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Depth Distribution of Bacterial Production in a Stratified Lake with an Anoxic Hypolimnion†

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The purpose of this study was to determine the depth distribution of bacterial biomass and production in a stratified lake and to test techniques to measure bacterial production in anaerobic waters. Bacterial abundance and incorporation of both [^{3}H]thymidine and [^{3}H]leucine into protein were highest in the metalimnion, at the depth at which oxygen first became unmeasurable. In contrast, [^{3}H]thymidine incorporation into DNA was highest in the epilimnion. The ratios of incorporation into DNA/protein averaged 2.2, 0.49, and 0.95 for the epilimnion, metalimnion, and hypolimnion, respectively. Low incorporation into DNA was not due to artifacts associated with the DNA isolation procedure. Recovery of added [^{3}H]DNA was about 90% in waters in which the portion of [^{3}H]thymidine incorporation into DNA was about 40%. At least some obligate anaerobic bacteria were capable of assimilating thymidine since aeration of anaerobic hypolimnion waters substantially inhibited thymidine incorporation. The depth profile of bacterial production estimated from total thymidine and leucine incorporation and the frequency of dividing cells were all similar, with maximal rates in the metalimnion. However, estimates of bacterial production based on frequency of dividing cells and leucine incorporation were usually significantly higher than estimates based on thymidine incorporation (using conversion factors from the literature), especially in anaerobic hypolimnion waters. These data indicate that the thymidine approach must be examined carefully if it is to be applied to aquatic systems with low oxygen concentrations. Our results also indicate that the interface between the aerobic epilimnion and anaerobic hypolimnion is the site of intense bacterial mineralization and biomass production which deserves further study.

The thymidine approach has been instrumental in demonstrating the importance of bacterioplankton in the trophic dynamics of aquatic systems (8). Several studies have used thymidine incorporation to estimate bacterial production and have found that bacterial production is often high (10 to 50%) compared with primary production in marine systems (see reference 4 for review) and freshwater lakes (21, 29, 36). Other measures of bacterial activity support the conclusions reached by the thymidine approach and also indicate that a large fraction of primary production is consumed by bacteria (19, 39).

Although the thymidine approach has been adopted as the standard method for measuring bacterial production (28), several potential problems have been identified. One problem is that not all bacteria may be able to assimilate thymidine. Microautoradiographic studies have shown that the number of bacteria assimilating amino acids can be substantially higher than the number assimilating thymidine (3, 38), although active but nondividing bacteria could explain these data (9). Dividing bacteria that are not capable of assimilating thymidine would lead to underestimates of the actual rate of bacterial production. Another problem is incorporation of [^{3}H]thymidine radioactivity into macromolecules other than DNA. Only [^{3}H]thymidine incorportion into DNA is the appropriate basis for calculating bacterial production (9), and nonspecific incorporation may necessitate isolation of DNA for each thymidine incorporation measurement. Finally, questions have been raised concerning the quantitative relationship between the rate of thymidine incorporation and bacterial production, due to the problem of isotope dilution (15).

We were interested in examining the depth distribution of bacterial biomass and production in a stratified lake (Lake Oglethorpe). Work on other lakes has shown that particulate matter, phytoplankton biomass, and primary production are sometimes maximal in metalimnion waters (18, 21, 22, 30, 31). The depth distribution of bacterial abundance and production in stratified lakes with anaerobic hypolimnions has not been examined thoroughly. Lovell and Konopka (21) found that thymidine incorporation rates were often highest in the metalimnion. In one experiment they also observed low incorporation of [^{3}H]thymidine into DNA in anaerobic hypolimnion waters, suggesting potential problems with the application of the thymidine approach in these waters. We examined this problem in further detail for Lake Oglethorpe and also compared thymidine incorporation rates with two other approaches for measuring bacterial production, the frequency of dividing cells (FDC) (10) and leucine incorporation (16). We found that all three measures of bacterial production indicated that rates are maximal in metalimnion waters, but that the thymidine approach must be examined carefully if it is to be used in anaerobic waters.

MATERIALS AND METHODS

Sample collection. Lake Oglethorpe is located 24 km from Athens, Ga., and has been studied extensively (14, 25–27). We collected samples where the lake was deepest (Z_{max} = 8 m). A peristaltic pump with a Nalgene plastic tube was used to collect water from each depth sampled. The borosilicate incubation tubes (13 ± 0.2 ml) were flushed with sample

