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Bacterioplankton in Lake Michigan: Dynamics, controls, and significance to carbon flux[1]

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Abstract

Lake Michigan bacterial production, based on [³H-methyl]thymidine (TdR) incorporation and empirically determined conversion factors ($5-25 \times 10^9$ cells $nmol^{-1}$), decreased with distance from shore ($\sim 2\times$ over 30 km), was higher at night ($1.4\times-2.2\times$), and decreased with depth ($\sim 10\times$ over 100 m). TdR-based growth rates were consistent with independent antibiotic- and dilution-based estimates. Population size varied little and appeared controlled by balanced growth (0.02–0.33 h^{-1}) and grazing (0.039–0.12 h^{-1}). Growth correlated with temperature only below 10°C. Cell size ranged from 0.015 to 0.072 μm^3. Carbon content averaged 0.154 ± 0.047 pg C μm^{-3}. Net annual carbon production was 142 g C m^{-2} yr^{-1}. Summer averages were 28.9 (epilimnion), 10.4 (10–35 m), 1.6 (hypolimnion) μg C $liter^{-1}$ d^{-1}, and 652 mg C m^{-2} d^{-1} for the water column. Flux to microconsumers averaged 8.4 μg C $liter^{-1}$ d^{-1} in the summer epilimnion.

Annual areal bacterial carbon demand is met by autotrophic production only if little of the latter is lost by other means. This suggests that external loads are needed, our conversion factors are high, or autotrophic production is underestimated. Although only small adjustments of those factors will satisfy the annual balance, the summer imbalance is still too large. We suggest that temporal and spatial disequilibrium of labile organic carbon supply and bacterial use is responsible for the apparent discrepancy during summer.

Recent bacterioplankton studies have emphasized rates of growth and grazing loss (e.g. Ducklow and Hill 1985a,b; Scavia et al. 1986). Results from these and other studies (e.g. Klug and Reddy 1984; Hobbie and Williams 1984; Lovell and Konopka 1985a,b; Cole et al. 1984) suggest that bacteria are important components of both marine and freshwater environments. Lake Michigan is a mesotrophic to oligotrophic lake of oceanic physical scale that provides a natural open-water pelagic environment from which to compare bacterial production and loss rates with both marine and smaller freshwater ecosystems.

Our previous work on heterotrophic bacterioplankton in surface waters from a 100-m station (26 km from shore) in southeastern Lake Michigan between May and November 1984 (Scavia et al. 1986) indicated that bacterial concentrations there were typical of oligotrophic environments ($0.67-1.04 \times 10^6$ cells ml^{-1}) and that bacterial growth rates, estimated from incorporation of [³H-methyl]thymidine (TdR), varied between 0.05 and 0.24 h^{-1}. The empirically derived conversion factors, used in that work to convert TdR incorporation to cell production, varied seasonally. This seasonal variation had not been reported for other environments. The validity of the conversion factors was tested by comparing

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resulting growth rates with independent estimates of growth rate in late fall and early spring. Those comparisons corroborated the estimates of growth rate and suggested that grazing loss may be important in controlling bacterial population size in those seasons. In other studies from the same region of Lake Michigan, uptake kinetics of added organic substrates provided evidence that grazing losses may be the major mechanism controlling bacterial population size throughout summer (Gardner et al. 1986). To verify seasonal variation in conversion factors and to test for grazer control of population size, we determined seasonally dependent, empirical conversion factors and independently determined growth and grazing loss rates in 1985. We report those results here.

Heterotrophic bacterioplankton production is a significant fraction of total carbon production in both marine and freshwater environments. As we suggested previously (Scavia et al. 1986) and corroborate herein, bacterial growth rates in southeastern Lake Michigan are high and grazing losses are often comparable. It implies a tight coupling between bacterioplankton and their predators and suggests that biomass produced by bacteria is passed rapidly to the next trophic level. Although heterotrophic flagellates are often suggested as major predators, recent evidence points to the potential importance of crustacean zooplankton (e.g. Riemann 1985) and chlorophyll-containing, mixotrophic flagellates (Bird and Kalff 1986; Estep et al. 1986).

Estimating carbon flow through the bacterial loop, based on bacterial growth rates, requires accurate estimates of bacterial size and carbon content. If literature values are correct, organic carbon sources other than contemporary algal excretion in Lake Michigan (Laird et al. 1986) are needed to account for observed bacterial production (Scavia et al. 1986). One explanation for this discrepancy is that the carbon requirement may be balanced by sources uncoupled in time and space from its use. If these sources are tied ultimately to algal production, then annual areal production of bacteria must be bounded by that of phytoplankton. To test this hypothesis, we measured bacterial abundance, cell size, and production throughout 1985 at several depths and combined them with new measures of bacterial carbon content to estimate both summer and annual production of bacterial carbon in the water column for comparison to autotrophic production. We also estimated carbon flux to bacterial consumers and tested the hypothesis that a major portion of bacterial production is passed to the next trophic level.

Because, as shown below, most observations were similar in 1984 and 1985, we combined our 1984 results (Scavia et al. 1986) with those reported here for 1985 to develop an overall picture of the seasonal dynamics and controls of Lake Michigan bacterioplankton.

Methods

Sampling stations and ambient conditions—Water samples from 2–4 depths were taken at a station 100 m deep (DS-7; 43°1'11"N, 86°36'48"W) 26 km west of Grand Haven, Michigan, on 23 dates in 1984 and 1985. Samples were also taken from five stations along a 40-km transect perpendicular to shore and including DS-7 and from five stations along a 180-km north–south transect from a station off Frankfort, Michigan, to DS-7. We obtained one sample on 23 January 1986 from a 100-m station off Milwaukee (Fox Point Station) from the RV *Neeskay* of the Center for Great Lakes Studies, University of Wisconsin. This sample was included as part of the 1985 data set to complete the annual cycle. All samples, except the one at DS-7 on the north–south transect (26 July 1985), were taken during daylight (usually between 0800 and 1400). Two diel studies were also carried out at or near DS-7; samples were taken at 1630, 2230, 0730, and 1230 hours on 15–16 August and at 1045, 1215, 1845, and 2000 hours on 9 September 1985.

Temperature profiles were determined from vertical casts of a TTD system (an electronic bathythermograph coupled to a 25-cm light-path Sea Tech transmissometer and pressure transducer) aboard the RV *Shenehon*. Water samples were preserved (2% formaldehyde final concn) for bacterial enumeration with the acridine orange direct count (AODC) method (Hobbie et al. 1977). Bacteria were stained with acridine orange (0.01% final concn) for 1 min and then filtered onto 25-mm-diameter, 0.2-μm pore size Nuclepore filters. The filters had previously been stained with Irgalan black, rinsed, dried, and stored. Eight microscope fields were counted on each of two slides (each prepared from duplicate subsamples from each depth) at 1,500× under a Leitz Laborlux 12 microscope equipped with a Ploemopak epifluorescent illuminator and filter cube I. The volume of water filtered was adjusted to obtain about 30 cells per field. This procedure yields a 4.6% counting error, assuming Poisson statistics. Unpreserved water was also filtered (100 ml) in triplicate onto 47-mm glass-fiber filters (Whatman GF/F) and extracted in 90% acetone for fluorometric Chl *a* analysis (Strickland and Parsons 1972).

