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# Primary and Bacterial Production in Two Dimictic Indiana Lakes

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**The relationship between primary and bacterial production in two dimictic Indiana lakes with different primary productivities was examined during the summer stratification period in 1982. Primary production rates were calculated from rates of $H^{14}CO_3^-$ incorporation by natural samples, and bacterial production was calculated from rates of [$^3$H-*methyl*]thymidine incorporation by natural samples. Both vertical and seasonal distributions of bacterial production in the more productive lake (Little Crooked Lake) were strongly influenced by primary production. A lag of about 2 weeks between a burst in primary production and the subsequent response in bacterial production was observed. The vertical distribution of bacterial production in the water column of the less productive lake (Crooked Lake) was determined by the vertical distribution of primary production, but no clear relationship between seasonal maxima of primary and bacterial production in this lake was observed. High rates of bacterial production in Crooked Lake during May indicate the importance of allochthonous carbon washed in by spring rains. Bacterial production accounted for 30.6 and 31.8% of total (primary plus bacterial) production in Crooked Lake and Little Crooked Lake, respectively, from April through October. High rates of bacterial production during late September and October were observed in both lakes. Calculation of the fraction of bacterial production supported by phytoplankton excretion implies an important role for other mechanisms of supplying carbon, such as phytoplankton autolysis. Several factors affecting the calculation of bacterial production from the thymidine incorporation rates in these lakes were examined.**

Recent advances in methodology have led to a reevaluation of the role of planktonic heterotrophic bacteria in aquatic ecosystems (1). Once believed to serve solely as mineralizers of organic compounds, these organisms are now widely thought to have a greater importance as producers of particulate organic carbon (1, 2, 5–7, 19). Results obtained by several methods (2–4, 7, 16, 23) have indicated that bacterial production can account for a quantity of particulate organic carbon equal to 25 to 50% of that produced through photosynthesis.

The quantification of bacterial production has been greatly facilitated by the thymidine incorporation method developed by Fuhrman and Azam (6). This method has been successfully used in water columns, both marine (6, 7, 13–15) and freshwater (2, 17), and in marine sediments (13, 14).

The measurement of bacterial production has been pursued predominantly in marine systems, whereas many interesting and important freshwater systems have yet to be studied further. Among these are the dimictic lakes of the northern latitudes. These bodies of water display a seasonal thermal stratification. Rates of primary production in the epilimnion are frequently high, providing an important carbon source for epilimnetic bacteria. Little or no primary production occurs in hypolimnetic waters because of insufficient light for photosynthesis. The metalimnion is typically about 10°C colder than the epilimnion and in some lakes is an important location for primary production. Here, we report the results of a study on the relationship of bacterial production to primary production in two dimictic lakes that have significant levels of metalimnetic primary production.

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## MATERIALS AND METHODS

This study was conducted on Crooked and Little Crooked Lakes (Noble County, Ind.) Temperature and oxygen measurements 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. 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 (18). The pH of water samples was determined with a pH meter (Digisense; Cole-Parmer, Chicago, Ill.) Direct counts showed the phytoplankton population to be dominated by cyanobacteria (C. R. Lovell and A. Konopka, Microb. Ecol., in press); consequently, chlorophyll *a* distribution was proportional to the distribution of cyanobacterial biomass.

Samples for bacterial production rate determinations were taken with an acid-washed Van Dorn bottle and transported to the Crooked Lake Biological Station in acid-washed polyethylene bottles in an ice chest. Experiments were initiated in the laboratory 10 min after sample collection. Subsamples (15 ml) were pipetted into each of three acid-washed 25-ml screw-cap glass tubes. One tube of each set received Formalin (1% final concentration), and each tube received [*methyl*-$^3$H]thymidine (Schwarz-Mann; specific activity, 60 to 71 Ci $mmol^{-1}$) to a final concentration of 2.5 nM. The tubes were then inverted for mixing and incubated in the dark at in situ temperatures or at 25°C for 20 min. In situ thymidine incorporation rates were calculated by correcting incorporation rates measured at 25°C with experimental data on the thymidine incorporation rate as a function of temperature for epilimnetic and metalimnetic samples. After incubation, Formalin was added, and the samples were filtered

