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Production of planktonic bacteria in Lake Michigan[1]

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Abstract

Bacterial production rates were estimated for the surface waters of a station 100-m deep in southeastern Lake Michigan during 1984. Production was calculated from incorporation of [^{3}H-methyl]thymidine and from empirical conversion factors determined from dilution experiments performed throughout the study. The conversion factors (with typical C.V. <40%) varied between 4.7 and 18.3 × 10^9 cells produced per nanomole of thymidine incorporated into ice-cold trichloroacetic acid extracts. Our estimates yielded bacteria exponential growth rates between 0.05 and 0.24 h^{-1} (C.V. typically <50%) based on the empirical conversion factors. The growth estimates are much lower (0.004–0.020 h^{-1}) when based on measured 47% thymidine incorporation into DNA and a theoretical conversion factor. The higher growth estimates appear more consistent with estimated grazing losses. Carbon flux estimates are less certain, due to the possible range of bacterial carbon content and growth efficiencies, but most of the higher growth estimates imply a bacterial carbon demand higher than concurrent ^{14}C-based primary production measurements. This may mean that a source other than recent primary production is needed to meet this demand.

Recent bacterial production estimates (e.g. Fuhrman and Azam 1982; Kirchman et al. 1982; Newell and Christian 1981; Jordan et al. 1978) support the hypothesis that bacteria play an important role in the energetics of marine and freshwater ecosystems. Much attention has been given to this heterotrophic production in coastal and open ocean environments (e.g. Klug and Reddy 1984; Hobbie and Williams 1984; Fasham 1984; Ducklow and Hill 1985*a,b*). In the few lakes where such measurements have been made, bacterial heterotrophic production appears to rival autotrophic production as a net (albeit de novo) source of particulate carbon available to consumers. For example, net bacterial carbon production in the water column ranges from 12 to 41% of total (autotrophic plus bacterial) production in several mesotrophic to eutrophic lakes (Pedros-Alio and Brock 1982; Riemann 1983; Lovell and Konopka 1985*a,b*; Cole et al. 1984).

The precise role of these heterotrophic microbes is unclear. It is not known whether bacterial biomass is consumed and transferred efficiently up the food chain or whether it is metabolized inefficiently, thus becoming an energy sink (Pomeroy 1984). If this "microbial loop" (Azam et al. 1983) is a significant carbon pathway, it provides a route for assimilation of otherwise lost dissolved organic carbon (DOC) into the food chain. If bacterial production is high and the loop is inefficient, then the loop becomes a major agent in nutrient recycling (Sherr and Sherr 1984). This latter role becomes particularly relevant for P-limited environments in light of recent reports of very active bacterial enzyme systems that liberate orthophosphate from 5′-nucleotides (Ammerman and Azam 1985).

Recent evidence has suggested the presence of microbial loops for Lake Superior (Fahnenstiel et al. 1986) and Lake Ontario (Caron et al. 1985); however, before their impact can be assessed for Lake Michigan, estimates of bacterial production rates are needed.

We have been investigating major carbon pathways in Lake Michigan (Scavia and Fahnenstiel unpubl. rep.), and in 1984 we did field experiments to assess the rate of bacterial secondary production. We report here the first estimates of bacterial production for Lake Michigan. We thank D. Lazinsky and L. Sicko-Goad for processing the electron microscopy samples, and J. J. Cole, H. W. Ducklow, J. A. Fuhrman, W. S. Gardner, and D. L. Kirchman for their suggestions and improvements to the manuscript.

[1] GLERL Contribution 463.

Methods

Samples were taken from 2–5-m depth with Niskin bottles from the RV *Shenehon* in 100 m of water about 27 km west of Grand Haven, Michigan, in 1984. Samples for bacterial counts were preserved with glutaraldehyde (2% final concn) in autoclaved 20-ml vials; bacterial abundance was determined by the acridine orange direct count (AODC) method (Hobbie et al. 1977). Acridine orange was added (2% final concn) for 1 min and the samples were then filtered onto 25-mm diam, 0.2-μm pore-size Nuclepore filters which had been stained with Iragalan black, rinsed, dried, and stored. Eight–ten microscope fields were counted at 1,500× on a Leitz Laborlux 12 microscope equipped with a Ploemopak epifluorescent illuminator and H2 filter cube. The volume of water filtered was adjusted to obtain about 30 cells per field, yielding about 6% counting error assuming Poisson statistics. Chlorophyll *a* samples (100 ml) were filtered in triplicate onto 47-mm Whatman glass-fiber filters (GF/F) and processed fluorometrically (Strickland and Parsons 1972).

Phytoplankton production was estimated from in situ incubations begun early in the day (0900–1000 hours) and kept for 24 h in 2-liter polycarbonate bottles suspended from a moored line. Estimates of primary production were also obtained from curves of photosynthesis vs. irradiance determined from 1-h incubations and integrated over depth and time for observations of vertical light extinction and time-variation of surface irradiance (Fee 1973).

Water samples for thymidine incorporation experiments were transfered to 4-liter linear polyethylene carboys and stored in dark coolers, for no more than 3 h, during transport to the shore laboratory. Thymidine incorporation into ice-cold trichloroacetic acid (TCA)-insoluble material was determined (Fuhrman and Azam 1982). Triplicate 10-ml samples of lake water were dispensed into autoclaved vials along with [^{3}H-methyl]thymidine (50–80 Ci mmol^{-1} New England Nuclear, 10–12 nM final concn of added unlabeled thymidine) and incubated for 40 min in the dark at ambient temperature. On each cruise date, 3–6 subsamples of the 4-liter carboys were processed. Incorporation of isotope was fixed after 40 min by adding unlabeled thymidine (final concn 5 μM). The samples were then chilled and 10 ml of 10% (wt/vol) ice-cold TCA added. After 10 min, the samples were filtered onto 25-mm-diam, 0.22-μm pore-size Millipore filters and rinsed five times with 1 ml of 5% (wt/vol) ice-cold TCA. The filters were then 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 by the external standard method. Blanks were determined by processing samples killed with glutaraldehyde (2% final concn) as above.