TdR incorporation—Water samples for TdR incorporation experiments were transferred to 4-liter linear-polyethylene carboys (usually one per depth) and either processed onboard or stored in dark coolers for no more than 3 h during transport to the shore laboratory. TdR incorporation into ice-cold trichloroacetic-acid (TCA)-insoluble material was determined as outlined by Fuhrman and Azam (1982). Concentration of added TdR and duration of incubation were established from uptake kinetics determined previously (Scavia et al. 1986). For each depth, two to three subsamples (30 ml) of the 4-liter carboys were processed. Each subsample was dispensed into autoclaved glass tubes along with [^{3}H-methyl]thymidine (70–80 Ci mmol^{-1}, New England Nuclear, about 20 nM final concn of added unlabeled TdR) and incubated for 40 min in the dark at ambient temperature. Isotope incorporation was stopped after 40 min by adding unlabeled TdR (final concn, 0.3 mM). After the samples were chilled to <4°C, 1.6 ml of 100% (wt/vol) ice-cold TCA (Sigma) was added (5% final concn) for 10-min extractions. Triplicate 10-ml subsamples from each tube were then filtered onto 25-mm-diameter, 0.2-μm pore size Gelman Metricel GA-8 filters and rinsed five times with 1 ml of 5% (vol/vol) ice-cold TCA. Filter funnels were then removed, and the filters were again rinsed three times with 1 ml of 5% ice-cold TCA. Filters were placed in scintillation vials with 12 ml of Filter Count (Packard) scintillation cocktail and radioactivity was assayed with a Packard Tri-Carb scintillation spectrometer. Counting efficiency was determined with external standards. Blanks were determined by processing samples killed with formaldehyde (2% final concn) as described above.

Conversion factors—A dilution-growth scheme was used to estimate the conversion of TdR incorporation to cell production (Kirchman et al. 1982). In that scheme, growth rates, determined by changes in cell counts, and thymidine incorporation rates are determined for diluted natural assemblages as they increase population size during 10–12-h incubations. These growth and TdR incorporation rates are then used as described below to determine conversion factors. About 100 ml of lake water were diluted with 900 ml of lake water, prepared by passing through sterile, 0.2-μm pore size, high-capacity (500-cm^2 effective filtration area) Gelman Mini Capsule filters. The diluted water was incubated in autoclaved growth flasks in the dark at ambient temperature and stirred manually at sampling times (about 0.5–2.0-h intervals). Initially, and at time intervals during the incubation, 20-ml subsamples were taken from the growth flasks and preserved to determine bacterial abundance (N). Thirty-milliliter subsamples were also taken over time for determination of TdR incorporation (V) as above. Additional samples were taken over the time-course, killed with formaldehyde, and processed as above for blanks. Bacterial growth rates from changes in cell counts and TdR uptake rates from these experiments were combined to determine conversion factors (C_i) in several different ways, as described by Kirchman et al. (1982) for C_1 and by Scavia et al. (1986) for C_2 (using

growth rates and pairs of abundances and TdR uptake estimates). Changes in cell counts were also used as independent estimates of growth.

Conversion factors (C_3) were also determined from measurements of the number of cells produced, $N_2 - N_1$, and moles of TdR incorporated, $V \cdot (T_2 - T_1)$, during successive time intervals, T_1 to T_2, of the experiment. Calculations of C_1 and C_2 require that all of the AODC population is growing, but calculation of C_3 does not. Natural population production rates (P, cells ml^{-1} d^{-1}) were calculated from

$$P = C_i V_r \tag{1}$$

where V_r is the rate of TdR incorporation by freshly collected, unaltered water samples and C_i is the conversion factor determined by drawing a smoothed seasonal curve through the values of C_1, C_2, and C_3.

Another method of converting TdR uptake to bacterial production is by estimating incorporation of TdR into DNA and using a derived conversion factor (Fuhrman and Azam 1980). We estimated the percent of isotope incorporated into DNA by incubating samples with and without the DNA-synthesis inhibitor Mitomycin C (Sigma). We have shown (Scavia et al. 1986) that this method produces results similar to the detailed macromolecular fractionations described by Fuhrman and Azam (1982) and Riemann and Sondergaard (1984).

Independent growth rates and grazing rates—Growth rates, estimated with a method independent of our TDR-based methods, were used to verify the latter technique. In these separate experiments, bacterial growth rates were determined from two 1,000-ml samples of lake water incubated for 8–10 h. One sample received the antibiotic gentamycin (Sigma, 20 μg ml^{-1} final concn) at the beginning of the incubation; the other sample was unaltered. Subsamples were taken over time and preserved to determine bacterial abundance (N) as described above. Bacterial growth is inhibited in the gentamycin-treated sample (Chrost 1978), but gentamycin alone does not cause AODC counts to decrease over time (Scavia et al. 1986). The slope of $\ln(N)$ vs. time in the untreated sample, r_R, is the realized population growth rate, equal to the difference between intrinsic growth (μ) and loss (m) rates:

$$r_R = \mu - m. \tag{2}$$

The slope of $\ln(N)$ vs. time in the antibiotic-treated sample, r_A, is equal to $-m$ because growth is inhibited ($\mu = 0$). 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

$$\mu = (r_R + m) = (r_R - r_A). \tag{3}$$

Bacterial cell size and carbon content—To measure bacterial size we made photographic slides of AODC preparations using 30–40-s exposures with Kodak Ektachrome professional slide film (EES 135-36) shot at ASA 1600. Slides were push-processed two stops and the images were projected onto white paper to effect a 10-fold enlargement. Rod volumes were determined from lengths and widths, measured to the nearest 0.5 mm with a clear plastic ruler on tracings of those photographic projections. Because we systematically overestimated the size of fluorescent microspheres (mean diam = 0.61 μm, SD = 0.01, Polyscience) calibrated to a photographic slide of a stage micrometer, we used the fluorescent microspheres to calibrate all new bacterial size determinations and to recalibrate our previous estimates (Scavia et al. 1986). Bead photographs were shot, developed, and sized with each roll of film. Although the beads can appear larger with longer exposure times, the size was consistent for exposures between 5 and 30 s; for longer exposures, the overexposed bead appeared larger. The beads, although slightly larger than the bacteria, appeared similar to them in brightness.