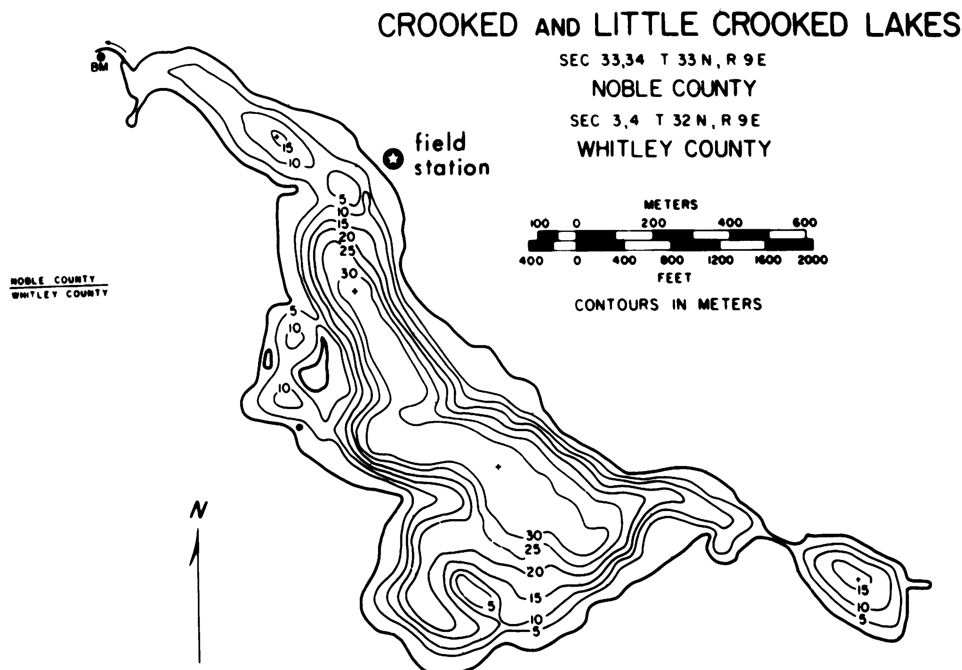


FIG. 1. Contour map of Crooked Lake and Little Crooked Lake.

onto 0.2-μm-pore-size polycarbonate filters (Nucleopore Corp., Pleasanton, Calif.). These filters were then placed in 5 ml of ice-cold 5% trichloroacetic acid (TCA) in 25-ml scintillation vials. The vials were incubated on ice for 30 min, the filters were replaced on the filtering manifold, and the TCA was poured through the filters. Two rinsings with 3 ml of ice-cold TCA followed, after which the filters were placed in plastic minivials and dissolved with a drop of phenethylamine (New England Nuclear Corp., Boston, Mass.). Three milliliters of ACS liquid scintillation fluid (Amersham, Arlington Heights, Ill.) was added, and radioactivity was determined with a Tracor Delta 300 liquid scintillation counter (Tracor Analytic, Austin, Tex.). We determined counting efficiencies using the channels ratio method and a quench curve constructed from a series of quenched standards. Corrections for isotope exchange with water were made by comparing counts obtained from the isotope solution with those obtained from isotopes from which all water has been removed. Our method of extracting the filters in scintillation vials by the direct addition of 5% TCA instead of by the addition of an equal volume of 10% TCA, as described in the procedure of Fuhrman and Azam (6), decreased the variability between replicate samples and reduced the filtration time required. Hydrolysis of DNA, carried out by heating filters at 100°C for 20 min after the addition of 0.5 ml of 0.5 N HCl, did not increase the counts obtained but rather prevented the complete dissolution of the filters with phenethylamine and thus reduced counting efficiency. DNA hydrolysis was therefore not included as a routine procedure.

Thymidine incorporation profiles of Little Crooked and Crooked Lakes were taken at biweekly to monthly intervals from April through November 1982. The lowest concentration of thymidine which supported maximum incorporation rates was determined by adding increasing quantities of isotope to the water samples and by processing as described above. We attempted to estimate isotope dilution by exogenous thymidine by supplementing samples with increasing concentrations of sterile, unlabeled thymidine and labeling and processing as described above (13).

Rates of thymidine incorporation into DNA were calculated by assuming that 20% of the cold TCA-insoluble label was not DNA. To test this assumption, samples from several depths were labeled as described above, and triplicate filters (with one Formalin-killed control) were (i) processed as described above to determine total TCA-insoluble label; (ii) placed in 5 ml of 0.5 N NaOH and heated at 60°C for 1 h, chilled, and acidified with 0.13 volumes of 6.12 M TCA for the determination of labeled DNA and protein; or (iii) placed in 5 ml of 5% TCA and steamed (100°C) for 1 h and then chilled for the determination of labeled protein. This is essentially the procedure used by Fuhrman and Azam (6) for the same purpose.

Bacterial production rates were calculated from thymidine incorporation rates by using the conversion factor $2.1 \times 10^{18}$ cells produced per mole of thymidine incorporated into DNA (17). A conversion factor of $1.20 \times 10^{-14}$ g C cell$^{-1}$ was used for the calculation of bacterial carbon production estimates (20).

Estimates of primary production by 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 different light intensities. In addition, vertical profiles of chlorophyll *a* concentration, termperature, and light extinction in the lakes were made. A numerical model was then used to calculate primary production (12). Data calculated with this model are plotted in 3-day intervals.

## RESULTS

The two lakes used in this study are physically connected by a shallow channel (Fig. 1). Thus, they have similar water chemistry but are sufficiently separated to differ in total productivity. Little Crooked Lake was about 2.6 times as productive on an areal basis as Crooked Lake during the

period of 1 April to 31 October 1982. Both lakes became thermally stratified by early May 1982. This stratification persisted throughout the summer, with fall overturn occurring in November. The oxygen concentration in the hypolimnion of Little Crooked Lake started to decrease in late May; by late June, anaerobiosis was established. Although some oxygen depletion was observed in the bottom 2 to 10 m of Crooked Lake from late June through the end of November, most of the hypolimnion remained oxygenated. The thermal stratification of these lakes resulted in the formation of physically, chemically and biologically distinct water layers (Fig. 2).

