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Seasonal Bacterial Production in a Dimictic Lake as Measured by Increases in Cell Numbers and Thymidine Incorporation

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Rates of primary and bacterial production in Little Crooked Lake were calculated from the rates of incorporation of $H^{14}CO_3^-$ and [*methyl*-^{3}H]thymidine, respectively. Growth rates of bacteria in diluted natural samples were determined for epilimnetic and metalimnetic bacterial populations during the summers of 1982 and 1983. Exponential growth was observed in these diluted samples, with increases in cell numbers of 30 to 250%. No lag was observed in bacterial growth in 14 of 16 experiments. Correlation of bacterial growth rates to corresponding rates of thymidine incorporation by natural samples produced a conversion factor of 2.2×10^{18} cells produced per mole of thymidine incorporated. The mass of the average bacterial cell in the lake was $1.40 \times 10^{-14} \pm 0.05 \times 10^{-14}$ g of C cell^{-1}. Doubling times of natural bacteria calculated from thymidine incorporation rates and in situ cell numbers ranged from 0.35 to 12.00 days (median, 1.50 days). Bacterial production amounted to 66.7 g of C m^{-2} from April through September, accounting for 29.4% of total (primary plus bacterial) production during this period. The vertical and seasonal distribution of bacterial production in Little Crooked Lake was strongly influenced by the distribution of primary production. From April through September 1983, the depth of maximum bacterial production rates in the water column was related to the depth of high rates of primary production. On a seasonal basis, primary production increased steadily from May through September, and bacterial production increased from May through August and then decreased in September.

The study of aquatic heterotrophic bacterial populations has been made easier and more precise by the introduction of new methods for studying bacterial growth and activity. The incorporation of [^{3}H-*methyl*]thymidine into bacterial DNA has been used to study bacterial production in several aquatic systems (1, 7–9, 13, 17–19). When coupled with the acridine orange direct count (AODC) method of enumerating bacteria (5, 11, 12), it can give estimates of both total production and specific growth rates.

The major shortcoming of this method is the imprecision in converting rates of thymidine incorporation into rates of bacterial production. Several factors must be determined experimentally or estimated qualitatively to make this conversion (7, 8, 13). The amount of label in DNA must be estimated by either extracting the DNA or assuming that it constitutes a constant portion of the radioactivity in cold trichloroacetic acid-insoluble material. The specific activity of deoxyribosylthymine triphosphate in the cells must be known or assumed to be that of the added label (i.e., no intracellular synthesis of thymidine). The percentage of nucleosides in the sample DNA accounted for by thymidine must be determined, or some assumption for this value must be made. The percentage of the microbial population capable of incorporating exogenous thymidine must be determined or assumed to be 100%. Finally, the quantity of DNA per cell must be determined or estimated. Fuhrman and Azam (7) initially used conservative estimates for the factors described above to convert thymidine incorporation rates to a range of estimated bacterial production rates. Results of later work (8) has corroborated these estimates and further refined the ranges of conversion factors. An average factor from this work was used by Riemann et al. (21) for estimating bacterial production rates in several freshwater lakes.

Another means of deriving a conversion factor is to determine independently and experimentally the rate of bacterial production in a sample for which the rate of thymidine incorporation is known. The direct measurement of increases in cell numbers in a sample from which predators have been removed can be used to determine bacterial growth rates (7, 26). A water sample is typically screened to remove most of the grazers and diluted with filter-sterilized water from the same sample to reduce nutrient limitations. Substantial increases in cell numbers in samples prepared in this manner are generally observed. This procedure has been used with undiluted samples to obtain a direct conversion from thymidine incorporation rate to bacterial production in a eutrophic lake in Sweden (1). Using a mathematical model to interpret data from dilution-incubation experiments in several aquatic systems, Kirchman et al. (13) have found a wide range of conversion factors between thymidine incorporation and biomass production. The question of variability in the conversion factor remains. It would be of interest to determine this factor within a given system over a course of several months and a wide range of bacterial growth rates.

We studied bacterial production in a dimictic eutrophic lake using the thymidine incorporation method of Fuhrman and Azam (7) and a dilution-incubation technique based on the method used by Fuhrman and Azam in the same study (7). In addition, primary production and total and active bacterial cell numbers were determined. The results were used to examine bacterial production and its relationship to primary production throughout the water column of this lake from spring to fall 1983.

MATERIALS AND METHODS

Sampling site. This study was conducted on Little Crooked Lake (Noble County, Ind.). Temperature and oxygen meas-

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urements were made with a YSI model 54 meter, (Yellow Springs Instruments, Kettering, Ohio). Water samples were collected with an acid-washed Van Dorn bottle (Wildco Supply Co. Saginaw, Mich.). The location of the phytoplankton population maximum was determined from chlorophyll *a* measurements of discrete samples from the water column. Direct counts showed that this population was dominated by cyanobacteria. Chlorophyll *a* concentrations were determined from the absorbance at 663 nm of dimethyl sulfoxide-acetone extracts (40:60) of organisms filtered onto glass fiber filters (22). The pH of water samples was determined with a pH meter (Digisense; Cole-Parmer, Chicago, Ill.). Experiments were initiated 10 min after sample collection in the laboratory of the Crooked Lake Biological Station.