Bacterial carbon content was determined from dilution experiments started on 2 July and 5 August 1986. We inoculated two 20-liter carboys filled with 0.2-μm filtered water, one from 5 m and one from 90 m, with 100 ml of 2.0-μm filtered (Nuclepore) epilimnetic water (200:1 dilution) and incubated at 14°C in the dark. Initially and 5–7 d later, particulate organic carbon (POC) measurements were made with an Oceanography International carbon analyzer after persul-

fate digestion (Golterman et al. 1978) of material collected on precombusted glass-fiber filters (Whatman GF/F). Subsamples of whole water and POC filtrate were preserved to determine bacterial concentration and cell volumes as described above. In the July experiment, the 5-m initial and final samples and the 90-m final samples contained two classes of cell size; therefore, total bacterial biovolume was determined from the sum of abundance-weighted cell volumes for the two populations. Refiltered filtrates served as carbon blanks, and bacterial biovolume (μm^3 ml^{-1}) was corrected for cells passing the glass-fiber filters. Subsamples (30 ml) of whole water were also taken at the time of final POC determination to estimate protozoan abundance. These subsamples were filtered onto 1.0-μm pore size, Irgalan-black-stained Nuclepore filters, stained with primulin (Caron 1983), and counted and sized on the Leitz microscope with Leitz filter cube A at 1,500×. Carbon content was determined from change in POC concentration (corrected for protozoan carbon) divided by the change in bacterial biovolume concentration over the incubation period.

Results

Ambient conditions, bacterial concentration, cell size, and carbon content — Surface temperatures (Fig. 1a) and the timing of thermal stratification were similar in 1984 and 1985. Epilimnetic chlorophyll concentrations (Fig. 1b) and bacterial abundances (Fig. 1c) were also similar during both years. Chlorophyll concentrations were highest in late winter and early spring. They decreased dramatically after thermal stratification began and rose slightly at the end of August. Bacterial abundances were lowest in late
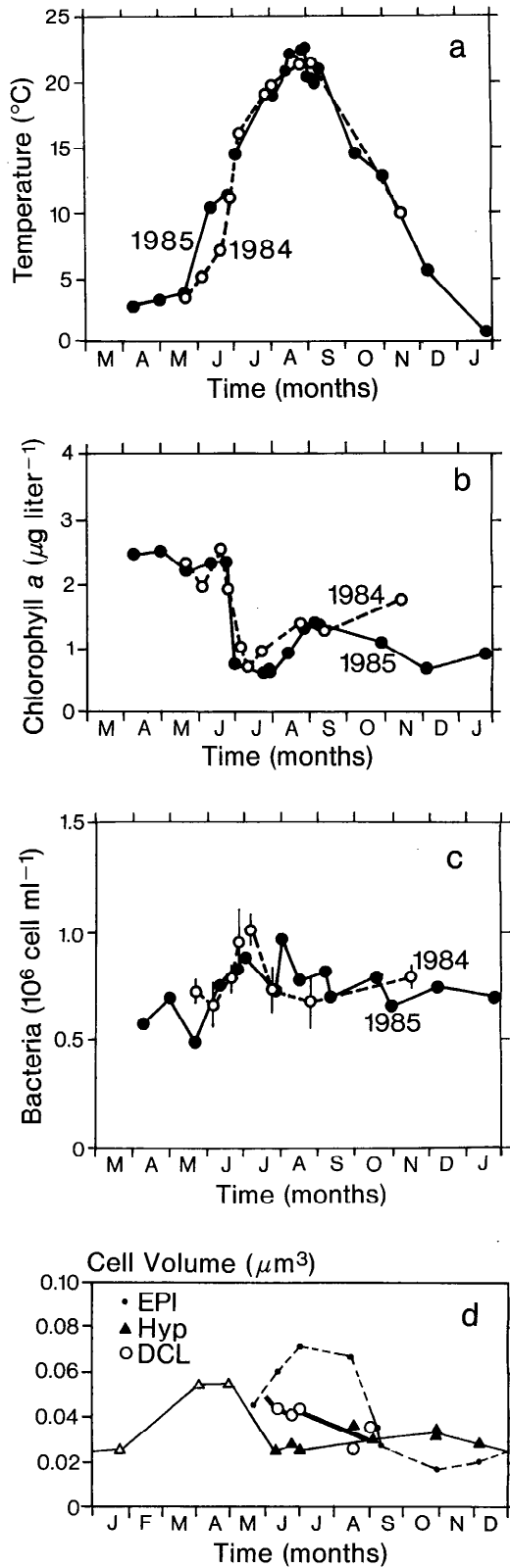
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Fig. 1. a. Temperature of surface waters for 1984 and 1985. b. Concentrations of Chl *a* in surface waters for 1984 and 1985. c. Bacterial abundances in surface waters for 1984 and 1985; error bars are ±SE (errors are similar for both years; 1985 bars left off for clarity). d. Seasonal variation in bacterial cell size for 1984 and 1985: epilimnetic values — dashed line and dots; deep chlorophyll layer values — heavy solid line and circles; isothermal (△) and hypolimnetic (▲) values — solid line and triangles.

Table 1. Results from carbon content dilution experiments (means ± pooled standard errors from replicate determinations) incubated 1 week in the dark at ambient temperature.

	Depth (m)	ΔPOC* (µg liter^{-1})	ΔBiovolume (10^7 µm^3 liter^{-1})	ΔPOC/ΔBiovolume (pg C µm^{-3})
7 Jul	5	9.7±8.6	16.5±3.2	0.059±0.054
7 Jul	90	19.6±17.0	9.48±1.8	0.207±0.184
12 Aug	90	11.5±7.4	5.90±0.7	0.195±0.127
			Mean	0.154±0.047

* Corrected for protozoan carbon (*see text*).

winter (about 0.5×10^6 cells ml^{-1}), increased to about 1.0×10^6 cells ml^{-1} by the onset of stratification (mid-June), and then decreased to about 0.75×10^6 cells ml^{-1} for the rest of the year. Bacterial abundances were uniform with depth both before and after the period of stratification, but decreased with depth during summer; concentrations in deep water were typically half of surface values. There was little horizontal variability in bacterial abundance for surface samples from five stations along the north–south transect (0.53–0.64×10^6 cells ml^{-1}) on 26 July with only slightly more variability among nearshore and offshore stations, all >2 km from shore (0.61–1.03×10^6 cells ml^{-1}), on 6 September.

Bacterial cell size varied with time and depth (Fig. 1d). Smallest sizes were observed during winter and in the hypolimnion (0.015–0.024 µm^3). Cells were larger in spring and reached maximum size during midsummer in the epilimnion (0.072 µm^3). During the period of thermal stratification, before the thermocline deepened to about 25–30 m (early September), bacteria sizes within the region of the deep chlorophyll maximum (10–35 m) were between those of the epilimnion and hypolimnion.

Results from the carbon-content dilution experiments were different for 5- and 90-m water in July and August (Table 1). The ratio of total POC to bacterial biovolume was high initially, indicating substantial nonbacterial carbon. To remove this bias, we estimated carbon content from changes in POC and biovolume. POC estimates at the end of the experiment were corrected for protozoan carbon determined from microscopic counts and biovolume estimates and an assumed ratio of carbon to wet weight of 0.08 (Sherr and Sherr 1984). Protozoan carbon represented 5.4, 5.9, and 11.1% of total POC at the end of the three experiments. Although estimation errors for individual experiments are large (Table 1), the mean and standard error of the combined estimates is 0.154 ± 0.047 pg C µm^{-3}.