Chlorophyll *a* concentrations in Little Crooked Lake were highest in the metalimnion throughout the period of thermal stratification, whereas those of Crooked Lake were more evenly distributed (Fig. 3A and 4A). Filamentous cyanobacteria dominated the phytoplankton populations of both lakes throughout the period of thermal stratification. Photosynthetic sulfur bacteria were not found in either lake. The depth at which maximum rates of primary and bacterial production occurred, however, varied seasonally in both lakes (Fig. 3B and C and 4B and C). Although production maxima were found in the metalimnion of Crooked Lake in late May and late July through early August and in the Little Crooked metalimnion in late May, early July, and mid-August, both lakes had epilimnetic primary production maxima through most of the period of thermal stratification. Periods during which primary production was very high were often

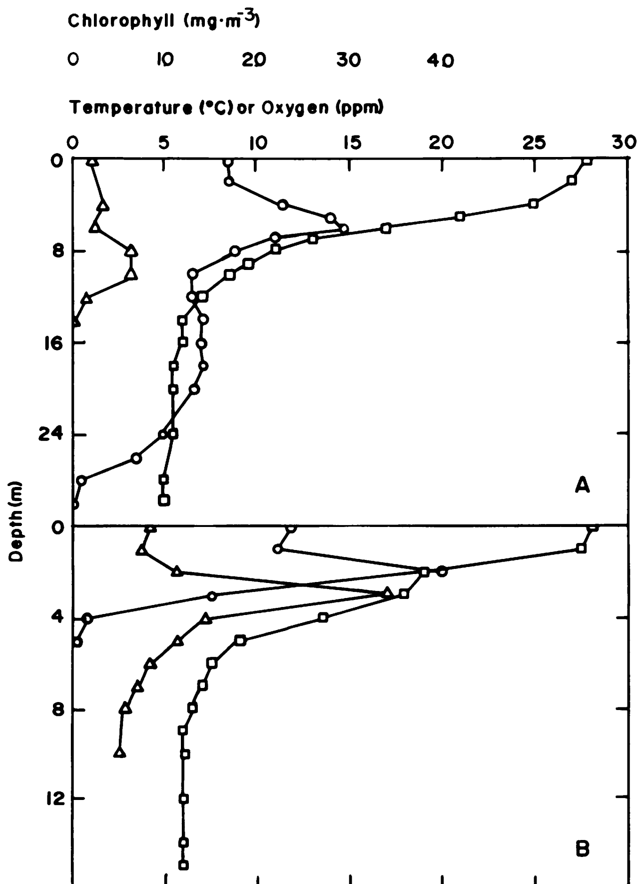


FIG. 2. Vertical profiles of temperature (□), oxygen concentration (○) and chlorophyll *a* concentration (△) from Crooked Lake (A) and Little Crooked Lake (B) on 19 July 1982.

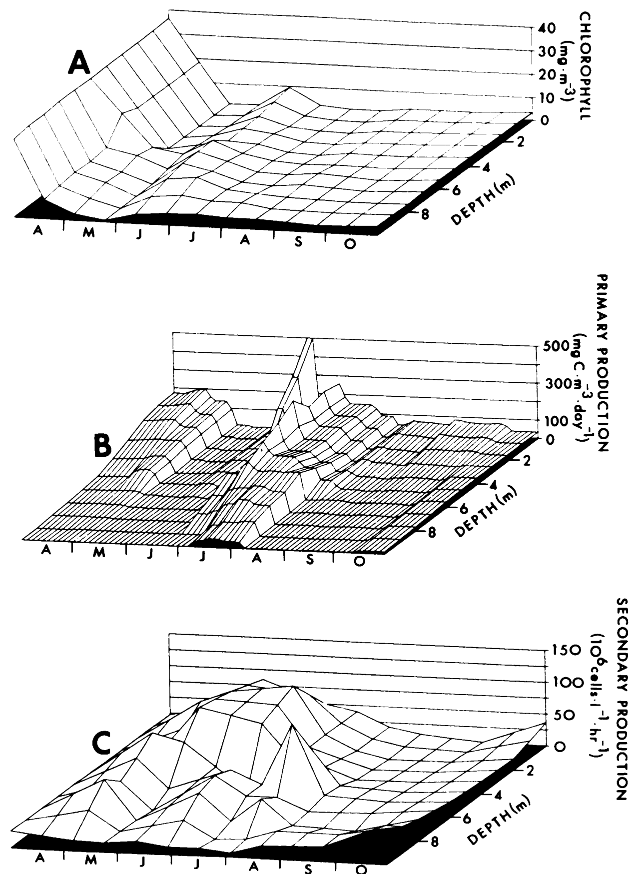


FIG. 3. Chlorophyll *a* concentration (A), primary production (B), and bacterial production (C) in Crooked Lake from 1 April to 31 October 1982 (letters on the x-axis indicate the month) from 0- to 10-m depths. The finer temporal scale in (B) is due to the use of 3-day intervals in plotting primary production values.

followed by increases in the rates of bacterial production at that depth (Fig. 5B and C and 6B and C). This trend was observed in both lakes. There was usually a lag of about 2 to 3 weeks between the establishment of a primary production maximum and the subsequent development of the bacterial production maximum. No strong relationship between depths of maximum bacterial production and chlorophyll *a* maxima was observed for either lake (Fig. 5A and 6A).

