos expériences, les bactéries hétérotrophes ne constituaient pas une source directe importante de carbone pour les organismes des niveaux trophiques supérieurs.

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The roles of both heterotrophic and autotrophic picoplankton in aquatic food webs are increasingly recognized as ecologically significant. For example, several studies have found that much of the carbon assimilated by heterotrophic bacteria and autotrophic picoplankton is grazed by nano- and microplankton populations (e.g. >3μm protozoans) (Wright and Coffin 1984; Fahnenstiel et al. 1986; Pace 1988; Gude 1988). In the Upper Great Lakes the importance of picoplankton may be especially signficiant because at times heterotrophic bacterial production rates rival autotrophic production in Lake Michigan (Scavia et al. 1986; Scavia and Laird 1987) and nearly half of autotrophic primary production passes a 3.0 μm screen in Lake Superior (Fahnenstiel et al. 1986). Marine studies have also found that a substantial proportion of the autotrophic production is associated with particles passing a 1.0 μm screen (Li et al. 1983). While there is significant activity in the microbial food web, it is still unclear if significant carbon is passing from this loop to the traditional food web (i.e. crustacean zooplankton, nano- and net phytoplankton). This question will have to be addressed to further understand the role of picoplankton in the ecosystem.

We suggested in earlier work (Scavia and Laird 1987) that grazing is the dominant process in controlling the size of the rather stable Lake Michigan heterotrophic bacteria population. Recently it was found that surface abundances of nanoflagellates peaked during mid-stratification (Carrick and Fahnenstiel 1989), a period when bacterial abundances decline (Scavia and Laird 1987). This further indicates a tight coupling between the nanoplankton and picoplankton populations.

Substrate availability appears to be an important factor controlling epilimnetic bacterial growth in Lake Michigan (Scavia and Laird 1987; Gardner et al. 1986, 1989). Because bacteria

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are considered to be the link between the dissolved organic pools and the traditional food web, the quantitative importance of bacterial substrate limitation would improve the overall understanding of the microbial food web.

In this study we made seasonal estimates of bacterial grazing rates. In 1987, fractionation experiments were also conducted to estimate the percentage of bacteria transferred to the mesoplankton via the microbial food web and also determine predator size. Finally, we used substrate addition experiments to determine if and when bacterial growth could be stimulated by increasing available organic carbon.

Methods

Sample location was at the 100-m depth contour, 26 km west of Grand Haven, Michigan (43°1′11″N, 86°36′48″W). Surface (5 m) water samples, taken aboard the R/V *Shenehon*, were transferred to a 4-L or 20-L carboys and processed either on board or stored in coolers in the dark for no more than 3 h before processing at shore.

All experimental treatments were incubated 8–10 h in the dark at ambient temperature and subsampled for bacterial abundance over time. Subsamples ($n = 3$–10) were preserved (2% formaldehyde final concentration), and bacteria were counted by acridine orange direct count (AODC) method (Hobbie et al. 1977).

In 1986 experiments were done eight times in 1-L and 2-L precombusted BOD bottles on raw lakewater. In June and August of 1987 they were done in duplicate 2-L sterile tissue culture flasks (Corning tissue culture bottles, 850 cm^2) and sample water was prefiltered through a 35 μm Nitex screen. Rates of change in bacterial cell abundance were determined from linear regression of ln (abundance) vs. time.

To estimate grazing loss rates, one treatment in each experiment received the antibiotic gentamicin (Sigma, cat. no. AA-5-18, 20 μm mL^{-1} final concentration) at the beginning of the incubation. The antibiotic stops bacterial growth; thus, rate of decline in cell abundance (r_A), which represents total bacterial mortality, is assumed to be due to grazing. For comparison, two separate treatments received 0.61 μm non-carboxylated fluorescence beads (mean volume = 0.12 μm^3, SD = 4×10^{-5} μm^3, final concentration 10^5 beads mL^{-1}, Polysscience). Bacterial cells in Lake Michigan summer epilimnetic regions are approximately 0.072 μm^3 in size (Scavia and Laird 1987). One of these two samples received the antibiotic gentamicin as well as beads. We compared the rate of disappearance of beads in these two samples to each other and to the independent estimate r_A. In 1987 we included a second control in which beads were added to 0.2 μm filtered (Nuclepore) water. The disappearance of beads in this treatment was attributed to abiotic factors such as sorption to the walls of the container.