Biomass and activity measurements. We used the AODC method of Hobbie et al. (11) with minor modification to enumerate bacteria in Formalin-fixed (2.5% final concentration) samples. A sample diluted to give approximately 20 cells per field was stained with acridine orange (Sigma Chemical Co., St. Louis, Mo.) (final concentration, 0.01%) for 2 min and filtered through a black 0.2-μm polycarbonate membrane (Nucleopore Corp., Pleasanton, Calif.). The procedure of Fliermans and Schmidt (4) was used to prepare the filters: (i) The filters were stained black by soaking them overnight in 2% Irgalan Black (Ciba-Geigy Corp., Reading, Pa.) in a solution of 2% (vol/vol) acetic acid. (ii) The stained filters were rinsed in distilled water and soaked for 60 min at 37°C in 50 mM Tris (pH 8.1) containing 200 μg of lysozyme (Sigma) per ml. (iii) The soaking solution was then brought to a concentration of 1 mM with disodium ethylenediaminetetraacetic acid, and incubation was continued for 2 h. (iv) Sodium dodecyl sulfate was added to give a final concentration of 1%, and the filters were rinsed in filter-sterilized 0.5 mM carbonate buffer (pH 9.6). Processed filters were stored between sheets of sterile filter paper in a petri dish. Filters treated in this manner gave control counts of less than one cell per field, whereas untreated filters had about five bacterial cells per field on their surfaces. A Zeiss Standard research microscope was fitted with an HBO 50-W mercury burner, BG12 exciter filter, and a barrier filter with a sharp cutoff at 500 nm. Two ×10 Kpl measuring eyepieces and a ×100 oil immersion Neofluar phase contrast objective constituted the lens system (Carl Zeiss, Inc., Thornwood, N.Y.). At least 20 randomly chosen fields containing a total of at least 400 cells were counted per filter. Owing to the wide variety of sizes and shapes of bacteria in freshwater samples, we found it most convenient to divide the bacteria into several size classes. The size classes used were (i) cocci, 0.5-μm diameter; (ii) cocci, 1.0-μm diameter; (iii) rods, 0.75- to 1.0-μm length by 0.5-μm width, (iv) rods, 1.5- to 2.0-μm length by 0.5- to 1.0-μm width; and (v) rods 2.5- to 5.0-μm length by 1.0-μm width. Cell volumes for each size class were calculated from cell size by using mathematical formulas for spheres and cylinders. The cell volume was converted to carbon biomass by multiplying by 1.2×10^{-13} g C μm^{-3} (27). This calculated value of average cell biomass was used in bacterial production estimates. Although Bowden (3) and Fuhrman (6) have found the accuracy of epifluorescence microscopy biomass estimates to be greater than that of scanning electron microscopy-based estimates, some of this precision is lost by placing cells in approximate size classes. Only fluorescing objects with the clear outlines of cells were counted.

We used the reduction of 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride (INT) to microscopically visible formazan crystals to determine the percentage of respiring cells in the populations. Samples were incubated in acid-washed, sterile screw-cap test tubes with a final concentration of 0.02% INT dye (Sigma) for 20 min in the dark at in situ temperatures (28). Formalin-fixed (2.5% final concentration) controls were included for each sample. After incubation the samples were fixed with Formalin. Twenty-minute incubations gave maximum formazan counts. For counting, samples were filtered onto 0.45-μm pore size nitrocellulose filters (Gelman Sciences Inc., Ann Arbor, Mich.), and the cells were suspended in a drop of filter-sterilized water on a freshly alcohol-flamed microscope slide. The smears were allowed to air dry and were then stained with 0.1% acridine orange for 1 min, rinsed, and covered with Cargille type B immersion oil and a cover slip. Since formazan crystals were not observable in bacteria capable of passing through a 0.45-μm pore size filter, possibly due to the relatively low magnification used, we considered bacteria retained by 0.45-μm pore size filters to be representative of the total population. To prevent excessive loss of formazan crystals due to dissolution in the immersion oil (24), slides were counted immediately after the oil was added (total time, ≤15 min). Only cells fluorescing under epifluorescence and containing a visible intracellular formazan crystal under bright-field microscopy were counted as being metabolically active. All cells in 10 fields were counted to obtain a total cell count of at least 200. We then calculated the percentage of cells which were INT active by substracting the INT-positive fraction of the Formalin-fixed controls (less than 5% in all cases) from the INT-positive fraction of the experimental samples.

Incorporation of [^{3}H]thymidine. Thymidine incorporation profiles of Little Crooked Lake were taken at biweekly to monthly intervals from April through October 1983. Our labeling procedure has been described previously (15a). AODC and INT counts were made using the same water samples as were used for thymidine incorporation measurements during the 1983 season. We have determined that 80% of the tritium label was in DNA (15a). Isotope exchange with water was corrected for by comparing a subsample of the isotope stock solution to a subsample from which all water has been removed by desiccation. Bacterial production values were calculated from rates of thymidine incorporation by using experimentally derived conversion factors for cells produced per mole of thymidine incorporated (see below) and for average carbon biomass per cell (see above).

Determination of bacterial growth rates from increases in cell members. Samples were taken from the depth of interest, and a subsample was gravity filtered through a 47-mm diameter 3.0-μm pore size, acid-washed, sterile polycarbonate membrane with an acid-washed, sterile glass filtration apparatus with a stainless steel screen filter support. This screened water was then diluted 10 to 50 times with 0.2-μm pore size filtered water from the same sample. The dilute suspension was then dispensed into either acid-washed, sterile biological oxygen demand bottles or acid-washed dialysis bags (molecular weight exclusion, 12,000 to 14,000; Spectropore 4 dialysis tubing; Cole-Parmer) and incubated in situ or at in situ temperatures in the dark in the laboratory. Bottles or bags were collected at timed intervals, and bacteria were enumerated by AODC. Most counts were done in duplicate, and at least 400 cells were counted for each sample. The logarithm of cell counts was plotted versus time, and a least-squares regression line was fitted to the data. In 14 of 16 experiments, the data plotted in this manner formed a straight line (i.e., exponential growth was ob-

served). Nonlinear data were not used. The instantaneous growth rate (μ) was calculated from the slope of the regression line for each experiment. Cell counts in these experiments were divided into size fractions, as described in the routine enumeration procedure, to check for any variation in average cell size during incubation. This served as a control against change in biomass which could not be accounted for by a change in cell numbers.