We determined substrate-dependent thymidine uptake kinetics for Lake Michigan water collected on 7 and 10 May. These experiments were similar to those described above except that different amounts of [^{3}H]thymidine (constant sp act) were added to a series of samples to achieve a range of added unlabeled substrate from 4.0 to 40.0 nM. Time-course experiments on 26 April and 7 and 14 May were also similar, except that larger volumes were inoculated and then subsampled at intervals before extraction.

A dilution-growth scheme was used to estimate the conversion of thymidine incorporation to production of cells (Kirchman et al. 1982). About 30 ml of lake water were diluted with 270 ml of sterilized lake water prepared by filtration through 90-mm-diam, 0.22-μm pore-size Millipore filters, and incubated in autoclaved 500-ml polycarbonate growth flasks in the dark at ambient temperature. Initially and at intervals during the incubation, 20-ml subsamples were taken from the growth flasks and preserved for bacterial counts. Triplicate 10-ml subsamples were also taken periodically for thymidine incorporation. Additional samples were taken, killed, and processed as above for blanks.

Kirchman et al. (1982) derived a relationship between the changes in thymidine incorporation (V) and cell number (N) which takes advantage of the (testable) exponential rate of change of both properties during growth of the diluted population. If

plots of ln(N) and ln(V) vs. t are linear, then the slopes determined from regression of those variables on time yield two estimates of growth (u_b and u_t). The intercept of the thymidine-incorporation regression (V_0) can be combined with an estimate of the growth rate (u_b or u_t) and the intercept of the cell-abundance regression (N_0) to yield the conversion factor C (cells produced per nanomole of thymidine incorporated):

$$C = uN_0V_0^{-1}. \quad (1)$$

Actually, any estimates of growth rate and simultaneous measure of V and N can be used in Eq. 1 if the above regressions are significant and $u_b = u_t$ (Kirchman et al. 1982). Natural population growth rates (u_r) can then be calculated from

$$u_r = CV_r \quad (2)$$

where V_r is the rate of thymidine incorporation by freshly collected, unaltered water samples.

Tests for the presence of nondividing cells were accomplished through nonlinear regression of N on time using the model

$$N = D + N_0\exp(ut) \quad (3)$$

where D is the population of nondividing cells and N_0 now is the population of dividing cells. Results from the nonlinear regression were compared with those of the linear regression of ln(N) vs. time and the existence of a sizable population of nondividing cells was discounted if the nonlinear model did not improve the explained variance.

Another method of converting the [^{3}H]thymidine uptake to bacterial production is by estimating the incorporation of [^{3}H]thymidine into DNA and using a derived conversion factor (Fuhrman and Azam 1982). For separation of incorporated thymidine into DNA, RNA, and protein fractions, we used the modification of the Fuhrman and Azam (1982) method suggested by Riemann and Sondergaard (1984). In addition to the ice-cold 5%-TCA extraction described above, a second set of subsamples was held at 60°C for 1 h after the addition of NaOH (1 N final concn) to hydrolyze RNA. The samples were then chilled and acidified with 0.28 volumes (2.8 ml for our 10-ml samples) of 1 g ml^{-1} TCA and filtered as above. A third set of subsamples was held for 30 min at 95°–100°C in 20% TCA (final concn) to hydrolyze both DNA and RNA. The samples were then chilled and filtered as above. Thymidine in the "purified" DNA fraction was calculated by difference between the second and third subsamples.

We also estimated the percentage of isotope incorporated into DNA by incubating samples with and without the DNA-synthesis inhibitor mitomycin C (Sigma). The inhibitor was added (10 μg ml^{-1} final concn) 30 min before the isotope and the difference between ice-cold TCA extracts of samples incubated with and without inhibitor was used to estimate ^{3}H incorporation into DNA.

The concentration of dissolved primary amines (PA) was measured in one experiment (14 November) after separating them from ammonium by cation exchange chromatography and reacting with o-phthalaldehyde (Gardner and Miller 1981).

In two experiments (November 1984 and April 1985) we estimated bacterial loss rates due to grazing. We incubated two 300-ml samples of lake water for 8 h, one with and one without the antibiotic gentamycin (Sigma, 20 μg ml^{-1} final concn). Subsamples were taken at intervals and preserved for AODC counts as before. Because bacterial growth is inhibited in the gentamycin-treated sample (Chrost 1978), the slope of ln(N) vs. time (corrected for the untreated sample) reflects loss rates attributable to grazing (Fuhrman and McManus 1984). With water collected from our 100-m station on 19 September 1985, we checked to see whether the addition of gentamycin alone would cause AODC counts to decrease. Water was prefiltered through a 47-mm-diam, 0.4-μm pore-size Nuclepore filter to remove grazers, dispensed (400 ml) into four glass bottles, and gentamycin added to achieve 0, 10, 20, and 30 μg ml^{-1} final concentrations. Initially, and during the 15-h dark incubation at 18°C, 20-ml subsamples were taken and preserved in Formalin. AODC preparations were made and counted.

Samples for electron microscopy were preserved with paraformaldehyde, glutaraldehyde, and sodium cacodylate (Lazinsky

and Sicko-Goad 1979). The TEM samples were concentrated by gentle centrifugation, rinsed four times in 0.05 M cacodylate buffer (pH 7.2), then postfixed with 1% OsO_4 in cacodylate buffer for 1 h at 4°C. The cells were then dehydrated in a graded ethanol and propylene oxide series and embedded in Epon (Luft 1961). Thin sections were cut with a diamond knife, collected on cleaned Formvar-coated 200-mesh copper grids, and stained with aqueous uranyl acetate (Watson 1958). Sections were examined with a JEOL JEM 100B electron microscope operating at 80 kV. Samples for SEM were filtered onto 0.2-μm Nuclepore filters and dehydrated in a graded ethanol-Freon 113 mixture. The wet filter was dried in a Bomar critical-point drier in Freon 13 and then fixed to an Al mount with carbon-conductive cement. The mount was sputter-coated with 20 Å of gold and examined in an ISI Mini SEM at 15 kV. Bacterial sizes were determined by measuring lengths and widths from photographic enlargements of electron micrographs (SEM corrected for gold-coating) and from projections of photographic slides made from AODC fluorescence micrographs. Volumes were calculated from $V = W^2(L - W/3)/4$, assuming rod shape.