We sometimes counted fewer bacteria in the refiltrate than in the original filtrate. In these cases, the second glass-fiber filter retained bacteria that passed the first one, possibly leading to an overestimate of the carbon blanks. Calculated bacterial biovolume in the original filtrate was between 2 and 6% of that for the total sample at the end of each experiment; the bacteria had increased in size over the 5-d incubation, and most were caught on the first filter. Because only a portion of the original filtrate bacteria would be caught on the second filter, large overestimation of the blanks is unlikely. The portion of biovolume passing the first filter at the beginning of each experiment was larger than at the end. However, initial total bacterial volume was <5% of that at the end of the 90-m July and 90-m August experiments and so overestimated blanks were not likely then. In contrast, initial biovolume was 20% of final biovolume in the 5-m July experiment. Therefore, we may have overestimated blanks and thus underestimated carbon content in that experiment. This bias may explain why that carbon content estimate was lower than in the other experiments.

TdR incorporation—TdR incorporation rates for surface waters followed similar patterns in both years (Fig. 2a); rates were lowest in winter, increased in spring until mid-June, and then were somewhat lower throughout summer and fall. This general pattern was observed in both years, but there was significant shorter term, within-season variability. During two diel studies (15 August and 9 September 1985), TdR incor-
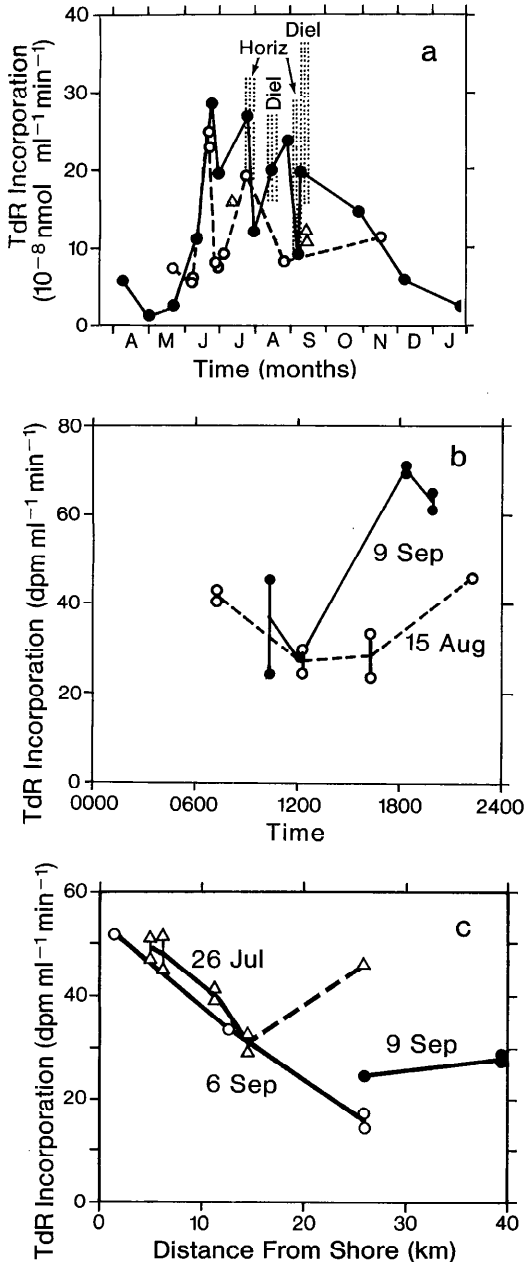
Fig. 2. a. TdR incorporation into ice-cold TCA extracts vs. time of year for surface samples in 1983 ($\triangle$), 1984 (O), and 1985 (●). Stippled boxes represent the ranges of measurements made during horizontal transects and diel studies during 1985. b. TdR incorporation into ice-cold TCA extracts vs. time of day during two diel studies in 1985. (Data at 1200 hours on 9 September are represented by two overlapping symbols.) c. TdR incorporation into ice-cold TCA extracts vs. distance from shore during three transects in 1985. Transect on 26 July was off Frankfort, Michigan, to off
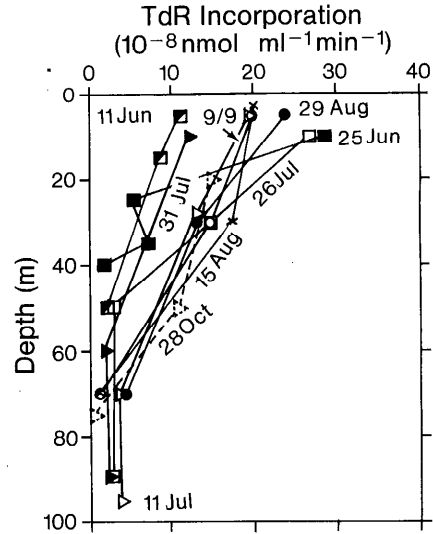

Fig. 3. TdR incorporation into ice-cold TCA extracts vs. depth during the period of stratification in 1985.

poration rates were about 1.7 and 2.2 times lower at midday than at night or early morning (Fig. 2b).

Incorporation rates also varied among stations on the north–south transect (26 July); the variation when expressed relative to distance from shore, however, was similar to results from our offshore transects on 6 and 9 September (Fig. 2c). The exception to this trend is from the 26-km station during the north–south transect (26 July). It was the only station along the three transects that was sampled late in the day (2000 hours) and, from our diel studies, we would expect higher rates then. Incorporation rates at a station 40 km offshore were similar to rates at our standard 26-km station on 9 September. Superimposing the range of diel and horizontal variability on the seasonal pattern of TdR incorporation (Fig. 2a) suggests that our seasonal trends are real, but day-to-day variations could be due to several unmeasured components.

Incorporation rates at the 100-m station decreased with depth (Fig. 3) during the 1985

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Grand Haven, Michigan. All samples were taken during the day except the 26-km station on 26 July.

Table 2. Regression results from dilution-growth experiments.

	μ_b*	ln(N)†	α‡	μ_t*	ln(V)†	α‡	C_1§	C_2§	C_3§
1985									
9 Apr	0.14	10.51	0.08	0.27	−18.89	0.002	13.8	5.3	8.9
1 May‖	0.06	11.16	0.003	0.48	−21.89	0.002	239.2	12.1	19.6
22 May	0.31	9.40	0.04	—	—	—	—	—	—
11 Jun	0.20	10.58	0.002	0.12	−18.48	0.030	14.2	25.4	21.0
25 Jun	0.15	11.94	0.013	0.22	−19.12	0.24	77.1	56.4	42.5
1 Jul	0.31	10.37	0.0002	—	—	—	—	—	—
29 Aug‖	0.19	11.05	0.057	0.57	−17.77	0.033	10.2	6.0	6.1
28 Oct	0.36	10.23	0.046	0.44	−18.42	0.009	16.7	15.1	13.3
6 Dec	0.10	11.70	0.038	0.50	−18.77	0.17	28.0	16.7	17.8
1986									
23 Jan	0.04	11.22	0.003	0.12	−20.04	0.31	25.2	23.9	14.6

* Slope of ln-transformed cell abundance (μ_b) and TdR incorporation (μ_t) curves (h⁻¹).
† Intercept of ln-transformed cell abundance (N) and TdR incorporation (V) curves.
‡ Attained level of significance for the regression slope.
§ Empirical conversion factors determined from three different calculation methods (*see text*, 10⁹ cells nmol⁻¹).
‖ Two-tailed t-test of H_0: $\mu_b = \mu_t$, not significant at $\alpha = 0.05$.

period of stratification. Rates from deep hypolimnetic (about 90 m) samples at this station, as well as at stations along the two transects and during the diel surveys, were typically a tenth of the surface rates.