Estimates of primary production by the phytoplankton in Crooked and Little Crooked Lakes were made from experimental measurements of the photosynthetic rates of epilimnetic and metalimnetic samples at a series of light intensities. In addition, vertical profiles of chlorophyll *a* concentration, temperature, and light extinction in the lake were made two to three times per month. A numerical model was then used to calculate primary production (15).

RESULTS

Physicochemical characteristics of Little Crooked Lake. Our study was carried out on a dimictic hard water lake in northern Indiana. Many of the lakes in the region, including Little Crooked Lake, are saturated with calcium carbonate (ca. 2.5 to 3.0 mM inorganic carbon and 0.8 to 1.0 mM Ca^{2+}). As a result, calcium carbonate precipitates in this lake, reducing phosphate levels in the water column (20). The combination of reduced nutrient availability in the epilimnion and adequate light penetration into the metalimnion causes the phytoplankton population to be concentrated in deeper waters where nutrient levels are higher.

Little Crooked Lake became thermally stratified by early May 1983. This stratification persisted throughout the summer, with fall the overturn occurring in November. The hypolimnion became anaerobic in late June as a result of this stratification and the ensuing absence of mixing. Thus, the thermal stratification of this lake results in the formation of physically, chemically, and biologically distinct water layers (Fig. 1).

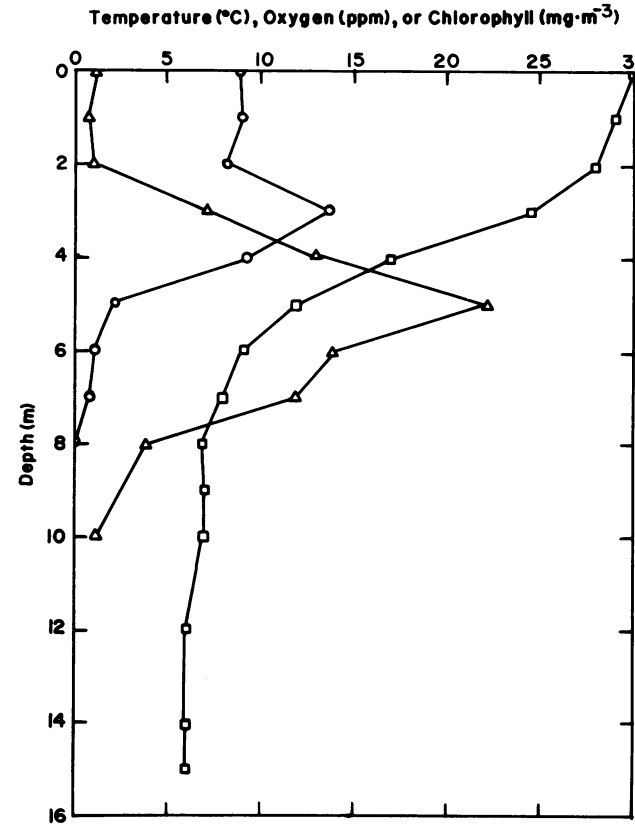

FIG. 1. Vertical profiles of temperature (□), oxygen concentration (○), or chlorophyll *a* concentration (△) from Little Crooked Lake on 18 July 1983.

Chlorophyll distribution and primary production. Primary production rates in Little Crooked Lake are controlled mainly by two factors, phytoplankton biomass and ambient light intensity. Maximum chlorophyll *a* concentrations were found in the metalimnion throughout the period of thermal stratification (Fig. 2A). However, the light intensities at these depths were only 1 to 10% of that at the surface. As a consequence, the maximum rates of primary production most often occurred in the epilimnion, whereas metalimnetic peaks were seen only in late June, early July, and mid-August (Fig. 2B). These metalimnetic primary production peaks were found during periods of good light penetration to the metalimnetic chlorophyll *a* maximum. The chlorophyll maximum concentration descended from 2- to 6-m depths in the water column from May through September, whereas primary production maxima changed location from the epilimnion to the metalimnion and back to the epilimnion during the June through August period. Monthly estimates of particulate organic carbon production are shown in Table 1.

Bacterial biomass and INT activity. The highest densities of planktonic bacteria were usually found in the metalimnion during 1983 (Table 2). These peaks tended to be found lower in the water column as time passed and were related to the location of the chlorophyll *a* concentration maxima from May through July. During August, the highest bacterial cell counts were found below the depth of maximum chlorophyll *a* concentration. The number of bacteria per milliliter at the peak was as much as twofold greater than in the rest of the water column, particularly from July through October. On a seasonal basis, total bacterial numbers per meter squared increased from May through September. Maximum bacterial numbers were found in April and September.

The average cell size (i.e., biomass per cell) of the bacterial populations in the three water layers during the summer of 1983 is shown in Fig. 3. The average cell size of epilimnetic bacteria was greatest in late May, but no overall trend of an increase or a decrease in the average cell size of epilimnetic bacteria was observed. Metalimnetic bacteria increased in size from late May through October. The size of hypolimnetic bacteria increased sharply from May through mid-August, dropped sharply in September, and increased again in October. August and October average cell sizes were noticeably larger in hypolimnetic samples than in metalimnetic samples. Increases of 39% in metalimnetic samples and up to 57% in hypolimnetic samples were observed from late May through late October. By pooling data from all three water layers, an average cell biomass of $1.40 \times 10^{-14} \pm 0.05 \times 10^{-14}$ g of C cell $^{-1}$ (mean ± standard error) was obtained.