Intrinsic growth rates (μ) can be calculated from the slope of ln(bacteria abundances) vs. time in the untreated sample, r_R, and the slope of ln(bacterial abundances) vs. time in the antibiotic treated sample, r_A. The realized population growth rate, r_R, is equal to the difference between intrinsic growth rate, μ, and loss (m) rate:

$$(1) \quad r_R = \mu - m.$$

Because growth is inhibited ($\mu = 0$), r_A is equal to $-m$; this loss rate has been attributed to grazing (Fuhrman and McManus 1984; Scavia et al. 1986). By determining r_R and r_A, we can calculate an intrinsic growth rate from

$$(2) \quad \mu = (r_R + m) = (r_R - r_A).$$

To explore the size range of potential predators, lake water was also gently filtered through 0.8, 1.2, 2.0–5.0, or 10.0 μm 47 mm-diameter Nuclepore filters prior to incubation.

In the substrate addition experiments, both glucose (final concn. 0.522 μM) and a mixture of 17 amino acids (Sigma, cat. no. AA-S-18, final concn., 0.522 μM) were added to raw lake water. Change in bacterial abundance was followed over time as above.

In 1987 experiments, the flux of bacterial cells into larger size fractions was determined using both radiolabeled planktonic bacteria derived from Lake Michigan and radiolabeled "minicells" derived from a mutant strain of *Escherichia coli*. During cell division of specific *E. coli*, a small spherical "minicell" is produced containing no DNA, as well as a normal cell with a full complement of DNA (Wikner et al. 1986). We used these two types of tracer bacteria to overcome some of the limitations of each. Radiolabeled planktonic bacteria, while accurately representing diversity of sizes and shapes of natural bacteria, would probably not become uniformly labeled in the short incubations we used (see below). Minicells, on the other hand, represent only a single size and shape, but offer the advantage of being uniformly labeled.

Planktonic bacteria for radiolabeling were concentrated from 6 L of lake water by first gently filtering through a 1.0 μm Nuclepore filter. Amino acids were added (Sigma, cat. no AA-S-18, 20 $\mu g \cdot mL^{-1}$ final concn.) to the 1.0 μm filtrate to ensure that ample substrate was available for bacterial growth. The filtrate was concentrated through reverse filtration using a 4-L pressure tank and a stirring cell filled with a 0.2 μm 76 mm Nuclepore filter resulting in a 6-fold concentration. The concentrate was incubated for 1 h at epilimnetic temperature before 1 mCi of [^{3}H-methyl]thymidine (70–80 $Ci \cdot mmol^{-1}$, New England Nuclear) was added and incubated for one additional hour. Two 20-L polycarbonate carboys were filled with unaltered lake water and unlabeled thymidine (final concentration 3.0 nM). The radiolabeled concentrate was split and added to both 20-L carboys (4% of ambient concentration).

We used a modification of the method of Wikner et al. (1986) to radiolabel minicells. Minicells were isolated from larger cells in overnight cultures using sucrose gradient centrifugation (Wikner et al. 1986). Minicell suspensions concentrated in a buffered saline solution (BSG — 0.85% NaCl, 0.03% KH_2PO_4, 0.06% Na_2HPO_4 100 μg gelatin$\cdot mL^{-1}$) were incubated with 250 μCi [^{3}H]methionine (80 Ci $mmol^{-1}$) in a shaking water bath at 37°C overnight. To remove unincorporated radioactivity, cells were sedimented by centrifugation and resuspended in BSG. Radioactivity per cell was determined by filtering 0.1 mL of the final minicell suspension on a 0.2 μm Nucleopore filter. Filters were washed twice with BSG, added to a scintillation vial, boiled for 1 h in 5% trichloracetic acid, and radioassayed. Minicell concentrations were determined by enumeration using the acridine orange direct count method (Hobbie et al. 1977). Minicells were added to single 20 L carboys at 7–8 × 10^5 minicells$\cdot mL^{-1}$ (= 2–4 × 10^3 DPM$\cdot mL^{-1}$).