In Crooked Lake, primary production in the water column from April through October amounted to 124.7 g C $m^{-2}$. Bacterial production during this period was 54.9 g C $m^{-2}$, which is 30.6% of the total ( primary plus bacterial) production in this lake. Both primary and bacterial production varied in total carbon produced through the period studied (Table 1). Primary production was lowest in May and September, with a strong peak in July. The highest percentages are due largely to periods of low primary production values, because bacterial production rates were relatively constant. It is interesting that the periods during which bacterial production accounted for the majority of total particulate organic carbon production in this lake were May, at the onset of thermal stratification, and October, the onset of fall overturn. These periods of high bacterial contribution to total production may be due to allochthonous carbon washed in during spring and algal senescence (12b) or macrophyte decomposition in the fall (22).

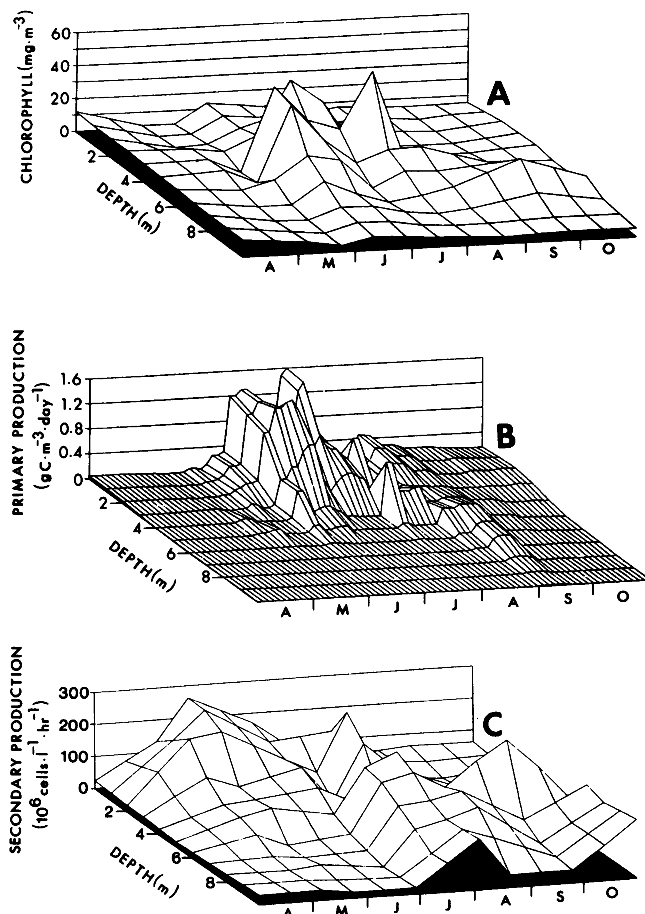


FIG. 4. Chlorophyll *a* concentration (A), primary production (B), and bacterial production (C) in Little Crooked Lake from 1 April to 31 October 1982 (letters on the x-axis indicate the month) from 0- to 10-m depths. The finer temporal scale in (B) is due to the use of 3-day intervals in plotting primary production values.

Both primary and bacterial production in Little Crooked Lake were about 2.5 times that of Crooked Lake. Bacterial production in Little Crooked Lake accounted for 31.8% of total production during the April through October period (Table 2). The low contribution of bacterial production in June and July was due to very high primary production during these months. Bacterial production increased steadily from May through August, with the highest values seen in August. Bacterial production in Little Crooked Lake was comparable to that in Crooked Lake during May and June and much greater than that in Crooked Lake during July, August, and September.

The seasonal trend of bacterial production rates in Crooked Lake seemed to bear no relationship to that of primary production rates. Bacterial production rates were highest in May and October, whereas primary production rates peaked in July. In Little Crooked Lake, the highest primary production rates were observed in June and July. Bacterial production rates increased steadily from April through August. A sharp decrease followed in September, and rates increased once again in October. It seems that high rates of bacterial production in July and August followed the high primary production rates in mid-July, whereas the October peak occurred at about the time of phytoplankton senescence.

We examined several factors that can affect bacterial production estimates made from thymidine incorporation data. These include uptake by photosynthetic microbes, isotope concentration, isotope dilution by endogenous thymidine, and the fraction of label incorporated into DNA. We observed no incorporation of thymidine by axenic cultures of *Merismopedia tenuissima* or *Synechococcus* sp., two unicellular cyanobacteria (data not shown). Although experiments with axenic cultures of filamentous cyanobacteria were not performed, filters which retained filamentous cyanobacteria but passed planktonic bacteria usually did not trap significant amounts of the [$^3$H]thymidine incorporated by natural populations (12b). The effects of the addition of increasing concentrations of [$^3$H]thymidine on the rates of incorporation into DNA were also examined. The rate of incorporation became saturated at 2.22 nM [$^3$H]thymidine. This pattern of saturation at relatively low isotope concentrations is similar to that found by Riemann et al. (17) for Fredriksborg Slotssø, Denmark, a Jutland lake with a productivity of about 500 g C m$^{-2}$ y$^{-1}$.