The percentage of bacteria capable of reducing INT to microscopically visible formazan crystals varied with depth during the period of thermal stratification (Table 3). Data were pooled for samples taken from different depths within the same region of the water column. Usually, three epilimnetic, four metalimnetic, and two hypolimnetic samples were analyzed. Although we do not have enough data to evaluate seasonal trends statistically, epilimnetic values were highest when maximum primary production rates were found in the epilimnion. The highest percentages of meta-

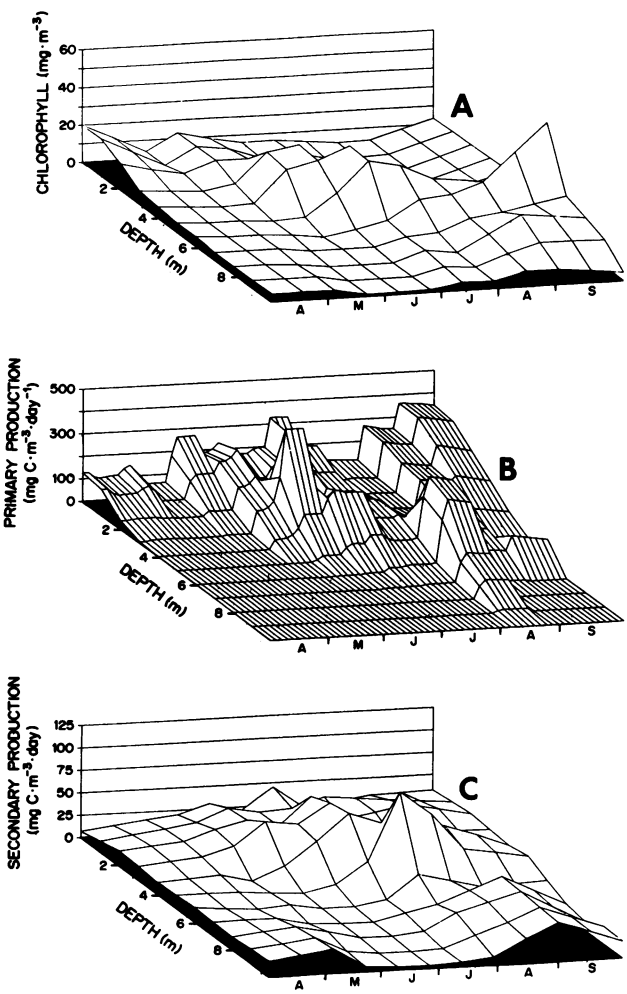

FIG. 2. Chlorophyll *a* concentration (A), primary production (B), and secondary production (C) in Little Crooked Lake from 1 April through 30 September 1983 (letters on the x-axis indicate the month). The finer temporal scale in (B) is due to the use of 3-day intervals in plotting primary production values.

limnetic and hypolimnetic bacteria seemed to be active when primary production rates were highest in the metalimnion. INT-positive cells ranged from 17.4 to 40.1% of the total bacterial population; the mean of all measurements made was about 30%.

Incorporation of [^{3}H]thymidine into DNA. Maximum rates of thymidine incorporation tended to occur in the metalimnion of Little Crooked Lake during 1983 (Fig. 2C). The depths at which the highest rates of secondary production occurred corresponded to the depths of peak chlorophyll *a* concentration through mid-July (Fig. 2A and C). From August onward, the location of secondary production maxima diverged sharply from chlorophyll *a* concentration maxima, with secondary production maxima occurring at shallower depths. This correlated with the high epilimnetic primary production rates observed throughout most of the August through September period (Fig. 2B).

Determination of bacterial growth rates. Bacterial growth rates in natural samples were determined to obtain a conversion factor for the calculation of secondary production rates from rates of thymidine incorporation. We diluted natural samples from which grazers had been screened with filter-sterilized lake water from the depth of sample origin. Changes in bacterial numbers in these samples were then measured. Four or five time points were used for each determination. Increases of from 30 to 250% in the numbers of bacterial cells in diluted water samples incubated in situ or at in situ temperatures in the laboratory for 12 to 14 h were observed (Table 4). No such increase was observed in undiluted, screened samples (data not shown). This may have been due to predation by micrograzers capable of passing through a 3.0-μm pore size filter (R. T. Wright, personal communication) or to nutrient limitation (7). Instantaneous growth rates (μ) were calculated from the slopes of the regression lines fitted to our data sets. The high proportion of samples with relatively short doubling times is due to our preferential use of samples which would give measurable increases in cell numbers within 10 to 14 h of incubation. The relative insensitivity of this method makes it very difficult to use when measuring long doubling times during short incubations.

No significant difference in bacterial growth rates was seen between identical samples incubated in dialysis bags or biological oxygen demand bottles. The small differences observed between bags and bottles fall within the 10 to 15% error which can be attributed to the counting procedure (data not shown).

The average cell biomass at each time point was calculated for several samples (Fig. 4). We observed no change in average cell biomass in samples incubated for less than 12 to 14 h. In longer incubations, the average cell biomass increased by about 15%, and in some cases the increase in the logarithm of cell numbers was clearly nonlinear (Fig. 4). To avoid these anomalous increases, we used only data from samples after <12 to 14 h of incubation.