Isotope incorporation into DNA, expressed as a fraction of that incorporated into ice-cold TCA extracts, varied between 39 and 80% for surface waters, with an increasing, but not significant ($P = 0.092$), trend from winter through summer. No clear trend occurred with depth. We use the overall mean of 59% (SE = 4.01, $N = 26$) to adjust the theoretical conversion factor determined for marine offshore waters (Fuhrman and Azam 1982) to 1.25×10^9 cells produced per nanomole of TdR incorporated into ice-cold TCA extracts.

Conversion factors, growth rates, and grazing rates—The empirical conversion factors calculated from the dilution-growth experiments (Table 2) were similar to those obtained in 1984 (Scavia et al. 1986). Differences among the three methods of calculation were related to the validity of the regressions and assumptions regarding linear vs. exponential growth, but were not large except on 1 May. Similar seasonal variability was seen for all methods (Fig. 4) and, except for 5 of the 45 estimates, conversion factors varied between 5×10^9 and 25×10^9 cells produced per nanomole of TdR incorporated into ice-cold TCA extracts, in contrast to the much lower theoretical factor (1.25×10^9 cells nmol⁻¹). Empirical factors were smallest in midsummer.

Epilimnetic growth rates (Fig. 5), determined from cell production divided by abundance, varied in a fashion similar to TdR incorporation (Fig. 2a). Rates, based on the empirical conversion factors, were lowest during winter, increased to values greater than 0.3 h⁻¹ at the onset of thermal stratification, and then decreased to about 0.05–0.15 h⁻¹ for the rest of the summer and into winter. Results of the independent, antibiotic-based (Table 3) and dilution-based (Table 2) experiments were generally consistent with these rates (Table 4). The antibiotic-based rates averaged 0.74 times higher, and the dilution-based rates averaged 1.95 times higher. Growth rates, determined from the theoretical TdR conversion factor, were much lower (0.001–0.027 h⁻¹). Comparison to the independent tests showed that the antibiotic-based rates averaged 9.68 times higher and the dilution-based rates averaged 24.1 times higher.

In most of the antibiotic-based experiments, the slopes of curves of ln(N) vs. time in the untreated sample (r_R) were not significantly different from zero (Table 3). For those cases, growth rates were equal to loss rates in those bottles, the latter being attributed to grazing. In the two cases where the slopes were significantly different from zero, grazing rates determined from the slope of the antibiotic-treated water only (r_A) were
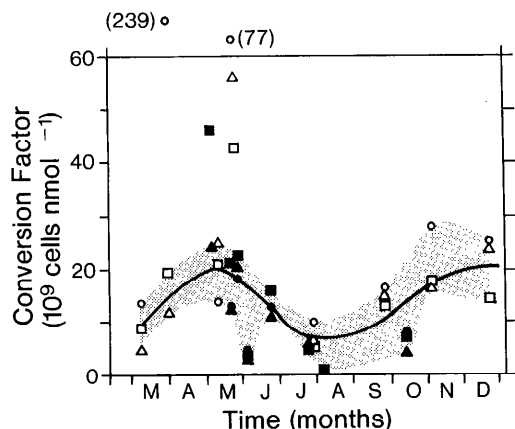
Fig. 4. Empirical conversion factors (cells produced nmol⁻¹ of TdR incorporated into ice-cold TCA extracts) for 1984 (closed symbols) and 1985 (open symbols). Three methods of calculation are used (*see text*): C_1 (circle), C_2 (triangle), C_3 (square). Smoothed curve drawn by inspection was used for interpolation.

Fig. 5. Growth rates for 1983 (×), 1984 (dashed line), and 1985 (solid line) calculated from TdR incorporation and empirical conversion factors. The shaded region includes all growth rates determined from TdR incorporation and the theoretical conversion factor (1.25×10^9 cells nmol⁻¹).

lower (22 May: 0.026 h⁻¹ vs. 0.045 h⁻¹) and slightly higher (6 December: 0.118 h⁻¹ vs. 0.099 h⁻¹) than growth rate estimates. Overall, grazing loss rates ranged between 0.039 and 0.118 h⁻¹ (mean = 0.055 h⁻¹, SE = 0.010, $N = 11$).

Bacterial carbon production—The product of TdR incorporation rates and empirical conversion factors yields cell production estimates for the combined 1984 and 1985 data sets (Fig. 6a). As with TdR incorporation (Fig. 3), cell abundance, and cell size (Fig. 1d), cell production varied with depth during the period of thermal stratification. During winter and early spring, production was uniform with depth (about 5×10^5 cells ml⁻¹ d⁻¹). Otherwise, production was highest in the epilimnion (up to 50×10^5 cells ml⁻¹ d⁻¹), intermediate in the region of the deep chlorophyll layer, and lowest in the cold hypolimnion (about 5×10^5 cells ml⁻¹ d⁻¹). Combining these estimates of seasonal and vertical cell production rates with bacterial cell size and average carbon content (0.154 pg C μm⁻³) yielded bacterial carbon production for the offshore region in southeastern Lake Michigan. The calculation rests on the following procedures and assumptions.

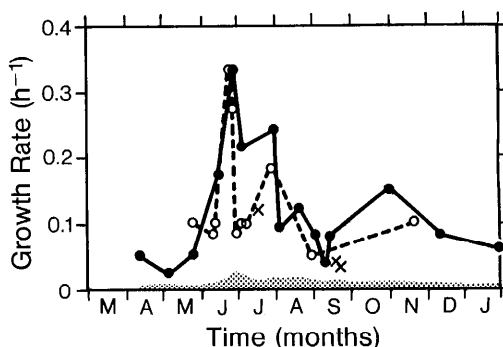Surface and bottom TdR incorporation rates were similar during isothermal pe-

riods, therefore a single production estimate was used for the entire water column. During summer, the water column was divided into a three-layer system representing the epilimnion, the deep hypolimnion, and the deep chlorophyll layer (DCL, 10–35 m) when DCL rates were different from those both above and below it (Fig. 6a). A two-layer system was used for other periods during summer. A similar scheme was used for variation in cell size (Fig. 1d). Cell production rates from both years were combined and an 8-point moving average was used to smooth day-to-day variation in epilimnetic incorporation rates between 23 May and 22 October. Data from single dates before and after that period were treated individually and visually connected to the 8-point average. This smoothing was used to "average out" diel and other poorly defined short-term variability (Fig. 2a). Empirical conversion factors were used and assumed to be constant with depth in Lake Michigan's aerobic water column. Horizontal variability was assumed to be minimal in the context of the annual calculation. Data were interpolated, digitized to 10-d intervals, and then integrated, resulting in $2.54 (\pm 0.81) \times 10^{16}$ cells m⁻² yr⁻¹ and 142 ± 48 g C m⁻² yr⁻¹. The 32% coefficient of variation (C.V.) on the cell production estimate and 34% C.V. on the carbon flux estimate are based on propagating variance from thymidine incorporation (mean C.V. = 20%, based on