Error estimates of various calculated properties are made by propagation of variance in the following manner. The variance of a calculated property (Y) is

$$\mathrm{var}(Y) = (\mathrm{d}f/\mathrm{d}Z_i)^2\mathrm{var}(Z_i) \qquad (4)$$

where Y is some function (f) of several variables (Z_i), each estimated with error represented by var(Z_i), and ($\mathrm{d}f/\mathrm{d}Z_i$) is evaluated at the mean value of Z_i.

Results

Preliminary tests and ambient conditions—Preliminary tests were carried out to establish the concentration of added thymidine and length of incubation required for Lake Michigan experiments. Incubations should be long enough to accumulate sufficient activity on the filter but short enough to ensure linear uptake throughout. In other environments, incubations have generally been between 10 and 60 min. In our time-course experiments, uptake was linear for up to 1 h so that we ended all incubations after 40 min. A second way to ensure a constant uptake rate throughout the incubation is to add enough thymidine to eliminate substrate-dependent rate changes as thymidine is removed from solution. Substrate-dependent uptake kinetics for Lake Michigan (Fig. 1) show saturation at about 7 nM of added thymidine. Thus all subsequent incubations received 10–12-nM additions. Both time-course and substrate-dependent kinetics suggest that 10–12-nM additions and 40-min incubations are sufficient to minimize external isotope dilution.

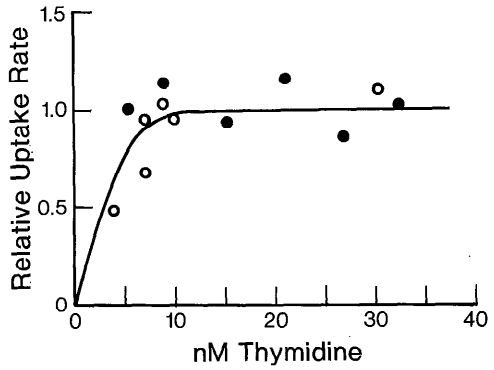

Fig. 1. Substrate-dependent uptake kinetics of thymidine: O—7 May; ●—10 May. Abscissa is added thymidine.

Bacterial abundance varied between 0.67 and 1.04 × 10^6 cell ml^{-1} and was highest in late June (Fig. 2). Surface Chl *a* concentrations varied between 0.7 and 2.6 mg m^{-3}, with highest concentrations during spring. Bacterial abundances were maximal during the period of rapid decline in surface chlorophyll concentrations which coincides with increased water temperature, the establishment of thermal stratification, and the subsequent loss of spring diatoms from the epilimnion (Fahnenstiel and Scavia unpubl.).

Bacterial size was determined by scanning and transmission electron microscopy and by fluorescence microscopy. Mean volumes of rods with lengths and widths measured by these methods are shown in Table 1. Results of fluorescence microscopy suggest that bacteria increase in size from spring (0.063 μm^3) to summer (0.087 μm^3) and then decrease in fall (0.042 μm^3). Size determinations by SEM and fluorescence varied, as

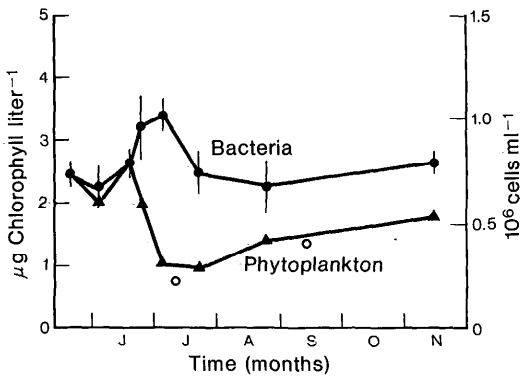

Fig. 2. Bacterial abundance and chlorophyll concentration. Data are for 1984 except open circles, which are chlorophyll from preliminary bacterial production experiments in 1983.

also found by others (e.g. Fuhrman 1981; Bratbak 1985). Because we examined thin sections under TEM, oblique sections were common; we therefore used the average length of the five largest cells observed as the length of the cells in the population. Because not all cells have the same dimensions, TEM used in this way will likely overestimate the average size. Therefore, while TEM distinguished between bacteria and the very small eucaryotes, results of this technique were not included in our overall estimate of mean size.

The antibiotic, gentamycin, had no effect on AODC counts. Our 0.4-μm filtrate (grazer-free) from 19 September 1985 initially contained 8.8×10^5 cell ml^{-1}; after 15 h of incubation concentrations were 7.9, 8.4, 7.5, and 8.5×10^5 cells ml^{-1} for 0, 10, 20, and 30 μg ml^{-1} of gentamycin. Since about 250 cells were counted from each AODC determination (~6% counting error), there was no indication that gentamycin alone at the 20 μg ml^{-1} concentration used would cause AODC values to decrease.

Conversion factors—Calculation of bacterial production or growth rates from thymidine incorporation requires an estimate of the number of cells produced per nanomole of thymidine incorporated. Fuhrman and Azam (1982), from experimental and theoretical evidence, suggested for their experimental area off the southern California coast a factor of $1.7–2.4 \times 10^9$ cells produced per nanomole of thymidine incorporated into the ice-cold TCA precipitate. The method requires estimating the portion of activity incorporated in the ice-cold TCA precipitate that is in the DNA fraction. (In their experiments, about 80% of the isotope in the ice-cold TCA precipitate was in the "purified" DNA fraction.) That conversion factor and variations of it have been applied to freshwater as well (e.g. Riemann et al. 1982; Riemann and Sondergaard 1984; Bell and Kuparinen 1984). Results from our macromolecular fractionations and DNA-synthesis inhibitor experiments (Table 2) in winter and spring 1985 suggest that 40–60% (mean = 47%) of the thymidine was incorporated into DNA. This is within the range reported for other freshwater environments (Riemann and Sondergaard 1984). Thus, for this method of conversion, we used a factor of 1.0×10^9 cells produced per nanomole of thymidine incorporated into the ice-cold TCA-insoluble material.