A linear regression was done between μ obtained in these experiments and specific rates of thymidine incorporation measured in samples collected from the same depth within 1 to 3 days of the growth rate measurement. The regression obtained had the form of picomoles of thymidine incorporated · $(10^9$ cells$)^{-1}$ hours^{-1} = 477 · μ −2.38 (r^2 = 0.89). Since the y-intercept was very close to zero, we felt justified in forcing the line through the origin (23), obtaining the relationship picomoles of thymidine incorporated · $(10^9$ cells$)^{-1}$ hours^{-1} = 457 · μ (r^2 = 0.84) (Fig. 5). The use of thymidine incorporation rates measured within 1 to 3 days of our incubation experiments was not an important problem, because rates measured over a 3-day interval were generally

TABLE 1. Monthly primary and bacterial production in the Little Crooked Lake water column from April through October 1983[a]

Mo	Primary production (g of C m^{-2} mo^{-1})[b]	Bacterial production (g of C m^{-2} mo^{-1})[b]	Total[c]	Percent[d]
April	8.0	7.0	15.0	46.7
May	10.3	7.3	17.6	41.5
June	27.8	7.3	35.1	20.8
July	30.9	13.9	44.8	31.0
August	39.4	19.8	59.2	33.4
September	42.8	11.4	54.2	21.0
October		5.2		
Total[e]	160.4	66.7		

[a] These values were calculated from data collected twice a month.
[b] To 15-m depth.
[c] Primary plus bacterial production.
[d] Bacterial production/total production × 100.
[e] April through September total.

TABLE 2. Vertical distribution of bacterial numbers in Little Crooked Lake (1983)

Depth (m)	No. of bacterial cells (10^9 cells liter^{-1}) on the following dates:										
	4 April 1983	9 May 1983	23 May 1983	6 June 1983	20 June 1983	5 July 1983	11 July 1983	18 July 1983	16 August 1983	21 September 1983	26 October 1983
0	2.38	1.70	3.92	2.83	3.70	2.98	3.09	2.26	2.40	2.88	2.79
1	5.78	2.06		2.36	4.72	2.79	3.32	2.22			
2		2.04	4.98	3.09	4.55	2.78	3.17	4.06	3.05	4.25	3.00
3	5.55	2.02	4.65	2.93	5.41	2.55	3.10	3.90	2.74		
4		2.05	3.98	2.69	4.91	4.24	3.72	4.36	2.54	3.71	3.71
5	5.04		3.33	2.73	5.40	5.91	6.25	3.45	2.56	4.07	3.48
6		2.19	1.91	1.88	4.15	3.33	6.03	5.97	2.14	5.14	3.89
7			1.87	1.96	3.79	3.00	2.84	4.56	4.38	3.38	3.95
8	5.69	1.98	1.85	2.79	2.53	2.88	3.14	4.07	4.40	5.02	4.70
9										3.50	4.72
10	4.68	2.18			1.97	2.72		2.20	3.84	4.42	4.19
12									4.08		4.22

similar to one another (Table 5). In addition, Konopka (14) has shown in a previous study of Crooked Lake that a physiological parameter of *Oscillatoria rubescens* ($^{14}CO_2$ incorporation into protein) was relatively constant over 3-day intervals. Data points that represent the highest thymidine incorporation rates are from experiments in which several samples from the same depth were incubated in parallel. There was considerable variation among the growth rates measured in these experiments, but these data do not represent a true plateau.

The relationship derived above was used to calculate doubling times of the heterotrophic populations from rates of thymidine incorporation. Doubling times ranged from 0.35 to 12.00 days (median, 1.50 days). Doubling times of bacterial populations in the water column of Little Crooked Lake varied greatly with depth. For example, on 20 June 1983, doubling times were 6 times longer in hypolimnetic samples than in metalimnetic samples. This profile was not exceptional; differences as great as 9.2-fold were seen between the most productive zone and the hypolimnion on 5 July 1983.

Bacterial production was calculated from rates of thymidine incorporation into cold trichloroacetic acid-insoluble material for vertical profiles of Little Crooked Lake taken from April to October 1983. Bacterial production estimates (in milligrams of carbon per meter cubed per day) were obtained by multiplying the number of cells produced by the average cell biomass of 1.40×10^{-14} g C per cell. Bacterial production varied with depth. It increased through the summer season, with maximum values occurring in the metalimnion through August (Fig. 2C). In September there was a decrease in bacterial production; surface waters were as productive as metalimnetic waters. As we observed for both Crooked and Little Crooked Lakes in 1982 (15a), the depth of the bacterial production maximum was related to the depth of the primary production maximum in the water column (Fig. 6B and C). Through early July, bacterial production maxima occupied depths immediately below or corresponding to depths of maximum primary production and showed a strong correlation with chlorophyll *a* maxima (Fig. 6A). After early July, primary production maxima were observed in the epilimnion until early August, when maximum primary production was again found in the metalimnion, where it remained for about 2 weeks. During this period, the bacterial production maximum ascended in the water column steadily, and the chlorophyll *a* concentration maximum remained at 6-m depth. Monthly estimates of particulate organic carbon production by heterotrophic bacteria from April through October are shown in Table 1. Bacterial production was not solely determined by cell numbers. Cell specific rates of thymidine incorporation ranged from 0.11×10^{-20} to 3.75×10^{-20} mol of thymidine incorporated cell^{-1} h^{-1} (median, 0.90×10^{-20} mol of thymidine incorporated cell^{-1} h^{-1}). When INT-active cell numbers were used to calculate cell specific rates of thymidine incorporation, the range was 0.39×10^{-20} to 14.94×10^{-20} mol of thymidine incorporated cell^{-1} h^{-1} (median, 3.37×10^{-20} mol of thymidine incorporated cell^{-1} h^{-1}). In several vertical profiles, depths having equal numbers of bacteria per unit volume varied by as much as a factor of three in specific thymidine incorporation. The highest specific rates of thymidine incorporation were found in the metalimnion. Values in the epilimnion were also frequently high. High specific rates of thymidine incorporation were found to be correlated with high rates of primary production, with specific thymidine incorporation rate maxima coinciding with or falling below primary production maxima in the water column.