Our attempt to determine the approximate thymidine pool size in epilimnetic and metalimnetic samples from Little Crooked Lake by the isotope dilution method of Moriarty and Pollard (13) gave very different results from those obtained by them from sediment samples (Fig. 7). The reciprocal of the rate of thymidine incorporation plotted against the total added thymidine did not yield the predicted

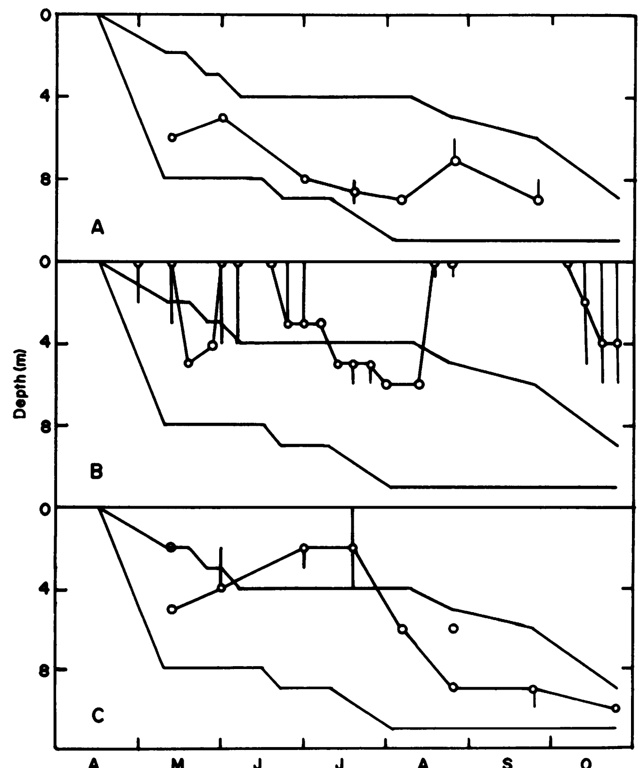


FIG. 5. Depths of maximum chlorophyll *a* concentration (A), maximum primary production (B), and maximum bacterial production (C) in Crooked Lake from 1 April to 31 October 1982 (letters on the x-axis indicate the month). The enclosed area represents the boundaries of the metalimnion. Bars represent the range of depths that have ≥90% of the maximum value. Dates with two points (i.e., 5C, 25 August) have two depths of high production (i.e., the unconnected point is ≥90% of the maximum value).

straight line. This procedure was therefore not used to correct for water column thymidine pool size.

The assumption that DNA accounts for 80% of cold TCA-insoluble material from labeled bacterial populations (6, 7) was tested, using water from several depths of Little Crooked Lake on 16 August 1983 (Table 3). The value of 80% was found to hold true for the epilimnion and metalimnion of Little Crooked Lake on this date. The distribution of label into macromolecules was clearly quite different for the hypolimnetic sample (8 m). This result is similar to that obtained by Hanson and Lowery (8) for marine bacterial populations at 500- to 2,000-m depths.

No adequate correction for thymidine incorporation into DNA in hypolimnetic populations can be made on the basis of this one experiment, so the 80% assumption was used in calculating hypolimnetic incorporation rates. The reduced amount of label in DNA from hypolimnetic samples does not greatly change our conclusions, because the hypolimnion is generally the zone of lowest productivity. It should, however, be borne in mind that actual hypolimnetic bacterial production rates, particularly late in the season in Little Crooked Lake, may be up to threefold lower than indicated by these data.

## DISCUSSION

Thermal stratification, with its associated effects on physicochemical parameters, is believed to exert a strong influence on the growth and activity of lake bacterial populations. Jones (9) has found that these effects were more important than the presence or absence of eucaryotic algae in determining the distribution of bacteria in the water column of Bletham Tarn, England, a shallow eutrophic lake. There was also such an effect in our system, but it was indirect. Primary production appears to be the most direct influence on bacterial production, but in Crooked and Little Crooked Lakes, metalimnetic primary production is often important. Because the epilimnia of Crooked and Little Crooked Lakes are fairly shallow (4-m depth for Crooked Lake 2-m, depth for Little Crooked Lake) and are poor in nutrients, adequate light penetration to the relatively nutrient-rich metalimnion occurs (11). As a consequence, high metalimnetic primary production rates are often seen.

Cyanobacterial biomass, as measured by chlorophyll concentration, was highest in the metalimnia of both lakes throughout the sampling season. Primary production rates in these lakes are most strongly influenced by ambient light intensity and cyanobacterial biomass (10). As a consequence, a moderate chlorophyll concentration in epilimnetic waters will frequently result in higher rates of photosynthesis than much higher chlorophyll levels in deeper waters, where light levels are lower. Thus, the depth of maximum primary production was often in the epilimnion, even though chlorophyll concentrations were highest in the metalimnion.