Table 3. Regression results from antibiotic experiments.

	r_R*	SE†	α‡	r_A*	SE†	α‡
9 Apr	−0.00078	0.011	0.95	−0.038	0.016	0.06
1 May	−0.00534	0.014	0.71	−0.040	0.0084	0.004
22 May	0.019	0.0044	0.005	−0.026	0.0096	0.041
11 Jun	0.0034	0.0078	0.69	−0.107	0.012	0.00027
25 Jun	0.0073	0.020	0.73	−0.079	0.022	0.035
1 Jul	−0.0034	0.011	0.77	−0.037	0.013	0.063
31 Jul	−0.0021	0.019	0.92	—	—	—
29 Aug	−0.0014	0.00012	0.80	−0.044	0.034	0.37
5 Sep	−0.032	0.0066	0.017	−0.029	0.011	0.047
28 Oct	−0.016	0.034	0.66	−0.031	0.020	0.22
6 Dec	−0.020	0.050	0.06	−0.118	0.044	0.12

* Slope of ln-transformed cell abundance curves from raw (r_R) and antibiotic-treated (r_A) samples (h⁻¹).
† Standard errors of the regression slopes.
‡ Attained levels of significance for the regression slopes.

3–9 replicates), conversion factors (25%, based on three calculation methods and temporal variation), and cell volumes (10%, based on population variation).

Bacterial net carbon production, integrated over the summer (9 June through 7 September) epilimnion, region of the deep chlorophyll layer, and hypolimnion were 28.9, 10.4, and 1.6 μg C liter⁻¹ d⁻¹, respectively. Water-column production for summer was 652 mg C m⁻² d⁻¹.

Carbon flux to grazers—The product of grazing loss rates (Table 3) and bacterial abundance yields the grazing loss flux of bacterial cells. Cell loss rates, determined from those values (Fig. 6b), ranged between 3.1 × 10⁵ and 21.2 × 10⁵ cells ml⁻¹ d⁻¹, with mid-June and late November being two periods of relatively high flux. Comparing grazing loss flux to production (Fig. 6a) suggests that the micrograzers included in those experiments are capable of cropping the production between November and June. However, grazing losses fall short of production during July through September, indicating that other grazers may also be significant at that time. Integrating the product

of cell loss rates, size, and carbon content over the period April–December yielded an average gross carbon flux of 5.5 μg C liter⁻¹ d⁻¹. Average for the summer epilimnion was 8.4 μg C liter⁻¹ d⁻¹.

Discussion

Spatial and temporal bacterial abundance and growth—Bacterial abundances in 1984

Fig. 6. Cell production (a) and grazing loss (b). Epilimnetic values—dashed line and dots; deep chlorophyll layer values—heavy solid line and circles; isothermal (△) and hypolimnetic (▲) values—solid line and triangles.

Table 4. Comparison of rates of TdR-based growth and independent antibiotic- and dilution-based growth. Values are means ± SE (range) of ratios of independent to TdR-based rates.

	Empirical TdR	Theoretical TdR	N
Antibiotic	0.74±0.18 (0.2–2.0)	9.68±3.7 (1.8–40.0)	10
Dilution	1.95±0.36 (0.5–6.3)	24.1±5.1 (5.7–77.5)	17

and 1985 at our 26-km station were similar to those reported for stations closer to shore in May and June 1980 (Moll and Brahce 1986), but lower than those reported for August and October 1980. Moll and Brahce showed decreasing bacterial abundances from Grand River plume stations through their nearshore and offshore stations (12.8 km). There was little variation along our transects for stations between 2 and 26 km from shore and even less variation among stations along a transect from our station to one 180 km north.

Maximal rates of increase in epilimnion cell abundance occurred just at the onset of stratification when chlorophyll concentrations were decreasing fastest (Fig. 1b, c). Maximal bacterial abundances were measured at the time of our observed chlorophyll minimum. Moll and Brahce (1986) reported that bacterial abundances were highest in fall when chlorophyll concentrations were lowest. They suggested that the overall low correlation ($r = 0.2$) between bacterial abundance and chlorophyll concentration indicated that release of algal metabolites may not be a major source of carbon for bacterial production. We have supported this suggestion by comparing estimates of summer bacterial production (Scavia et al. 1986) with measurements of algal carbon release rates (Laird et al. 1986).

Results of diel studies (Fig. 2b) show bacterial growth rates in Lake Michigan were lower during midday than at either night or early morning. This pattern is similar to that in Lake Erken (Bell 1984) but opposite to those observed in several oceanic studies (*cited by* Fuhrman et al. 1985). In both coastal marine and lake waters (total of 17 diel studies: Riemann et al. 1984; Riemann and Sondergaard 1984), no systematic diel pattern evolved; higher night rates were evident, however, in some diel cycles.

Our results illustrating bacterial production rates decreasing with distance from shore (Fig. 2c) are similar to those reported for measures of bacterial activity in several coastal marine environments (e.g. Ferguson and Palumbo 1979; Fuhrman et al. 1980; Ducklow and Kirchman 1983) and in Lake Michigan (Moll and Brahce 1986). It may imply a coupling of fluvial inputs and bacterial production nearshore. It is not clear, however, to what extent that influence is felt at stations tens of kilometers offshore.

TdR incorporation and growth rates followed seasonal patterns similar to those of bacterial abundance in both years. Rates were lowest in winter and early spring. They increased rapidly during the initial heating period until the onset of stratification and then decreased through summer and fall. Our independently determined (antibiotic and dilution experiments) growth estimates are similar to the TdR-based growth rates determined from our empirical conversion factors (Table 4). Antibiotic-based rates tend slightly lower; these estimates may be low if gentamycin is <100% effective as an antibiotic or if it affects grazers. Dilution-based rates tend slightly higher; the relatively large manipulations and filtrations involved in those experiments could result in spillage of labile organics (Fuhrman and Bell 1985; Goldman and Dennett 1985) and lead to the occasional high estimates. Although the independent rates and TdR-based empirical rates differ, on average, by factors of 0.74 and 1.95, the independent rates are 9.68 and 24.1 times higher than TdR rates based on the theoretical conversion factor (Table 4).