The 20-L carboys containing either radiolabeled minicells or concentrated radiolabeled planktonic bacteria were incubated at shore under screened natural light and epilimnetic temperatures. After 24 h, the contents of the 20-L carboys were filtered sequentially through 100 μm and 35 μm Nitex screens and

TABLE 1. Ingestion rate of florescent beads, d^{-1}. NS — Non-significant regression.

	Lakewater		Antibiotic treated		
Date	Rate	SE	Rate	SE	Average[a]
4/30/86	0.42	0.17	0.54	0.19	0.48
5/27/86	1.20	0.43	1.50	0.38	1.35
6/12/86	0.34	0.15	0.63	0.04	0.49
7/2/86	0.65	0.20	NS		0.65
7/28/86	NS		0.32	0.16	0.32
8/21/86	1.45	0.60	1.12	0.26	1.29
10/3/86	0.67	0.21	0.33	0.21	0.50
10/23/86	1.70	0.84	1.64	0.93	1.67

[a]Slopes with and without antibiotic treatments were not significantly different ($\alpha = 0.10$).

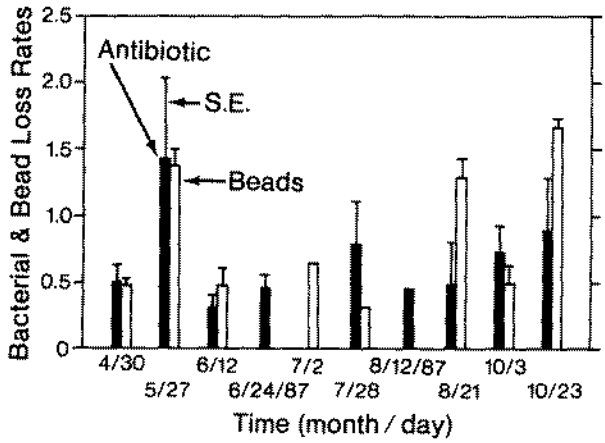

FIG. 1. Bacterial and bead loss rates (d^{-1}) in 1986 and 1987. Bead rates represent the means of treatments with and without antibiotic. Error bars represent standard error of regression.

through 10.0, 2.0, and 0.2 μm Nuclepore filters. An additional filter (0.8 μm) was used in the August 1987 experiment. The Nuclepore filters and subsamples of the 35 μm filtrate were placed in liquid scintillation vials. Material caught on the Nitex screens was washed onto 10 μm Nuclepore filters and placed in vials.

Twelve mL's of Filter Count (Packard) scintillation cocktail were added to each scintillation vial and radioactivity was assayed with a Packard Tri-Carb scintillation spectrometer. Counting efficiency was determined with external standards.

Results

Our method of estimating grazing rates assumes that the antibiotic, gentamicin, does not effect the eucaryotic grazers. To evaluate this, we measured the disappearance rates of bacteria-sized fluorescence beads in lake water with and without gentamicin. These measurements may underestimate actual rates because our incubation times (8–10 h) were significantly greater than gut retention times (20–60 min) of protozoa (Sherr et al. 1988). However, our estimates are based on regressions of time-course measurements and we observed no systematic decrease in rate estimates over time. In general, the disappearance rates with and without gentamicin were not significantly different (Table 1). Therefore, for further comparisons (see below) we averaged the two rates. In 1986 disappearance rates ranged from 0.32 to 1.67 d^{-1} (Table 1, Fig. 1). As a control, beads were also added to 0.2 μm filtrate during the 1987 experiments. The rate of bead disappearance in these filtrates was 0.70 d^{-1} on June 24 and 0.37 d^{-1} on August 12. These rates were subtracted from the disappearance rates of beads in untreated lake water (0.96 d^{-1} and 0.77 d^{-1}, respectively). Because the filtrate loss rates in these experiments were high, we repeated these filtrate-control experiments both in the combusted glass BOD bottles, used in 1986, and in the tissue culture flasks, used in 1987. In both cases there was no significant rate of change in bead abundances ($\alpha > 0.40$); therefore, we assume that the 1987 results were specific to that experiment. Bacterial loss rates, based on the antibiotic treatment, ranged from 0.32 to 1.43 d^{-1} in 1986 and were 0.48 d^{-1} on both June 24 and August 12, 1987 (Table 2, Fig. 1).