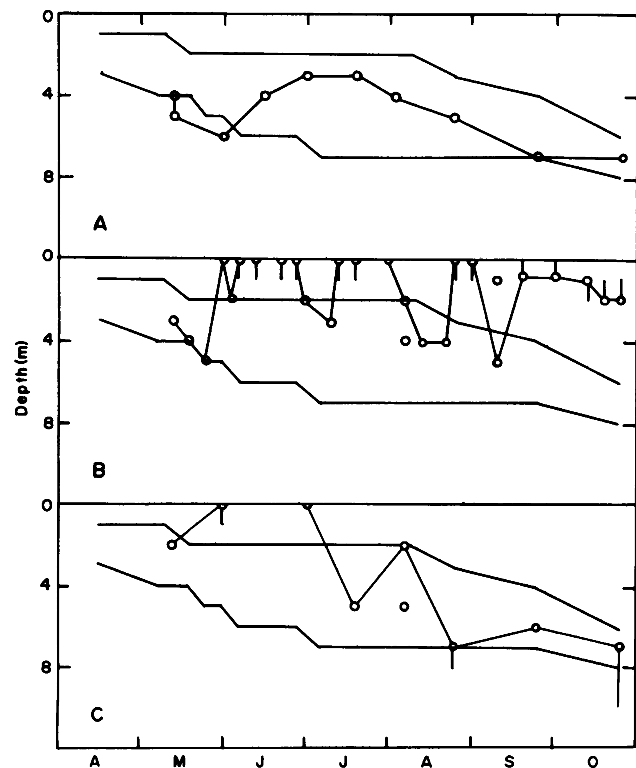


FIG. 6. Depths of maximum chlorophyll *a* concentration (A), maximum primary production (B), and maximum bacterial production (C) in Little Crooked Lake from 1 April to 31 October 1982 (letters on the x-axis indicate the month). The enclosed area represents the boundaries of the metalimnion. Bars represent the range of depths that have ≥90% of the maximum value. Dates with two points (i.e., 6C, 8 August) have two depths of high production (the unconnected point is ≥90% of the maximum value).

TABLE 1. Monthly primary and bacterial production in the Crooked Lake water column from April through November 1982[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 | 29.4 | 10.0 | 39.4 | 25.4 |
| May | 5.0 | 11.2 | 16.2 | 69.1 |
| June | 17.7 | 9.1 | 26.8 | 34.0 |
| July | 46.5 | 5.4 | 51.9 | 10.4 |
| August | 14.6 | 3.8 | 18.4 | 20.6 |
| September | 6.2 | 4.0 | 10.2 | 39.2 |
| October | 9.2 | 11.4 | 20.6 | 55.3 |
| November | | 6.5 | | |
| Total[e] | 124.7 | 54.9 | | |

[a] Calculated from data collected twice a month.
[b] To 10-m depth.
[c] Primary plus bacterial production.
[d] Bacterial production/total production × 100.
[e] April through October total (total mean percent, 30.6).

TABLE 2. Monthly primary and bacterial production in the Little Crooked Lake water column from April through November 1982[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 | 3.7 | 3.2 | 6.9 | 46.4 |
| May | 5.6 | 10.6 | 16.2 | 65.4 |
| June | 84.2 | 10.9 | 95.1 | 12.6 |
| July | 100.1 | 14.4 | 114.5 | 11.1 |
| August | 53.6 | 22.3 | 75.9 | 29.4 |
| September | 49.0 | 11.3 | 60.3 | 18.7 |
| October | 30.0 | 19.1 | 49.1 | 38.9 |
| November | | 10.0 | | |
| Total[e] | 323.8 | 91.8 | | |

[a] Calculated from data collected twice a month.
[b] To 10-m depth.
[c] Primary plus bacterial production.
[d] Bacterial production/total production × 100.
[e] April through October total (mean total percent, 31.8).

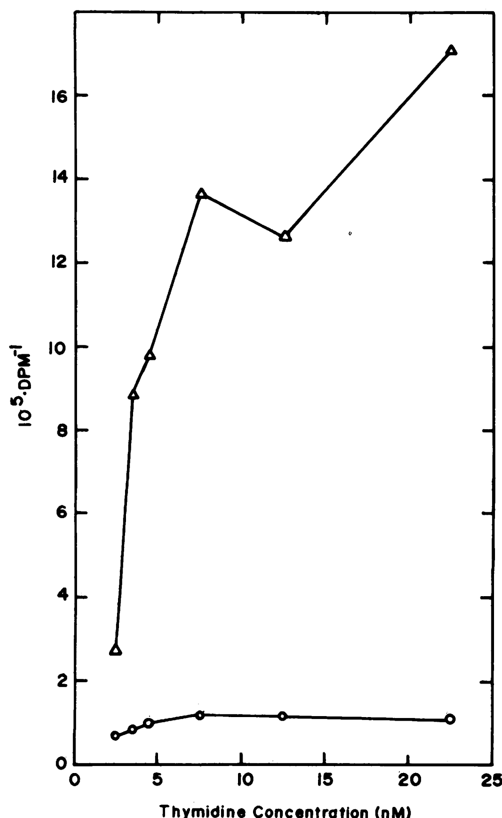


FIG. 7. Isotopic dilution plot of incorporation of [$^3$H-*methyl*]thymidine by epilimnetic (○) and metalimnetic (△) samples. Samples were taken from Little Crooked Lake on 25 May 1983. Each point represents the mean ± standard error for three replicate samples.

A stronger relationship was observed between rates of bacterial production and rates of primary production than between rates of bacterial production and the distribution of cyanobacterial biomass.

The development of a bacterial production peak in the water column seemed to follow a period of intense primary production at that depth. A lag of about 2 to 3 weeks between a burst of photosynthetic activity and an increase in bacterial production was observed in both lakes. We interpret this to be the time required for the naturally occurring bacteria at the depth of peak primary production to respond to the increase in carbon availability.