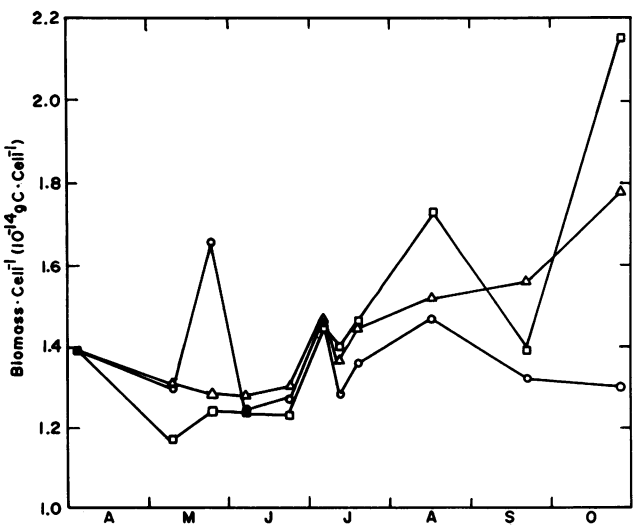

FIG. 3. Average bacterial cell size in the water layers of Little Crooked Lake from 4 April through 26 October 1983 (letters on the x-axis indicate the month). Symbols: ○, epilimnion; △, metalimnion; □, hypolimnion.

DISCUSSION

In our study of the bacterial populations in Little Crooked Lake, we found that the bacterial population undergoes changes in numbers and growth rates with depth and time.

TABLE 3. Epilimnetic, metalimnetic, and hypolimnetic INT activity in Little Crooked Lake[a]

Zone	Percent INT active on the following date:					
	4 April 1983	20 June 1983	5 July 1983	11 July 1983	16 August 1983	Avg
Epilimnion	29.7 ± 2.3[b]	20.4 ± 0.8	40.1 ± 6.4	34.7 ± 1.7	25.5 ± 1.1	30.1 ± 3.4
Metalimnion		28.2 ± 4.0	22.6 ± 4.0	27.9 ± 2.3	33.2 ± 1.9	28.0 ± 2.2
Hypolimnion		34.6 ± 0.4	17.4 ± 4.0	29.4 ± 4.6	39.9[c]	30.3 ± 4.8

[a] Mean values for the three water layers ± standard error.
[b] Water column completely mixed.
[c] Only one hypolimnetic sample taken on this date.

Bacterial numbers were typically greatest in the metalimnion; the depth of the maximum occurred at or below the depth of maximum chlorophyll *a* concentrations. Maximum rates of bacterial production also coincided with maximum chlorophyll *a* concentrations during the April to mid-July period but occurred higher in the water column from mid-July to October. This was probably a response to epilimnetic primary production maxima during this period and is similar to previous observations for Crooked Lake, as well as Little Crooked Lake (15a).

The fluctuations in bacterial numbers and rates of production that we observed in the water column of Little Crooked Lake were reminiscent of the changes in cell numbers observed in Kiel Fjord, Germany (16) and the Humber Estuary, England (2). Although bacterial numbers in estuaries are believed to be strongly influenced by water flow, a major factor controlling nutrient influx; and because there is no such flow in Little Crooked Lake, the observed changes in bacterial numbers in this lake may have a similar cause. The production of photosynthetically fixed organic carbon also varied with depth and time. Because this probably constitutes a major nutrient source for pelagic bacteria, the changes in bacterial numbers and rates of bacterial production are likely to reflect these changes. Bacterial numbers did not show a strong relationship to thymidine incorporation rates. This is because cell-specific thymidine incorporation activity of the lake bacteria varied considerably with depth.

Bacterial growth rates increased in response to increasing primary production from May through August. Doubling times were shortest in water layers with high rates of primary production, with maximum growth rates often being found in the metalimnion. It is known from pure-culture studies that bacterial cell size increases with increasing growth rates (10). This relationship was reflected by increases in the average cell size in metalimnetic samples from late May through October. It is not clear why the average cell size of hypolimnetic bacteria was comparable to that of metalimnetic bacteria and, at some times, higher. Doubling times of hypolimnetic bacteria were significantly longer than those of metalimnetic bacteria throughout the period of thermal stratification. Although it is true that growth rates of

TABLE 4. Bacterial growth rates in diluted natural samples from Little Crooked Lake, 1982 to 1983[a]

Date	Depth (m)[b]	Percent increase in AODC[c]	T_D[d]	μ (h^{-1})	r^2[e]
21 July 1982	3	31.5	18.8	0.037	0.850
9 August 1982	2	93.2	8.6	0.081	0.979
	4	205.0	5.5	0.126	0.992
	6	183.1	6.4	0.108	0.972
10 August 1982	6	214.3	4.8	0.146	0.977
	6	265.8	4.5	0.154	0.942
	6	263.2	3.6	0.192	0.930
14 June 1983	3	54.4	7.8	0.089	0.999
21 June 1983	3	39.2	19.2	0.036	0.895
18 July 1983	0	55.0	16.6	0.042	0.998
	5	119.4	10.9	0.064	0.916
19 July 1983	0	48.0	17.8	0.039	0.959
	5	43.5	17.6	0.040	0.948
17 August 1983	2	138.1	17.6	0.040	1.000

[a] The logarithm of cell numbers was plotted against incubation time for diluted natural samples. Instantaneous growth rates were calculated from the slope of the line. Slope and coefficient of determination were found by linear regression. Four or five time points were used for each regression.
[b] Samples from 0 to 2 m were from the epilimnion, those from 3 to 5 m were from the metalimnion, and those from 6 m were in the hypolimnion.
[c] $[(N_t - N_0)/N_0] \times 100$.
[d] T_D, Doubling time.
[e] Coefficient of determination.