To determine the size of potential predators, lake water was prefiltered through several different filter pore sizes prior to incubation. The increase in bacterial abundance, above that found in the unaltered treatments, was attributed to predator elimination. In 1986, when filtrate sizes ranged from 2–5 μm, a significant increase in bacterial population occurred only in October. On June 24, 1987 several filter sizes were used. In the 2.0 μm-filter, 10.0 μm-filter, and untreated samples, the rate of change in abundances was not significantly different from zero. In the 0.8 μm-filter and 1.2 μm-filter treatments, growth rates of 0.48 ± 0.07 d^{-1} and 0.66 ± 0.18 d^{-1} (rate ± SE) were found, respectively, On August 12, 1987 growth rates of 0.35 ± 0.10 d^{-1} and 0.14 ± 0.10 d^{-1} (rates ± SE) were obtained in replicate 0.8 μm filtrates. Generally, we found that only in the less than 2.0 μm-filtrates was there a significant positive increase in bacterial abundances above those of the unaltered samples. This suggested that the predators are in the less than 2.0 μm size category.

These results were confirmed with the tracer experiments in which radiolabeled bacteria traced consumption into various size fractions of the food web. After 24 h, very little isotope was found in size fractions greater than 2.0 μm either in the case of treatments using concentrated lake bacteria or minicells. On June 24, 97% (concentrated bacteria) and 85% (minicells) of the label remained in the 0.2–2μm fraction (Fig. 2a). On August 12, for the concentrated bacteria treatment, 80% remained in the 0.8 μm fraction (Fig. 2b). Similarly, for the minicell treatment in August, 95% of the label remained in the smallest two size fractions (Fig. 2b).

We inferred substrate limitation if added substrate stimulated bacterial growth rates above the unaltered controls. In 1986 growth rates ranged between 0.29 and 1.11 d^{-1} in the lake water samples receiving substrate (Table 2). In 1987 growth rates in two separate experiments were 0.48 and 1.20 d^{-1} on June 24, and 0.33 d^{-1} on August 12. These rates are all greater than those in the respective controls.

Discussion

Substrate Limited Growth

We have shown previously for Lake Michigan that below 10°C bacterial growth rate increases logarithmically with increasing temperature (Scavia and Laird 1987). These conditions exist for subthermocline regions all year and for the entire water column between early fall and spring. When temperatures are warmer, growth appears to be limited more by substrate availability. Gardner et al. (1987) evaluated this for Lake Michigan bacteria by comparing seasonal

TABLE 2. Grazing and growth rates (d^{-1}). r_R = Control, bacterial growth rates; r_A = antibiotic treatment, bacterial mortality rates; r_S = substrate addition, bacterial growth rates; SE = standard error of regression slope; P = attained level of significance of regression; NS = non-significant regression.

	Untreated			Antibiotic			Substrate addition		
Date	r_R	SE	p	r_A	SE	p	r_S	SE	p
4/30/86		NS		0.51	0.09	0.03	1.11	0.39	0.10
5/27/86		NS		1.43	0.59	0.14	0.52	0.35	0.28
6/12/86		NS		0.32	0.10	0.05	0.49	0.29	0.19
7/2/86		NS			NS		0.29	0.15	0.15
7/28/86		NS		0.79	0.31	0.09	0.48	0.03	0.005
8/21/86		NS		0.49	0.30	0.24	1.10	0.11	0.06
10/3/86	−0.28	0.02	0.05	0.74	0.19	0.06	0.55	0.37	0.28
10/23/86				0.90	0.38	0.14			
6/24/87		NS		0.48	0.07	0.001	0.48	0.24	0.08
							1.20	0.14	0.0003
8/12/87	0.17	0.10	0.14	0.48	0.07	0.001	0.50	0.24	0.11
		NS		0.48	0.19	0.06			