In an earlier study (Lovell and Konopka, in press), we estimated the rates of excretion of photosynthetically fixed organic carbon by the Little Crooked Lake phytoplankton. Excreted carbon which was assimilated by heterotrophic bacteria amounted to about 5% of total primary production. If we assume that 50% of the excreted carbon assimilated by bacteria is respired (7), the percentage of the carbon required to support bacterial production that comes from excretion can be calculated. In Crooked Lake excreted carbon supported 3.4% of bacterial production (range, 0.5 to 10.8%). In Little Crooked Lake, an average of 5.1% of bacterial production was supported by excreted carbon, ranging from a low of 0.7% in May to a maximum of 9.7% in June. Thus, other mechanisms, such as phytoplankton autolysis and decomposition, must supply most of the carbon for heterotrophic bacteria. If autolysis occurred rather slowly, and as cyanobacterial filaments were sinking, this might explain why increases in bacterial production rates lag behind bursts of primary production and generally occur deeper in the water column. First-order decay constants measured for the aerobic decomposition of organic matter in aquatic systems showed that the half-life of the quickly degraded fraction was 14 days (21), a time period which is similar to the lag time we observed between increases in primary and bacterial production.

Thus, rates of bacterial production were related to primary production with respect to both their vertical and seasonal distributions. A strong correlation between depths of high primary production rates and bacterial production maxima was also observed in the Southern California Bight (7). In our system, bacterial production maxima were near or below the depth of maximum primary production and phytoplankton abundance. On a seasonal basis, bacterial production was relatively high compared with primary production in May and October. The results in May were possibly due to allochthonous carbon washed in by spring rains. The elevated rates of bacterial production seen in October may be a response to algal senescence (12b) or to carbon released by decaying macrophytes (22). Senescence of both planktonic algae and macrophytes was presumably due to lower light levels in the fall.

To calculate rates of bacterial production from thymidine incorporation rates, several assumptions are generally made (6). These include the following: (i) only bacteria incorporate thymidine added at nanomolar concentrations, (ii) all actively growing bacteria are capable of incorporating exogenous thymidine during growth, (iii) a constant percentage (usually taken to be 80%) of cold TCA-insoluble label is in DNA, (iv) the specific activity of added thymidine is unaffected by endogenous thymidine, (v) the thymidylic acid residues constitute a constant portion (about 25 mol%) of sample DNA, and (vi) the average DNA content per cell is about $2.78 \times 10^{-15}$ g (average of reported values).

Most of these assumptions have been tested by Fuhrman and Azam (6, 7) in marine waters and have been found to be reasonably valid in the systems tested. Size fractionation studies (6, 12b), as well as autoradiography (7), have shown no significant thymidine incorporation by organisms other than nonphotosynthetic bacteria. A comparable percentage of bacterial cells capable of assimilating glucose or amino acids also took up thymidine (7). Approximately 80% of epilimnetic and metalimnetic label in surface marine (7) and in our epilimnetic and metalimnetic samples was in DNA. The results for marine samples at 500- to 2,000-m depths (8) and our hypolimnetic samples indicate a much greater incorporation of label into protein or RNA. This assumption should be tested in any system in which thymidine incorporation rates are measured for the purpose of estimating bacterial production.

The remaining assumptions are much more difficult to test in natural waters. It is effectively impossible to achieve complete separation of bacteria from detritus and algal cells.

TABLE 3. Distribution of label in macromolecules of [$^3$H]thymidine-labeled bacterial populations of Little Crooked Lake, 16 August 1983

| Depth (m) | % of total label in the following: | | |
|---|---|---|---|
| | DNA | RNA | Protein |
| 0 | 79 | 12 | 9 |
| 2 | 80 | 7 | 13 |
| 6 | 81 | 11 | 8 |
| 8 | 28 | 50 | 22 |

Consequently, the thymidine content of bacterial DNA and the quantity of DNA per bacterial cell are difficult to assess. Owing to the difficulty in separating algae from bacteria, the specific activity of deoxyribosylthymine triphosphate in natural bacteria cannot be adequately assessed. A kinetic procedure devised by Moriarty and Pollard (13) for determining isotope dilution in sediment systems did not yield any useful information in our experiments. This procedure was also used unsuccessfully in Lake Norrviken (Sweden) (17). It is possible that the low bacterial biomass in our water column samples resulted in isotope dilutions below the level of sensitivity of this method. Our use of saturating levels of [$^3$H]thymidine (2.22 nM) may also have some bearing on our results.

Although the importance of these last three assumptions is as yet inadequately tested, it is noteworthy that the calculated conversion factor of $2.1 \times 10^{18}$ cells produced per mole of thymidine incorporated by Fuhrman and Azam shows good agreement with production estimates obtained from bacterial growth in diluted samples (2, 7, 12b). This relationship between the calculated conversion factor and empirically derived production estimates implies that the assumptions given above are valid in several aquatic systems.

The concentration of isotope required to saturate the rate of incorporation seems to vary with the trophic state of the water body studied. Riemann et al. (17) found the required thymidine concentration to vary among several freshwater lakes with differing trophic states. Results found by Bell et al. (2) for eutrophic Lake Norrviken (Sweden) fall within the range found by Riemann et al., as do our data from Crooked and Little Crooked Lakes. It would be interesting to establish this relationship more precisely because it could offer valuable information on the importance of isotope dilution in these systems.