Overall, these data confirm our previous tentative findings (Scavia et al. 1986) that estimates of rapid growth, based on empirically determined TdR conversion factors, are valid for Lake Michigan. We draw this conclusion from the fact that three of the four growth estimates agree, but all three determinations were based on experiments requiring 8–10-h incubations, and the influence of potential bottle effects (Ferguson et al. 1984) is unknown. The relatively long incubations may have produced inflated growth rates in the dilution and antibiotic experiments or altered the relationship between TdR incorporation and cell production. However, even the theoretically based TdR rates were subject to some "preincubation" during sample transport (1–3 h) to the shore laboratory. Perhaps, in light of comparisons of carbon fluxes (*see below*), the true conversion factors of TdR incorporation to cell production lie between the theoretical one and those determined empirically.
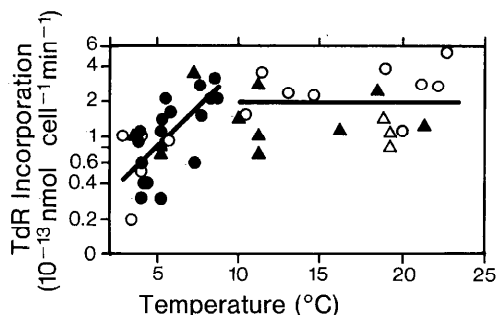
Fig. 7. Cell-specific TdR incorporation rates vs. incubation temperature for all experiments in 1983 (△), 1984 (▲), and 1985 (epilimnion—○, hypolimnion—●).

Control of seasonal growth rates—Grazers seem to control bacterial *abundance* (Scavia et al. 1986; Gardner et al. 1986). The control of *growth rates* can be explored further here. Below about 10°C, ln-transformed rates increase linearly with temperature (Fig. 7). Above 10°C, no relationship with temperature is apparent. Hobbie and Cole (1984) also showed a strong correlation between bacterial production and temperature, as did Pomeroy and Deibel (1986). Although covariation with other variables confounds a simple relationship, growth rates appear strongly related to temperature below 10°C in Lake Michigan. Above 10°C, the relationship with temperature is weak, and it is likely that substrate supply controls growth. The breakpoint in the rate-temperature plot (8°–10°C) is coincident with surface-water temperatures just after the onset of thermal stratification (mid-June 1984 and 1985). Decreased TdR incorporation (Fig. 2a) and growth (Fig. 5) rates after that time imply that production is limited by substrate supply then.

Grazing loss rates and bacterial population size control—Grazing loss rates between 0.039 and 0.118 h^{-1} and growth rates between 0.02 and 0.3 h^{-1} suggest that growth and grazing loss are often in balance, a conclusion also drawn from analysis of the kinetics of substrates added to samples from the same region (Gardner et al. 1986). It is also consistent with our observations that bacterial abundance does not change dramatically during the year, even though growth may be as high as 0.3 h^{-1}. Although

our overall trends in bacterial abundance and production show gradual changes seasonally, substantial short-term variability is also evident. This sort of variability is expected for predator-prey systems (e.g. Andersen and Fenchel 1985) as active and tightly coupled as our data suggest. This tight coupling of growth and grazing is also consistent with observations made in 1981 with uptake experiments with size-fractioned radioactive glucose (Moll and Brahce 1986). In those experiments, substantial isotope accumulation was found in size fractions >1 μm. Moll and Brahce interpreted the results as evidence of significant algal osmotrophy, but an alternative interpretation is that bacteria assimilated the glucose and subsequent grazing by "algae-sized" predators passed the isotope to the large size-fractions. Their 2-h experiments would certainly allow for this transfer. Thus, Lake Michigan bacterial biomass is likely transferred to the next trophic level, a conclusion reached in several studies on other systems (e.g. Fenchel 1982*b*; Riemann 1985; McManus and Fuhrman 1986).

Our specific loss rates range between 0.039 and 0.118 h^{-1} (Table 3, mean = 0.055, SE = 0.010). Epilimnetic heterotrophic flagellate abundances range between 3,000 and 1,000 individuals ml^{-1} from spring to summer at a station in western Lake Michigan (M. E. Boraas pers. comm.). Combining our estimates of winter and summer loss rates with those abundances yields clearance rates between 22 and 44 nl flagellate^{-1} h^{-1}. These values are similar to those determined for some larger marine species (2–40 nl flagellate^{-1} h^{-1}: Fenchel 1982*a*; 15–20 nl flagellate^{-1} h^{-1}: Andersen and Fenchel 1985) but higher than those for species similar in size (about 22.4 μm^3: M. E. Boraas pers. comm.) to Lake Michigan flagellates (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; 0.3–1.0 nl flagellate^{-1} h^{-1}: Sherr et al. 1983). Our higher estimates of clearance rates could be due to overestimated specific loss rates, to uncertainty in the absolute abundance and taxonomy of the heterotrophic flagellates in our experiments, or to the fact that organisms other than the flagellates

(Riemann 1985; Bird and Kalff 1986; Estep et al. 1986) may contribute to the specific loss rate. The discrepancy between July–September production of bacterial cells and micrograzer loss fluxes (Fig. 6a, b) suggests that the latter factor may be significant.

Carbon content — Bacterial volumes ranged between 0.015 and 0.072 μm^3. Estimation error, potentially caused by fluorescence fading or halos at the edges of cells, was reduced by calibration with 0.61-μm-diameter fluorescent beads, whose measurements are influenced by the same subjective decisions used for bacterial cells. Using this method, rather than standard calibration with stage micrometer and transmitted light, reduced cell volume estimates by a factor of about 1.4. The most likely source of error in determining carbon per unit volume is in estimating cell volume (Bratbak 1985). However, even if our estimates of cell volume are in error, the same error would be present in our estimates of carbon content and would thus cancel when converting from cell abundance to carbon concentration. Our estimates of carbon content averaged 0.154 pg C μm^{-3} (range 0.059–0.207) and are similar to the value most commonly used (0.121: Watson et al. 1977), to the one proposed for cases where "true" bacterial size is known (0.22: Bratbak and Dundas 1984), and to those determined seasonally with natural populations (0.039–0.188, mean = 0.106: Nagata 1986), but lower than an estimate suggested for use if cell size was underestimated (0.56: Bratbak 1985).

Comparison of annual bacterial and phytoplankton production — Summer epilimnetic bacterial carbon demand was compared to measures of contemporary algal excretion (Scavia et al. 1986), and it was concluded that other carbon sources are necessary to balance the summer demand if our assumptions and conversion factors were correct. We suggested two additional carbon sources, both based on decoupling rates of supply and use. One source is late-winter and early-spring phytoplankton production and the other is labile organic compounds produced within the near-bottom nepheloid layer. Both potential sources are related ultimately to autotrophic primary production, but on annual rather than seasonal scales.