An alternative approach (Kirchman et al. 1982) is to estimate the conversion factor empirically by observing rates of change in thymidine incorporation (V) and cell abundance (N) during incubation of diluted samples. If plots of $\ln(V)$ and $\ln(N)$ vs. time are both linear (e.g. Fig. 3) and nondividing cells

Table 1. Estimates of bacterial size (N is sample size, V is μm^3, SE is standard error of the mean). April sample from 6 km offshore, all others from our 100-m station, 27 km offshore.

	Fluorescence			SEM			TEM	
1985	N	V	SE	N	V	SE	N	V
9 Apr	73	0.063	0.007	62	0.162	0.011	47	0.640
22 May	120	0.070	0.009	48	0.029	0.004	130	0.129
1 Jul	73	0.087	0.008	80	0.084	0.011	—	—
16 Aug	168	0.070	0.006	—	—	—	—	—
9 Sep	118	0.042	0.006	—	—	—	—	—
Weighted mean	533	0.066	—	190	0.096	—	177	0.260

Table 2. Percentage of [^{3}H-methyl]thymidine incorporation into the ice-cold TCA precipitate isolated in the DNA fraction.

1985	Fractionation*	Mitomycin†
11 Apr	61	60
1 May	41	57
22 May	39	39
11 Jun		49

* From macromolecular fractionation scheme.
† From DNA-synthesis inhibitor experiments.

are not particularly abundant, then the relationship between thymidine incorporation and growth is constant and the conversion factor can be calculated as described above and by Kirchman et al. (1982). Our data fit the linear model well ($\alpha < 0.06$) in 16 cases; two experiments yielded somewhat less significant ($\alpha = 0.14, 0.16$) regression coefficients (Table 3). We tested for the presence of a nondividing population by fitting bacterial abundance over time from the dilution experiments to the growth models with and without a nondividing population. In no case did including the nondividing population increase the explained variance by more than 3%. We thus assume that throughout the season most of the population counted by AODC was actively growing and that direct application of Eq. 1 is valid.

In some instances u_b was higher than u_r, the unaltered, thymidine-based growth rate (*see below*). We used the measured higher growth rates (u_b) to calculate the relationship between thymidine incorporation and cell production in the dilution experiments only where both were measured on the same sample. We assume that the relationship between thymidine incorporation and cell production is the same for unaltered lake water.

Our calculated conversion factors varied between 4.7 and 18.3 × 10^9 cells produced per nanomole of thymidine incorporated into ice-cold TCA precipitate. Linear regression analysis provides standard errors of the slope and intercept estimates, which can be combined as defined in Eq. 4 to estimate the error propagated through our calculations of conversion factors. With two exceptions (26 June 1984 and 9 April 1985), the coefficients of variation for our estimates are <40% (Table 3). The two larger error estimates can be traced to the larger standard errors for the regression intercept estimates.

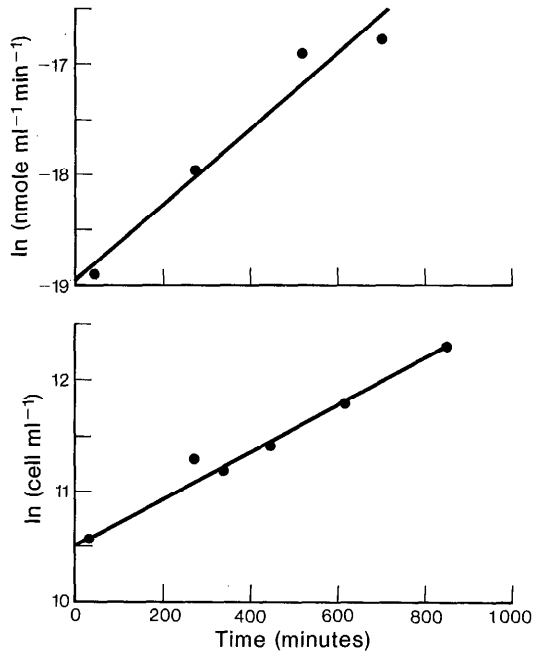

Fig. 3. Natural-log transformed thymidine uptake (above) and bacterial abundance (below) vs. time in dilution-growth experiment of 24 July 1984.

One does not have to rely on both regressions (u_r, u_b) to estimate conversion factors. C can be calculated from Eq. 1 using u_b and paired determinations of cell abundance and thymidine incorporation. These values are also shown in Table 3, both for the initial times only and for the mean of all times during the dilution experiments. These results are similar to those using the regression slope and both intercept estimates in Eq. 1.

Production and growth rates—Thymidine incorporation into ice-cold TCA precipitate from unaltered water samples varied between 3 and 15 pmol liter^{-1} h^{-1}. Incorporation rates were high in late June, at the time of the highest measured bacterial abundances, and again in late July (Fig. 4). Although incorporation rates varied over the season by a factor of 5, abundances varied by a factor of only about 1.6 (Fig. 2); therefore differences in incorporation rates mainly reflect differences in growth rates.

Table 3. Regression results from dilution and grazing experiments. (NT—not testable.)