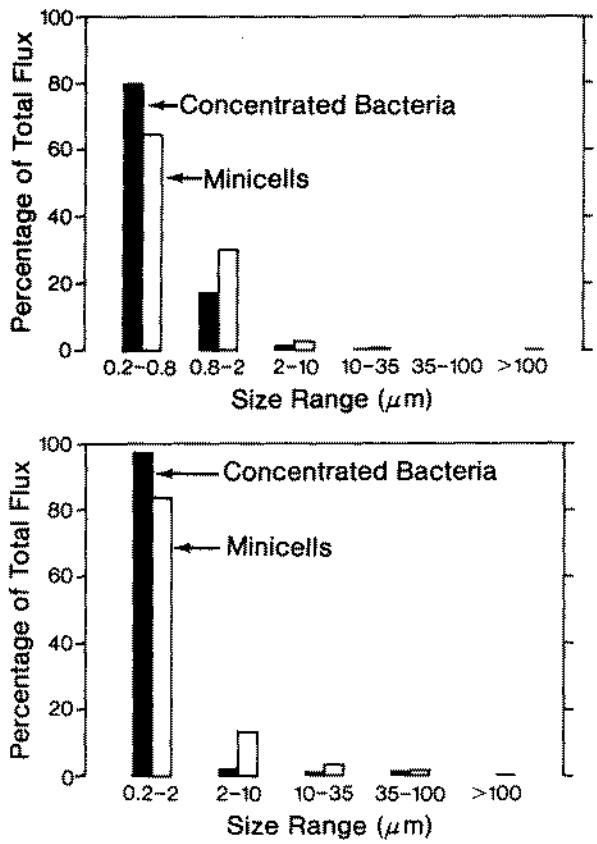

FIG. 2. Percent of total radiolabeled bacteria found in various size fractions after 24 h incubation. (a) June 24, 1987 and (b) August 12, 1987.

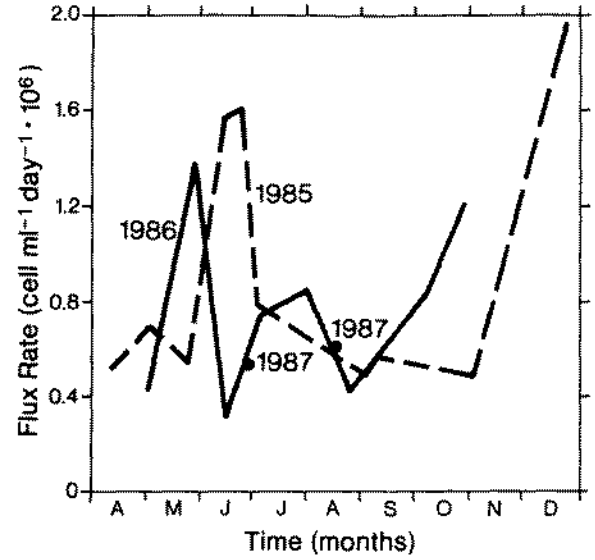

FIG. 3. Bacterial flux rates (cell·mL^{-1}·day^{-1}) calculated from specific loss rates and ambient cell concentrations in 1985, 1986, and 1987.

"maximum" and "actual" dissolved free amino acids (DFAA) turnover rates. In their analysis, the greater the difference between the two rates the greater the substrate limitation. They found the greatest differences in summer and early fall, suggesting that bacteria are more limited by substrate in the summer epilimnion than during spring and fall. In contrast, we have shown lowest response of bacteria to added organic substrates (glucose plus DFAA) to occur in midsummer (Table 2). Both of these methods rely on following a response to presumably important substrates. More recently, Laird and Scavia (1990) have followed the seasonal development of bacterial growth potential on natural organic supplies and found, similar to Gardner et al. (1989), that the substrate supply appears to dwindle over summer.

Grazing Losses

Use of antibiotics to inhibit bacterial growth and estimate grazing loss presumes that the decreases in population size, which represents total bacterial mortality, are mostly due to grazing and that the antibiotic is specific to prokaryotes. Previously, we showed that gentamicin alone does not cause a decline in bacterial cell abundance (Scavia et al. 1986). In this study, we demonstrated that the ingestion of bacterial-size fluorescence beads was not influenced by the addition of gentamicin. Therefore, we conclude that the predators in the presence of gentamicin continued to ingest bacteria normally.