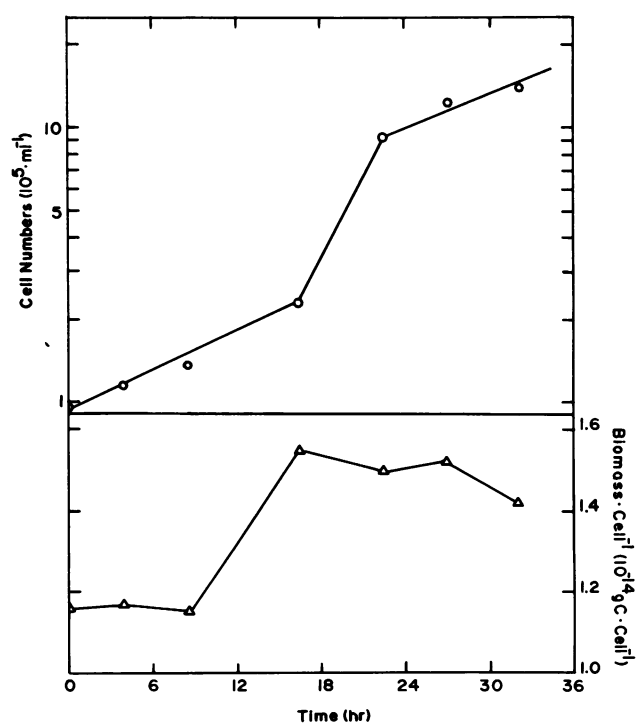

FIG. 4. Cell numbers (○) and average cell biomass (△) in a diluted natural sample during prolonged incubation. The sample was from Little Crooked Lake (2-m depth) on 17 August 1983.

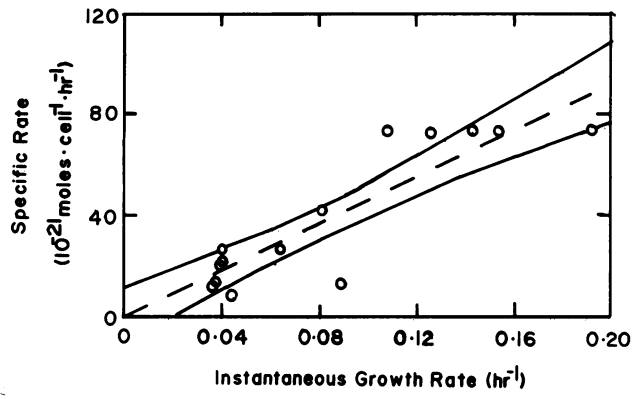

FIG. 5. Regression of specific rate of thymidine incorporation versus μ for 1982 and 1983 Little Crooked Lake samples. The equation of the line is pmol $(10^9$ cells$)^{-1}$ h^{-1} = 457 μ; r^2 = 0.835. Solid lines represent 95% confidence limits.

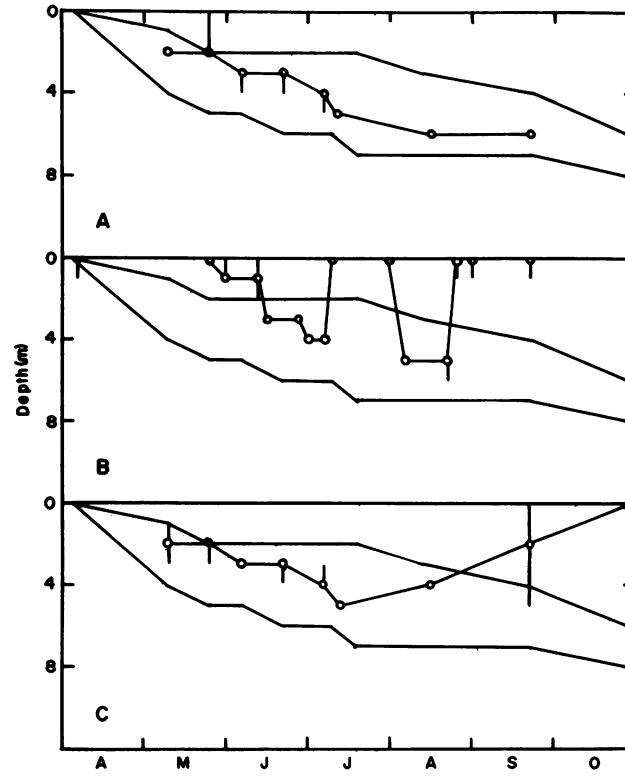

FIG. 6. Depths of maximum chlorophyll *a* concentration (A), primary production rate (B), and secondary production rate (C) in Little Crooked Lake from 4 April through 26 October 1983 (letters on the x-axis indicate the month). The enclosed area represents the boundaries of the metalimnion in the water column. Bars represent the range of depths with ≥90% of the maximum value.

hypolimnetic bacteria increased from late June through mid-August, the increase in the average cell size observed in October coincided with a period of very slow growth. Doubling times for metalimnetic bacterial populations in October were twice as long as those in September, but the average cell size increased by about 15% from September to October. In addition, growth rates of epilimnetic bacteria in August were about 4.5 times as high as those found in April, but the average cell size in August was only slightly greater than that in April. The observed increase in cell size for metalimnetic bacteria during periods of rapid growth fits well with the positive correlation between average cell size and growth rate which has been observed in laboratory cultures. However, our other data do not fit this correlation. This may occur because these are heterogeneous samples, not pure cultures, and dominant species of different sizes could be selected as conditions in the lake change.