Our estimate of annual bacterial carbon requirement is similar to estimates of annual primary production. Although additional contemporary measurements of primary production are available for Lake Michigan (Fahnenstiel and Scavia 1987a), a 1970–1971 study (Fee 1973) is the only data set sufficiently detailed in time to allow an estimate of annual production. Recent analysis of spring–summer primary production in Lake Michigan (Fahnenstiel and Scavia 1987a) illustrates that phytoplankton production in the water column has not changed significantly since the early 1970s. Fee (1973) estimated average daily production for five stations across Lake Michigan, based on surveys between 25 June 1970 and 9 February 1971. On the basis of these daily averages, annual production ranged between 121 and 247 g C m^{-2} yr^{-1} (mean ± SE = 167 ± 23). These estimates are low for at least two reasons. High rates of production in the water column were likely missed because the period between February and June was not sampled and rates of production are high in spring (Parker et al. 1977; G. L. Fahnenstiel pers. comm.; Fahnenstiel and Scavia 1987a). The 1970–1971 estimates are also low because the vertical structure of phytoplankton abundance was not taken into account. If a deep chlorophyll maximum was present in summer 1970, as has been observed during every study since, then the 1970 summer estimates likely are low by about 35% (Fahnenstiel and Scavia 1987a). Thus, if we raise summer values by that factor and winter–spring values similarly to account for higher-than-average production during those months, we calculate annual areal primary production to be 225 ± 31 g C m^{-2} yr^{-1}. If picoplankton are an important component of the current Lake Michigan algal assemblage, then autotrophic production may be underestimated further.

Annual net production of bacterial carbon (142 ± 48 g C m^{-2} yr^{-1}) is about 63% of estimated autotrophic production. Estimated bacterial carbon demand (236 ± 79

g C m^{-2} yr^{-1}, assuming 60% growth efficiency: Cole et al. 1982, 1984; Calow 1977) is very close to autotrophic production. Eadie et al. (1984) suggested that because only a small amount of annual primary production accumulates in the sediment of Lake Michigan each year (about 8 g C m^{-2} yr^{-1}), most of the production is recycled within the water and surface sediments on an annual basis. From our analysis it appears that much of that recycled material passes through heterotrophic bacteria. Because respiratory losses along the traditional autotrophic food web cannot be ignored and because allochthonous sources are small (about 7 g C m^{-2} yr^{-1}, if fluvial inputs are diluted throughout the entire southern basin: Eadie et al. 1984), it is clear that bacterial demand must be lower than autotrophic production. Although underestimated autotrophic production (e.g. the influence of picoplankton), overestimated bacterial carbon demand (e.g. high conversion factors), and underestimated allochthonous inputs (e.g. focused offshore transport) are all potentially possible, only very large errors would endanger our conclusion that a major portion of autotrophic production passes through heterotrophic bacteria in Lake Michigan on an annual basis.

Spatial and temporal disequilibrium— With only minor adjustments, phytoplankton production and allochthonous inputs appear to satisfy bacterial carbon requirements on an annual, areal basis, but there is a large discrepancy between demand and supply during summer. Average bacterial net production in summer is 652 mg C m^{-2} d^{-1}. Primary production for summer 1983 and 1984 averaged 627 mg C m^{-2} d^{-1} (Fahnenstiel and Scavia 1987a). Net bacterial production is similar to autotrophic production, but bacterial carbon demand is not. If bacteria are 60% efficient, they require 1,087 mg C m^{-2} d^{-1}; only about half of that is potentially available directly from the autotrophs. Yet, net autotrophic production also has other immediate fates. Typically, 60–70% of net epilimnetic production is shunted to crustacean zooplankton or sedimented from the epilimnion (Scavia and Fahnenstiel 1987) and, undoubtedly, some carbon is also consumed below the epilim-

nion. Thus, the discrepancy between phytoplankton supply and bacterial requirement during summer is substantial. The fact that summer rates do not balance, yet annual rates are much closer, supports our original contention (Scavia et al. 1986) that disequilibrium between organic carbon supply and demand on seasonal and spatial scales is significant.

Because bacterial production is lowest at all depths during winter and spring and in deep water during summer (Fig. 6a), these are two likely places in Lake Michigan where production of dissolved organic carbon exceeds its use and labile organic substrate concentrations increase. Phytoplankton carbon production and chlorophyll concentrations are maximal before the onset of thermal stratification (Fahnenstiel and Scavia 1987a,b; Scavia and Fahnenstiel 1987). If excretion of organic compounds is proportional to production, then release of metabolite is probably greatest then. Because bacterial production is low then, concentrations of labile organic carbon may increase. The labile pool may also build as autotrophically formed POC is processed via the traditional food web at that time because bacterial production is more strongly suppressed at lower temperatures than is autotrophy (Fig. 7). This notion was also suggested from observations off the coast of Newfoundland (Pomeroy and Deibel 1986) where bacterial production was more severely limited by temperature than was phytoplankton production.

A second possible source of labile organic compounds is related to stratification in the preceding year. Each year, Lake Michigan develops a nepheloid layer (Chambers and Eadie 1981; Eadie et al. 1984) with exponentially increasing particle concentrations within the bottom boundary (5–10 m off the bottom). This layer includes organic material produced during the spring diatom bloom and subsequently settled from the epilimnion (Fahnenstiel and Scavia 1987b; Scavia and Fahnenstiel 1987), material resuspended locally from the top few centimeters of sediment (Eadie et al. 1984), and allochthonous inputs from the Grand River sedimented nearshore and transported downslope (Chambers and Eadie 1981). If

labile organic carbon is produced or transported to this deep region faster than it is used by the slowly growing bacteria (Fig. 6a), then it should become available for subsequent production after winter mixing in the same way that phosphorus is apparently remineralized and subsequently made available to the next year's algal crop (Eadie et al. 1984).

Three sets of observations are consistent with the notion of a winter–spring carbon source dwindling during summer. Data reported here indicate that rates of epilimnetic bacterial carbon production (Fig. 6a) and cell size (Fig. 1d) decrease after stratification begins. Both are expected to occur if the supply of organic substrate is decreasing. Turnover times of primary amines (PA) determined from long-term (6–14 d) rates of PA net removal and concentrations (corrected for about 0.07 μM PA overestimation) for the same location in Lake Michigan in 1984 (Gardner et al. 1986) averaged 32.8 d (SE = 5.9, N = 20, omitting three extremely high values of 125, 195, and 389 d) in March–June and 9.4 d (SE = 0.97, N = 19) in July–August. These slow net turnover times indicate the potential for a buildup and subsequent utilization of organic carbon pools. Decreased turnover times in summer, relative to spring, also suggest an increased demand, relative to supply, after stratification. Total DOC concentration decreased from 2.52 (SE = 0.074, N = 5) and 2.43 (SE = 0.058, N = 5) mg liter^{-1} on 10 June and 21 July 1980 to 1.95 (SE = 0.041, N = 5) and 1.97 (SE = 0.020, N = 3) on 1 and 15 October 1980 at stations 8–29 km offshore from Grand Haven (W. S. Gardner unpubl. data). Although total DOC concentration is always high and of unknown composition, this significant decrease over summer suggests that at least some of the pools are being depleted.

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