In this study, we also used selective filtration to estimate predator size. Problems with this technique potentially include the introduction of dissolved organic compounds in the filtrate, presumably as a result of cell breakage (Goldman and Dennett 1985), removal of phytoplankton that supply organic carbon to the bacteria, and removal of some of the bacterial population.

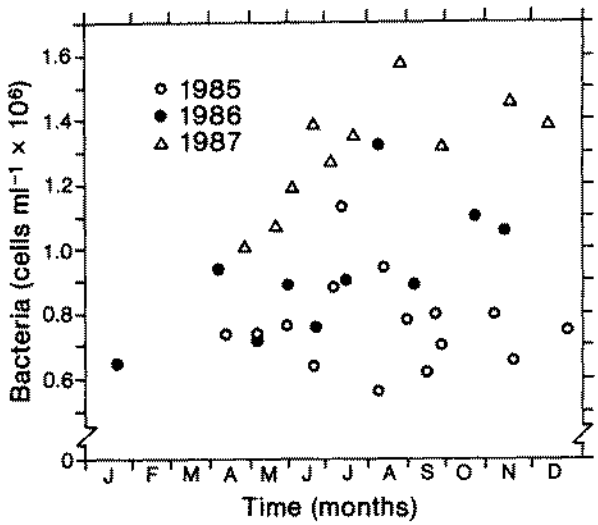

FIG. 4. Surface water bacteria abundances in 1985, 1986, and 1987.

TABLE 3. Flux and clearance rates for 1986 and 1987

	Clearance rates nL·cell^{-1}·h^{-1}	Flux rates 10^5 cell·mL^{-1}·d^{-1}
4/30/86	9.4	4.40
5/27/86	15.9	13.91
6/1/86	2.4	3.13
7/28/86	3.8	8.61
8/21/86	2.9	4.55
10/3/86	8.2	8.42
10/23/86	10.0	11.97
6/24/87	3.6	5.42
8/12/87	2.8	6.19

Initial bacterial abundances in the filtrate experiments were similar to those in lake water; therefore, the latter problem did not seem major. Further, it has been demonstrated that there is no increase in measurable primary amine or ammonium concentration in Nuclepore-filtered Lake Michigan water (Gardner et al. 1987). Removing algae could potentially reduce bacterial production; however, we could not assess that effect with the methods used in this study.

Grazing loss rates ranged between 0.32 d^{-1} and 1.43 d^{-1} between April and October, 1986, and were 0.48 d^{-1} on both June 24, and August 12, 1987. While these rates ranged slightly lower than those previously reported for Lake Michigan (0.62 d^{-1} – 2.8 d^{-1}; Scavia and Laird 1987) they were generally consistent with rates of loss of fluorescent beads (Fig. 1) measured concurrently. It is curious to note that while the specific loss rates in 1986 and 1987 were lower than those in 1985, higher bacterial abundances in 1986 and 1987 (Fig. 4) compensated and resulted in consistent calculated flux rates. These calculated flux rates, determined from epilimnetic bacterial abundances and experimental loss rates (r_λ) (Fig. 3), ranged between 3.13 and 13.90 × 10^5 cells ingested·mL^{-1}·day^{-1} in 1986 and 1987. Flux rates in 1985 were 4.95 – 21.2 × 10^5 cells ingested·mL^{-1}·day^{-1} (Scavia and Laird 1987). Flux rates peaked during late spring when bacterial production is also highest (Scavia and Laird 1987), declined during summer, and increased again in the fall.