	u_b*	ln(N_0)†	$\alpha(N)$‡	u_r*	ln(V_0)†	$\alpha(N)$‡	C_1§	C.V.‖	C_2§	C_3§	C_4§	u_b vs. u_r
6 Jun 84	0.0006	12.22	0.02(6)	—	—	—	—	—	24.3	—	46.1	NT
22 Jun 84	0.0031	10.67	0.04(5)	0.0022	−18.41	0.14(4)	13.2	30	12.2	12.7	21.5	¶
26 Jun 84	0.0049	9.65	0.16(4)	0.0048	−19.30	0.05(4)	18.3	96	8.9	20.6	22.8	¶
4 Jul 84	0.0028	10.48	0.01(5)	0.0060	−17.66	0.03(4)	4.7	37	4.1	3.0	4.5	
24 Jul 84	0.0020	10.55	0.0002(6)	0.0034	−18.96	0.02(4)	13.1	27	12.3	11.1	16.1	
22 Aug 84	0.0024	10.72	0.004(6)	—	—	—	—	—	8.3	5.8	5.1	NT
5 Sep 84	0.0034	9.86	0.02(5)	—	—	—	—	—	0.4	0.8	1.1	NT
14 Nov 84	0.0021	10.82	0.001(9)	0.0046	−18.20	0.03(4)	8.4	34	5.8	4.1	7.3	
14 Nov 84	0.0017#	11.60	0.005(5)	—	—	—	—	—	—	—	—	NT
14 Nov 84	−0.0010**	13.90	0.005(8)	—	—	—	—	—	—	—	—	NT
9 Apr 85	0.0030	10.38	0.06(6)	0.0034	−18.32	0.02(5)	8.7	65	2.9	4.1	5.4	¶
9 Apr 85	−0.0006**	13.80	0.06(7)	—	—	—	—	—	—	—	—	NT

* Regression slope (min^{-1}).
† Intercept of cell-abundance (N) and of thymidine-incorporation (V) regressions.
‡ Significance level of regression slope and number of data points in regression.
§ Conversion factor calculated from Eq. 1 (10^9 cells nmol^{-1})—C_1; conversion factor calculated from u_b and first pair of N and V from dilution experiment—C_2; as C_2, but averaged over all pairs during the dilution experiment—C_3; average conversion factor calculated from change in N and V between sample times after dilution—C_4.
‖ C.V. of conversion factor calculated from Eq. 4.
¶ Slopes are statistically indistinguishable (t-test, α = 0.1, two-tailed).
\# Results of gentle filtration experiment.
** Gentamycin-treated grazing experiment.

Thymidine incorporation rate multiplied by the empirical conversion factors (Table 3) yield bacterial cell production rates (P_b) from 0.44 to 1.98 × 10^8 cells liter^{-1} h^{-1}. Rates of net algal production, determined from 24-h in situ incubations integrated over the epilimnion, varied between 13.6 and 39.8 mg C m^{-3} d^{-1}. The estimates of epilimnetic autotrophic and heterotrophic production track well during summer 1984 (Fig. 5).

Bacterial cell production rates, normalized by cell abundances, can be used to calculate exponential growth rates for the bacterial community. Our calculated rates (u_r) varied between 0.05 and 0.20 h^{-1} (Fig. 6) based on our empirical conversion factors (generation times of 3–15 h). Growth rates determined from changes in cell abundance in the dilution experiment (u_b) varied between 0.036 and 0.29 h^{-1} and are in the same range; however the relation of u_r to u_b varied seasonally. The two estimates of growth were similar in June and July, but on occasion, as the season progressed, thymidine-based estimates decreased to values below those of the dilution experiments. Growth rates varied from 0.004 to 0.020 h^{-1}, based on the conversion factor suggested by Fuhrman and Azam (1982). These rates are much lower than both u_b and u_r.

Error estimates for growth rates calculated from thymidine incorporation, cell abundance, and the empirical conversion factors yield C.V. <43% for all days except two dates in June when they are 101 and 103%. The larger errors are due to the larger error estimates for the conversion factors (Table 3). Error estimates for bacterial counts were calculated assuming 6% counting error. Error estimates for thymidine incorporation were based on a mean C.V. of 20%, determined from means and variances of 3–6 subsamples (each with triplicate assays) for each cruise date.

The higher growth rates from the dilution experiments (Fig. 6) may have been caused by sample manipulation. Fuhrman and Bell (1985) found increased dissolved free amino acids in filtrates when more than 50 ml were filtered through 25- or 47-mm filters. Vacuum pressure had little effect. Goldman and Dennett (1985) reported release of a

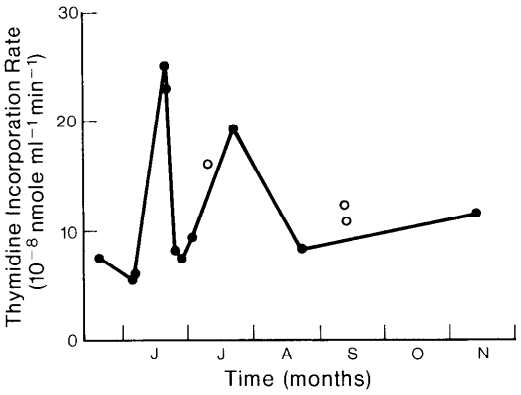

Fig. 4. Thymidine incorporation rates measured in 1984 (●) and in preliminary experiments in 1983 (○).

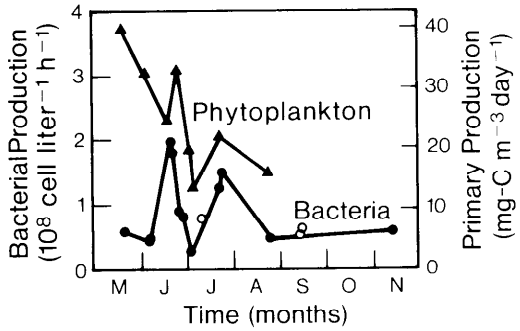

Fig. 5. Bacterial cell production (●) and phytoplankton net production (▲) in the epilimnion during 1984. Bacterial rates from preliminary experiments in 1983—○.

significant portion of recently fixed carbon into the filtrate of short term primary production experiments when typical vacuum pressure differentials are used. We routinely filtered 500–1,000 ml through 90-mm-diam, 0.2-μm pore-size Millipore filters but may inadvertently have stimulated growth by introducing labile organic substances. In one experiment when we found substantially different values for u_b and u_r (15 November), we measured primary amines before and after our standard filtration and could not detect any increase in the filtrate. However, we also did a parallel dilution-growth experiment in November using 100 ml of gently filtered dilution water. The growth rate determined from changes in cell counts in this case was lower than the one calculated from our routine protocol (0.10 vs. 0.13 h^{-1}) and closer to the growth rate of the unaltered sample (u_r = 0.07 h^{-1}).