In 1982, the vertical distribution of bacterial production was related to the location of high rates of primary production in Little Crooked Lake, as well as Crooked Lake (15a). A movement of the primary production maximum to a new location in the water column resulted in the development of high rates of bacterial production at that depth after a lag of 2 to 3 weeks. The vertical distribution of bacterial production in Little Crooked Lake followed a similar trend in 1983 but did not follow the movements of the primary production maximum as closely as was observed in this lake in 1982. Since total primary production in Little Crooked Lake in 1983 was about one-third that of 1982, the allochthonous carbon input may have been relatively more important in determining the vertical distribution of bacterial production in 1983 than it was in 1982. Seasonal changes in primary and bacterial production rates in 1983 were different from those in 1982. In 1983, water column primary production increased steadily from May through August, as did secondary production. In 1982, primary production was greatest in June, decreasing through October. Bacterial production in 1982 peaked about 3 weeks after this primary production maximum was attained and decreased from roughly August through September. High rates of bacterial production were also observed in October 1982, when primary production rates were low. In 1983, primary production rates were high

TABLE 5. Variability between thymidine incorporation rates measured on different dates

Depth (m)	Date	Rate[a]	Date	Rate[a]
0	25 September 1982	30.1 ± 11.2	28 September 1982	32.9 ± 7.3
6	25 September 1982	45.7 ± 6.6	28 September 1982	45.9 ± 1.1
0	25 October 1982	30.5 ± 12.2	26 October 1982	30.8 ± 4.0
7	25 October 1982	87.1 ± 1.5	26 October 1982	77.8 ± 2.2
0	29 November 1982	9.5 ± 3.3	30 November 1982	12.1 ± 2.3
0	5 April 1983	21.2 ± 4.4	6 April 1983	24.7 ± 2.3
3	6 June 1983	74.5 ± 4.2	7 June 1983	39.8 ± 8.2
6	6 June 1983	8.9 ± 2.0	7 June 1983	4.6 ± 0.6
0	5 July 1983	58.7 ± 5.5	6 July 1983	54.7 ± 9.4
4	5 July 1983	101.4 ± 0.2	6 July 1983	72.9 ± 2.9

[a] Picomoles of thymidine incorporated per liter per hour ± standard error of the mean.

in September, but the corresponding bacterial production rates in September and October were lower than the values in August.

The use of increases in cell numbers in diluted natural samples to study the growth rates of natural bacterial populations is well documented (for a recent review, see reference 26). Use of this method is becoming more frequent (1, 7, 13). Exponential growth of natural bacteria was observed in this study, with little evidence of a lag period. Similar results were obtained by Fuhrman and Azam (7) and Kirchman et al. (13). Such a lag has been interpreted as reflecting the presence of dormant (i.e., nongrowing) bacterial cells in the sample. The fact that in almost all of our experiments no lag was observed suggests that dormant cells may make up a very small portion of the bacterial population. Similar low proportions of dormant cells were observed by Tabor and Neihof (24, 25) in samples from the Chesapeake Bay. The high percentage of active cells implied by our growth rate experiments is puzzling in view of INT results which indicate that the majority (~70%) of the bacterial population in Little Crooked Lake lack electron transport activity. Samples were typically not taken for the determination of cells numbers in the incubation experiments until after 3 to 4 h. Thus, it is possible that a lag of short duration may have been overlooked. It is also possible that formazan crystals in the small cells (rods with lengths of 0.75 to 1.0 μm by 0.5 μm width), which constituted the numerically largest fraction of the bacteria in this lake, were not readily observable with the optics used in this study.

The increases in cell numbers and average cell size observed after prolonged (>14 h) incubations in some of our growth rate experiments have important implications for users of this method. The observed increase in the average cell size reflects the fact that cells increase in size when their growth rate increases, but the reason for this burst of growth after 16 to 20 h is not at all clear. The common procedure of taking only zero time and endpoint cell counts (26) for growth rate determinations could easily overlook this effect. Endpoint incubations should not be used without testing for this phenomenon.

The calculation of rates of bacterial production from rates of thymidine incorporation has previously involved making several assumptions or experimentally determining several factors (7, 8, 15a). One means of simplifying the conversion of thymidine incorporation rates to rates of bacterial production is the standardization of thymidine incorporation rates to experimentally determined bacterial growth rates. We related bacterial growth rates to thymidine incorporation rates and obtained a conversion factor very close to that obtained in another system (1). These factors show very good agreement with the range of conversion in factors calculated by Fuhrman and Azam (8).

A more complex model involving the thymidine incorporation rates of a sample as a function of incubation time was used by Kirchman et al. (13) to determine conversion factors. Factors calculated by this method showed considerable variability between the different systems studied but were the same order of magnitude as our factor for bay, marsh, and pond samples. Neretic marine samples covered a range of three orders of magnitude but were listed as not fitting the model.

The data from which we derived our conversion factor covered a broad range of bacterial growth rates determined at several depths in a lake water column and at several times during the periods of thermal stratification of 2 years. Because the data obtained from such a diverse sampling program show a linear relationship between rates of thymidine incorporation and independently measured bacterial growth rates and because the factors obtained by a similar method in a lake in Sweden (1) and through calculation (8) agree well with our own, we think it likely that a constant relationship between rates of thymidine incorporation and rates of bacterial growth exists, at least in epilimnetic and metalimnetic waters. This supposition requires further testing in other aquatic ecosystems to confirm its validity.

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