Predators appeared to pass through the 2–5μm filter used in 1986. This is consistent with filtrate experiments in 1987 when significant increases in cell abundances occurred only in the

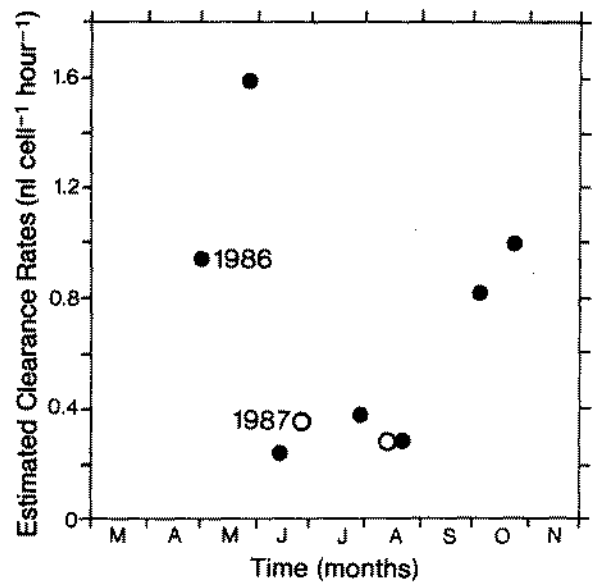

FIG. 5. Estimated clearance rates (nL·flagellate^{-1}·day^{-1}) calculated from specific loss rates and heterotrophic flagellate abundance estimates from Carrick and Fahnenstiel (1989).

less than 2.0 μm size filtrate. Marine studies have also suggested that the major consumers of bacteria are small heterotrophic flagellates (Fenchel 1982a; Fenchel 1986). A recent experimental study by Wikner and Hagstrom (1988) found results similar to ours for coastal marine environments. In their study, predation on bacteria was measured in a series of size fractionated water samples. Predation was generally reduced only by filtration though 1.0 and 0.6 μm filters, implying that the primary predators were flagellates in the size range of 1–3 μm.

In our tracer, bacteria experiments we found <5% of the label in size classes greater than 2.0 μm after 24 h (Fig. 2a, 2b). Both types of tracers, concentrated lake bacteria and minicells, showed the same pattern, suggesting our conclusion is not limited by methodological concerns over labeling natural communites or using a single type of tracer cell. A similar study followed the fate of ^{14}C-labeled bacterioplankton and found that only 2% of the labeled bacterial cells were present in the larger organisms (Ducklow et al. 1986) after 13 d. Both studies indicate that heterotrophic bacterial carbon may not be passed significantly up the food chain, and that the primary bacteria consumers, the nannoflagellates, provide a formidable block in the carbon flow. Further investigations are needed to substantiate this conclusion.

To explore the notion that the heterotrophic flagellates are responsible for the bactivory in Lake Michigan, we calculated clearance rates from our estimated specific grazing rates and flagellate abundances determined in Lake Michigan surface waters in 1987 (Carrick and Fahnenstiel 1989). These estimates for summer ranged between 2.4 and 3.8 nL·flagellate^{-1}·hr^{-1} and are similar to those reported elsewhere (0.3-1.0 nL·flagellate^{-1}·h^{-1}, Sherr et al. 1983; 2.3–4.4 nL·flagellate^{-1}·h^{-1}, Caron et al. 1985; 4.2–12.9 nL·flagellate^{-1}·h^{-1}, Goldman and Caron 1985). Clearance rates in spring and fall 1986 were higher (8.2–15.9 nL·flagellate^{-1}·hr^{-1}) and similar to those of larger marine species (2–40nL·flagellate^{-1}·h^{-1}, Fenchel 1982b; 15–20nL·flagellate^{-1}·h^{-1}, Anderson and Fenchel 1985) (Table 3; Fig. 5). It thus seems reasonable to assume that the flagellates enumerated by Carrick and Fahnenstiel (1989) are

responsible for the bacterial grazing loss rates, although the possibility of consumption of bacteria by small mixotrophic algae (Sander and Porter 1988) cannot be excluded at present.

In conclusion, Lake Michigan epilimnetic bacterial growth rates can be stimulated by organic substrate between April and October. Bacterial growth is often in balance with bacterial loss and the immediate bacterial grazers fall into the picoplankton size category (0.2 μm – 2.0 μm). We found that bacterial carbon does not pass quickly to higher trophic levels, within a 24 h period, indicating that bacterial production may be a sink rather than a significant source of carbon for the upper food web in Lake Michigan.

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