Discussion

Conversion factors and bacterial growth— Our empirically derived conversion factors are similar to those determined for freshwater Ice House Pond (3.0 and 5.9 × 10^9 cells nmol^{-1}) by Kirchman et al. (1982); their empirical factors ranged from 1.9 to 68 × 10^9 cells nmol^{-1} in three diverse environments. Our values are also similar to those determined empirically (ca. 14 × 10^9 cells nmol^{-1}) in mesocosms simulating a eutrophication gradient in coastal marine waters (Hobbie and Cole 1984) and higher than those determined in a stimulation-growth experiment on eutrophic Lake Norrviken (Bell et al. 1983). Although our conversion factors tend to be higher than those suggested previously on theoretical grounds (*see below*), they are not as high as those determined to be invalid on empirical grounds (i.e. $u_b \neq u_r$) for open ocean samples (e.g. 1 × 10^{10}–3.9 × 10^{22} cells nmol^{-1}: Ducklow and Hill 1985*b*). The variability across environments of conversion factors determined with this method is to be expected because it is an empirical approach that integrates the effects of both biochemical and environmental variability on the relationship between thymidine incorporation and cell production. The fact that our value varied during the study suggests further that even within one water body, changes in

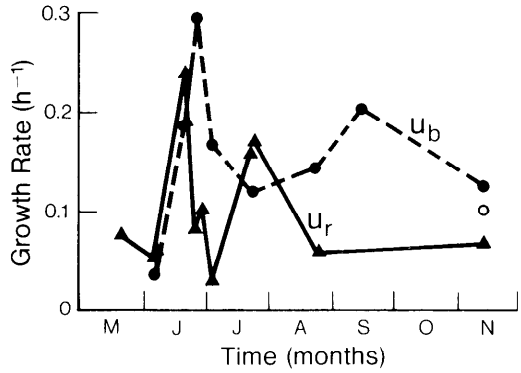

Fig. 6. Bacterial growth rates determined from cell counts in dilution experiments (u_b) and from conversion of unaltered-water thymidine incorporation (u_r). u_b from the gentle-filtration dilution experiment—○.

physical, chemical, and biological conditions may affect the conversion factor. If, as our 1985 data suggest (Table 1), cell volumes change considerably with season, then this too may be an important variable influencing empirical conversion factors.

Slopes of ln(V) and ln(N) vs. time (u_t and u_b) from the dilution experiments should be equal; however, differences have been reported (Kirchman et al. 1982; Ducklow and Hill 1985*a,b*). We also observed these differences in three of six of our experiments (Table 3). Kirchman et al. (1982) suggested that the difference may be caused by u_t being sensitive to increases in biomass as well as to cell number. In our experiments, the average difference in growth rates translates to only a 46% increase in cell diameter or an equivalent change from about a 0.40- to 0.56-μm-diam coccus. We could not verify this change in cell size with fluorescence microscopy. Ducklow and Hill (1985*a,b*) explored further the relationship between u_t and u_b and concluded that, in their open ocean environment, thymidine incorporation may not be tightly coupled to growth in longer incubations. In their studies, dilution-based growth rates determined from thymidine incorporation and cell counts resulted in the ratio $u_t : u_b$ ranging from 0.9 to 15.8 (mean = 4.6). Our values of u_t and u_b were much closer (ratio between 0.7 and 2.1, mean = 1.5) and it seems reasonable to assume that uptake and growth were closer to being in balance during our incubations, even for the cases of statistically significant differences between u_b and u_t (Table 3). However, one need not rely on any regression results or tests of significantly different slopes. If we calibrate thymidine incorporation during given intervals of the dilution experiment to the change in cell abundance during the same intervals, as Fuhrman and Azam (1982) did for 3-μm filtrates, our conversion factors range from 1.1 to 46.1 $\times$ 10^9 cells nmol^{-1} (Table 3). These are like those calculated from Eq. 1, about 5–20 $\times$ 10^9 cells nmol^{-1}.

Our conversion factors are about 5–20 times those suggested by Fuhrman and Azam (1980, 1982). Fuhrman and Azam (1980) derived the commonly used conversion factor from a set of conservative assumptions. They later (Fuhrman and Azam 1982) refined the factor in light of new measurements of bacterial DNA and comparisons with ^{32}P-based DNA synthesis. They stated that the method is conservative, but for their environment, probably good within a factor of two. Bell and Kuparinen (1984) found good agreement between thymidine incorporation and other measures of bacterial production and came to the same conclusion as Fuhrman and Azam (1982). Hagstrom (1984) also compared thymidine incorporation and frequency-of-dividing cells methods and found good agreement.

Bell et al. (1983) compared thymidine incorporation and $^{14}CO_2$ dark uptake methods and found good agreement when bacterial activity was high (growth rates of 0.06–0.15 h^{-1}), but thymidine-based production was about 10 times slower than $^{14}CO_2$-based production when activity was low (0.01–0.02 h^{-1}). Riemann and Sondergaard (1984) reported good correlation (r^2 = 0.74) between thymidine-incorporation and frequency-of-dividing-cells (FDC) methods; however the thymidine-based estimates were 7–21 times lower than those based on FDC or dark $^{14}CO_2$ uptake. This bias was not evident in their marine samples. Newell and Fallon (1982), however, also found good correlation between thymidine incorporation and FDC-based production (r^2 = 0.97), but thymidine-based estimates were 2–7 times lower than those based on FDC in a salt water environment. All of the above investigators (except Bell et al. 1983) converted thymidine incorporation to production or growth rates via the derived, conservative conversion factor suggested by Fuhrman and Azam (1982). Bell et al. (1983) determined a conversion factor based on the Kirchman et al. (1982) method except that they stimulated growth by adding substrate rather than by diluting the bacterial population. Their conversion factor was very similar to that of Fuhrman and Azam (1982). The accumulated comparisons thus far suggest that production estimates based on the conversion factor of 2.1–3.0 $\times$ 10^{18} cells per mole of thymidine incorporated into DNA (Fuhrman and Azam 1982) are indeed conservative.