The results of this study indicate that bacterial production is related to primary production, with respect to both vertical and seasonal distribution in the water columns of Crooked and Little Crooked Lakes. The vertical distribution of bacterial production and its response to primary production in a freshwater system have not been examined in any detail in other studies. The indication that a lag may exist between a pulse of primary production and the stimulation of bacterial production suggests that mechanisms of slow carbon release, for example, algal autolysis, may be important in these lakes.

## ACKNOWLEDGMENTS

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## LITERATURE CITED

1. **Azam, F., T. Fenchel, J. G. Fields, J. F. Fenchel, J. S. Gray, L. A. Meyer-Reil, and F. Thingstad.** 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. **10**:257–263.
2. **Bell, R. T., G. M. Ahlgren, and I. Ahlgren.** 1983. Estimating bacterioplankton production by measuring [$^3$H]thymidine incorporation in a eutrophic Swedish lake. Appl. Environ. Microbiol. **45**:1709–1721.
3. **Cole, G. A.** 1979. Textbook of limnology, 2nd ed. Mosby, St. Louis, Mo.
4. **Derenbach, J. B., and P. J. LeB. Williams.** 1974. Autotrophic and bacterial production: fractionation of plankton populations by differential filtration of samples from the English Channel. Mar. Biol. **25**:263–269.
5. **Ducklow, H. W., and D. L. Kirchman.** 1983. Bacterial dynamics and distribution during a spring diatom bloom in the Hudson River plume, USA. J. Plankton Res. **5**:333–355.
6. **Fuhrman, J. A., and F. Azam.** 1980. Bacterioplankton bacterial production estimates for coastal waters of British Columbia, Antarctica, and California. Appl. Environ. Microbiol. **39**: 1085–1095.
7. **Fuhrman, J. A., and F. Azam.** 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Mar. Biol. **66**:109–120.
8. **Hanson, R. B., and H. K. Lowery.** 1983. Nucleic acid synthesis in oceanic microplankton from the Drake Passage, Antarctica: evaluation of steady-state growth. Mar. Biol. **73**:79–89.
9. **Jones, J. G.** 1977. The effect of environmental factors on estimated viable and total populations of bacteria in lakes and experimental enclosures. Freshwater Biol. **7**:67–91.
10. **Konopka, A.** 1981. Influence of temperature, oxygen, and pH on a metalimnetic population of *Oscillatoria rubescens*. Appl. Environ. Microbiol. **42**:102–108.
11. **Konopka, A.** 1982. Buoyancy regulation and vertical migration by *Oscillatoria rubescens* in Crooked Lake, Indiana. Br. Phycol. J. **17**:427–442.
12. **Konopka, A.** 1983. Epilimnetic and metalimnetic primary production in an Indiana hardwater lake. Can. J. Fish. Aquat. Sci. **40**:792–798.

12a. **Lovell, C. R., and A. Konopka.** 1985. Seasonal bacterial production in a dimictic lake as measured by increases in cell numbers and thymidine incorporation. Appl. Environ. Microbiol. **49**:492–500.

12b. **Lovell, C. R., and A. Konopka.** 1985. Thymidine incorporation by free-living and particle-bound bacteria in a eutrophic dimictic lake. Appl. Environ. Microbiol. **49**:501–504.

13. **Moriarty, D. J. W., and P. C. Pollard.** 1981. DNA synthesis as a measure of bacterial productivity in seagrass sediments. Mar. Ecol. Prog. Ser. **5**:151–156.
14. **Moriarty, D. J. W., and P. C. Pollard.** 1982. Diel variation of bacterial productivity in seagrass (*Zostera capricorni*) beds measured by rate of thymidine incorporation into DNA. Mar. Biol. **72**:165–173.
15. **Newell, S. Y., and R. D. Fallon.** 1982. Bacterial productivity in the water column and sediments of the Georgia (USA) coastal zone: estimates via direct counting and parallel measurement of thymidine incorporation. Microb. Ecol. **8**:33–46.
16. **Pedrós-Alió, C., and T. D. Brock.** 1982. Assessing biomass and production of bacteria in eutrophic Lake Mendota, Wisconsin. Appl. Environ. Microbiol. **44**:203–218.
17. **Riemann, B., J. Fuhrman, and F. Azam.** 1982. Bacterial secondary production in freshwater measured by $^3$H-thymidine incorporation method. Microb. Ecol. **8**:101–114.
18. **Shoaf, W. T., and B. W. Lium.** 1976. Improved extraction of chlorophyll a and b from algae using dimethylsulfoxide. Limnol. Oceanogr. **21**:926–928.
19. **Sieburth, J. M.** 1976. Bacterial substrates and productivity in marine ecosystems. Annu. Rev. Ecol. Syst. **7**:259–285.
20. **Watson, S. W., T. J. Novitsky, H. L. Quinby, and F. W. Valois.** 1972. Determination of bacterial number and biomass in the marine environment. Appl. Environ. Microbiol. **33**:970–976.
21. **Westrich, J. T., and R. A. Berner.** 1984. The role of sedimentary organic matter in bacterial sulfate reduction: The G model tested. Limnol. Oceanogr. **29**:216–224.
22. **Wetzel, R. G.** 1975. Limnology. The W. B. Saunders Co., Philadelphia, Pa.
23. **Wolter, K.** 1982. Bacterial incorporation of organic substances released by natural phytoplankton populations. Mar. Ecol. Prog. Ser. **7**:287–295.