We believe that our empirically based es-

timates produce less conservative growth rates and that the actual rates may approach our values. This is tentatively substantiated by our grazing experiments in November 1984 and April 1985. Grazing loss rates from those experiments were 0.06 and 0.036 h^{-1} (Table 3). Bacterial abundance in control samples for those experiments changed little over time (as was the case for most unaltered samples); thus growth rates would have to be comparable to grazing rates if the latter were the only process removing cells. Growth rates based on thymidine incorporation and the empirical conversion factor were 0.070 h^{-1} in November and 0.030 in April. These rates are more consistent with the "balanced" grazing losses than are growth rates calculated from the Fuhrman and Azam (1982) factor (0.008 and 0.004 h^{-1} for November and April). This difference becomes even more important because two possible difficulties with our grazing experiment could lead to underestimates of grazing: if gentamycin is not completely bacteriostatic and if gentamycin affects the grazers. Thus, bacterial growth must be at least equal to our grazing estimates if the population is to remain balanced.

Although there may be a slight increase in epilimnetic bacterial abundance in late June, overall our abundance data are quite uniform. This is particularly striking in light of exponential growth rates of the order of 0.1 h^{-1}. Such a temporal uniformity of abundance in the face of high production rates has led many investigators (e.g. Davis and Sieburth 1982; Sherr and Sherr 1984; Fenchel 1984, Sieburth 1984; Fuhrman and McManus 1984) to implicate tightly coupled grazing pressure by microzooplankton (predominantly flagellates) as a major control of these populations. Certain metazoa filter bacteria and thus derive some nutrition (Porter 1984; Peterson et al. 1978), but they do not seem able to consume sufficient quantities to affect bacterial populations (Porter 1984; Fenchel 1984). Little is known of the Great Lakes protozoan populations; however, heterotrophic protozoans can make up a significant portion of the nanoplankton biomass in Lake Ontario (Caron et al. 1985) and Lake Michigan (Fahnenstiel and Scavia unpubl.). Caron et al. (1985) observed cyanobacteria in the food vacuoles of heterotrophic microflagellates and the guts of rotifers from Lake Ontario and suggested that these organisms may be important consumers of the procaryote population. Fahnenstiel et al. (1986) found both cyanobacteria and heterotrophic bacteria in food vacuoles of protozoa from Lake Superior and estimated protozoan grazing pressure on the picoplankton from observed nonpigmented protozoan densities and typical filtering rates for flagellate protozoa (Fenchel 1982). They calculated grazing loss rates (0.76–0.95 d^{-1}) sufficient to combat measured cyanobacterial growth rates (0.8–1.5 d^{-1}). Results from our grazing experiments in November 1984 and April 1985 (*see above*) suggest that Lake Michigan bacterial populations may also be controlled by predation, at least during these times. This must be evaluated further because relatively high flagellate growth efficiencies (24–54% biomass produced per food biomass consumed: Sherr and Sherr 1984) imply the beginning of a significant "microbial loop" in the food chain; however, the number and efficiencies of subsequent links are also critical but are not known.

Carbon flux comparisons—Bacterial cell production and net primary production follow similar patterns after the onset of thermal stratification (Fig. 5). This relationship between algal and bacterial production has been noted in lakes (e.g. Pedros-Alio and Brock 1982) and the sea (e.g. Lancelot and Billen 1984) and indicates that recently fixed photosynthate may be a significant source of carbon for the microheterotrophs. It is tempting to calculate carbon flux through the bacterial population and compare it to various sources of organic carbon. To do so, we must know the carbon content and growth efficiency of the bacteria. The implication of estimates for these two factors is discussed below.

Carbon content is most often determined by measuring cell size and assuming a constant weight of per unit volume. Although few measurements of bacterial carbon in aquatic systems have actually been made, the value most used is 1.21×10^{-7} μg C μm^{-3} (Watson et al. 1977). Bratbak and

Dundas (1984) suggested that 2.2×10^{-7} may be a more appropriate value if the "true" size of native bacteria is known and that 5.6×10^{-7} should be used if the sizes are determined by microscopy of fixed samples (Bratbak 1985). Bias in the carbon content will translate linearly to production. Thus, if carbon per unit volume is between 1.21 and $5.6 \times 10^{-7} \mu g$ C μm^{-3}, bias from this source can be as much as 4–5-fold. Error in carbon content, when carbon per cell has been measured, is due mainly to difficulties in determining cell volume (Bratbak 1985). The reason is obvious. Errors in estimating linear cell dimensions propagate to biovolume by the third power. Thus, for example, the small difference between 0.4- and 0.5-μm-diam cocci would change carbon estimates by a factor of 2. Most investigators have determined cell size by measuring dimensions under epifluorescence microscopy. Because the limit of resolution of light microscopy is 0.1–0.2 μm (Brock 1984), it is difficult to assess the accuracy of sizes determined in this way. Marine and freshwater bacteria typically have linear dimensions of 0.2–1.0 μm and recent investigations emphasize the importance of bacteria at the lower end of this size range. We determined size from transmission and scanning electron microscopy (TEM, SEM) and fluorescence microscopy photographs, which are also subject to error. Fuhrman (1981) reported smaller volumes for cells measured by SEM than by epifluorescence and attributed the shrinkage to sample drying for SEM preparation. Bratbak (1985) demonstrated an overall but unsystematic variation in cell volumes estimated by SEM, epifluorescence microscopy, and electronic particle counting. Our current best estimate of volume for Lake Michigan bacteria, based on a weighted mean ($N = 723$) of estimates from SEM and fluorescence microscopy, is 0.074 μm^3 (Table 1).

If we assume that our volume estimates are unbiased and apply the range of volume-specific carbon contents above, we obtain carbon contents of $8.5–39.2 \times 10^{-9}$ μg C cell^{-1}. Mean cell production rates from our study are 0.18×10^9 cells liter^{-1} d^{-1} based on the Fuhrman and Azam (1982) conversion factor and 2.32×10^9 cells liter^{-1} d^{-1} based on the empirical conversion factors derived herein. Combining the range of carbon contents with these cell production estimates yields an almost two-order-of-magnitude range in carbon production (1.53–90.0 mg C m^{-3} d^{-1}). If the uncertainty of bacterial production efficiency (50–60%: Cole et al. 1982, 1984; Calow 1977; 10–30%: Newell 1984) is included, then the range of carbon supplies required to balance bacterial secondary production becomes 2.55–454.5 mg C m^{-3} d^{-1}.

Comparison to autotrophic production is tenuous, at best, at this point; however, we can place bounds on some of the potential sources of organic carbon supporting bacterial production. Daily autotrophic production estimated by integrating curves of production vs. irradiance over time and depth varied from 0.5 to 0.9 g C m^{-2} d^{-1} for the ~25-m photic zone, and the mean for our two 1984 summer experiments is 0.5 g C m^{-2} d^{-1}. Although we made these short term autotrophic measurements only four times during the study, estimates for this year are similar to measurements made in 1983 (Scavia and Fahnenstiel unpubl.). In an earlier, more detailed study (Fee 1972), the mean of daily estimates for three open-water stations during June through November was 0.47 g C m^{-2} d^{-1}; the seasonal mean varied from 0.25 to 0.80 g C m^{-2} d^{-1}. This type of production estimate is based on 1-h incubations which probably underestimate gross production (Harris 1978); however, even if we assign a generous portion of this production to excretory loss (20%), the resulting direct supply of dissolved organic carbon (DOC) to the 25-m photic zone is 0.1 g C m^{-2} d^{-1}. This algal release, which is in fact ~10 times the rates of release of recently fixed ^{14}C measured during the same study (Laird et al. 1986), can balance all of the bacterial carbon requirement (0.064 g C m^{-2} d^{-1}, assuming uniform bacterial production in the top 25 m) based on the Fuhrman and Azam (1982) conversion factor, the lowest carbon content, and the highest bacterial growth efficiency.

However, for any other combination of factors, phytoplankton extracellular release could provide only 1–52% of the calculated demand (11–52% based on the Fuhrman

and Azam 1982 conversion factor; 1–12% based on the empirical conversion factor). These latter comparisons, although representing a broad range, are consistent with observations from other lakes summarized by Brock and Clyne (1984) in which only 14–80% of bacterial production (usually <50%) could be balanced with phytoplankton exudate. Some combinations of conversion factors also lead to unreasonably high carbon requirements. For example, the empirical conversion factor, the higher carbon content, and a 20% growth efficiency yield a bacterial carbon requirement of about 20 times phytoplankton production. It may not be required that bacterial carbon demand be met solely by recent phytoplankton production (*see below*), but it seems unlikely that bacterial production could be that high. It is not possible at present to assign the cause of such high estimates to any one of the three calculation factors.

Although allochthonous sources to Lake Michigan cannot be discounted entirely, they are probably not an important source of available DOC. From data of Strand (*cited by* Eadie et al. 1984), the atmospheric input of DOC can be calculated as only 0.014 g C m^{-2} d^{-1}. We calculated TOC loads from the Grand River, the largest single source of nutrients to Lake Michigan, to be of the order of 0.005 g C m^{-2} d^{-1} (Chambers and Eadie 1980) if the load is diluted by the entire southern basin. The TOC load would have to be concentrated into only 5% of the basin and all of it be available to bacteria (0.1 g C m^{-2} d^{-1}) for this allochthonous source to become important. It thus seems likely that internal sources have to balance bacterial secondary production. If, as estimated above, direct release by actively growing algae is not sufficient, other pathways must be. Although we have no data to evaluate the hypothesis, DOC inputs from dying and grazed algae (Cole et al. 1984) and zooplankton excretion may be significant (Sharp 1984). This pathway is also dependent on primary autotrophic production and, in the steady state or on average, could transfer only a small fraction of that production to the bacteria.

The concept of a steady state system for Lake Michigan may be too restrictive. For example, the notion that the extant DOC pool is composed entirely and consistently of refractory material is arguable (Wright 1984). It appears from our 1 year of data that bacterial production may be relatively low in spring when temperature is low (Fig. 5); yet surface phytoplankton production is high and may reach maximum rates during this spring bloom. If release of organic carbon is also high during this time, then a reserve of DOC may be built up and used subsequently. This pool of relatively labile compounds may be masked by the high concentration of total DOC, typically near 3 mg $liter^{-1}$. A similar process may also occur in the flux of DOC from deeper water, especially from the dynamic nepheloid layer and sediment porewater (Chambers and Eadie 1981; Eadie et al. 1983, 1984). Intense vertical mixing of the water column during winter and early spring when temperatures are <4°C may set the stage for subsequent bacterial production, much in the same way as this winter resuspension appears to provide phosphorus for subsequent new phytoplankton production (Eadie et al. 1984).

It seems likely that no one source of carbon satisfies the bacterial demand but that different sources may be important at different times. This is not surprising. Lake Michigan is dynamic physically and ecologically. It can have temperatures <4°C, can be isothermal at 4°C, and can be stratified with epilimnetic temperatures >20°C. Its flora and fauna also change dramatically from spring to summer (Fahnenstiel and Scavia unpubl.; Scavia et al. 1986). There is little reason to expect a fixed relationship between bacterial production and a single steady source of organic carbon.

In conclusion, we have presented results from several dilution experiments that lead to conversion factors, with C.V. typically <40%, for converting thymidine incorporation rates to bacterial production for Lake Michigan. Our conversion factors are within the range of those in the literature based on an empirical approach. Thymidine uptake from unaltered lake samples multiplied by these conversion factors resulted in growth rate estimates (C.V. typically <50%) between 0.05 and 0.24 h^{-1}. These rates are higher than those based on the theoretical

conversion factor of Fuhrman and Azam (1982) but more in line with our estimates of grazing loss rates and growth rates of diluted Lake Michigan bacterial populations. Finally, we showed that, based on the range of values for conversion factors, bacterial carbon content, and bacterial growth efficiency, between 1 and 100% of bacterial carbon requirements can be balanced by organic carbon release from phytoplankton. While this range demonstrates the uncertainty in making such calculations, omitting only the most conservative set of factors reduces the range to 1–50%, implying that a source other than the recent release of photosynthate may be necessary to balance the bacterial demand.

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