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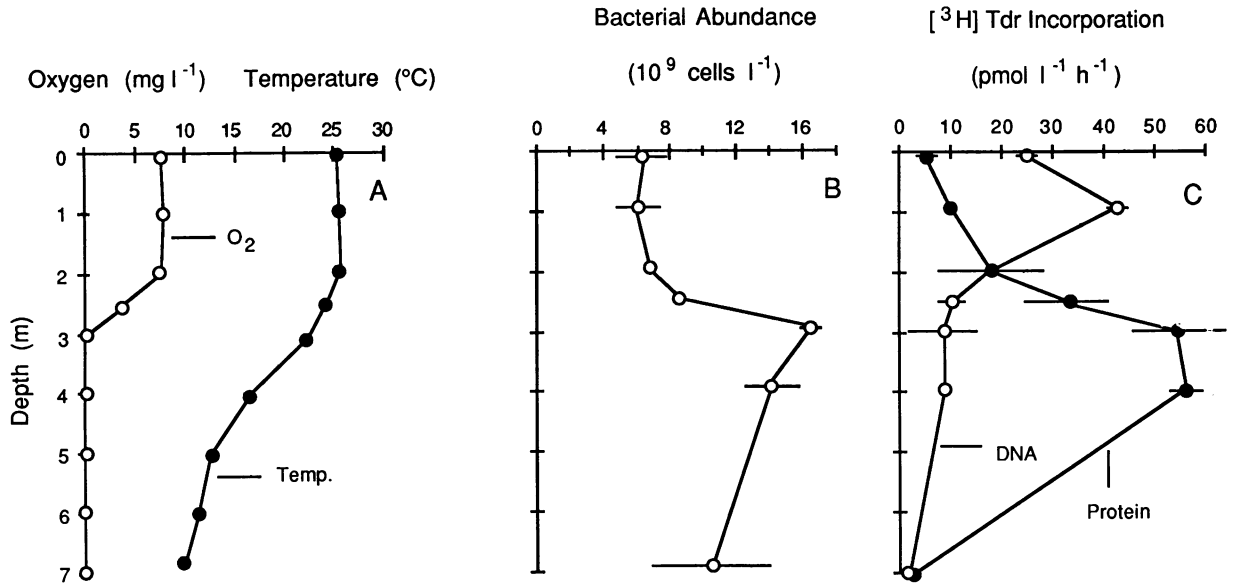

FIG. 1. Depth profiles of oxygen and temperature (A), bacterial abundance (B), and [^{3}H]thymidine (Tdr) incorporation into DNA and protein (C), July 1984.

water, filled, and then capped with a rubber stopper into which a syringe needle was inserted. The needle allowed excess water to be displaced from the incubation tube during capping so that no headspace was present. The tubes were filled without introducing air into the water sample, and no oxygen was detected in hypolimnion water when samples were collected by this method. All isotopes and inhibitors (see below) were injected into the incubation tubes through the syringe needle, which was then removed. Depth profile incubations were conducted in situ at the depth from which the sample was taken. Temperature and oxygen concentrations were measured with a YSI model 57 oxygen meter and 5739 probe (Yellow Springs Instrument Co.).

Bacterial abundance was measured by the direct-count method (12, 33) from samples preserved in 4% Formalin and stored at 2°C (<2 weeks). The FDC was measured by the method of Hagström et al. (10). Three subsamples were examined per depth (17) and 250 bacteria per sample were examined to determine the FDC. The FDC values were converted to bacterial growth rates by using the equation provided by Christian et al. (2), which was determined from experiments in a salt marsh estuary. Growth rates were multiplied by the bacterial abundance to obtain rates of bacterial cell production.

Incorporation of [^{3}H]thymidine. The incorporation of [*methyl*-^{3}H]thymidine (50 to 90 Ci/mmol; Amersham Corp.) into DNA, RNA, and protein was measured during <1-h incubations. Time course experiments with samples from both the epilimnion and hypolimnion showed that incorporation of radioactivity into each macromolecule was linear during 1-h incubations. For routine depth profiles, we used 5 nM [^{3}H]thymidine. Bacterial production was calculated by multiplying [^{3}H]thymidine incorporation into both DNA and protein by 2.0×10^{18} cells per mol of thymidine incorporated (9). This conversion factor was derived from experiments in southern California coastal waters (9).

The incorporation of [^{3}H]thymidine into RNA, DNA, and protein was determined by acid and base hydrolysis (9, 23). All isotope incubations were stopped by chilling samples in ice. They were then poured into scintillation vials containing 1.45 ml of 50% trichloroacetic acid (TCA) or 5 N NaOH (final concentrations, 5% TCA and 0.5 N NaOH). Abiotic adsorption was accounted for with killed controls. Filters (Gelman GA-8, 25 mm, 0.22-μm pore size) and macromolecules were solubilized in 1 ml of Protosol (New England Nuclear Corp.) at 45 to 50°C overnight. Samples were then neutralized with acetic acid and radioassayed in 10 ml of Scintiverse 1 (Fisher Scientific Co.).

The effect of isotope dilution on the incorporation rate of [^{3}H]thymidine was examined by the approach of Moriarty and Pollard (24). The incorporation of [^{3}H]thymidine (2 nM) in the presence of different concentrations of nonradioactive thymidine (2, 5, 10, and 20 nM) was tested with duplicates according to the sampling procedure described above.

The effects of metabolic inhibitors on thymidine incorporation were examined by measuring the incorporation of thymidine into DNA and protein over time in the presence of cycloheximide and chloramphenicol (final concentration, 0.1 mg/ml). Subsamples were taken at time zero, and at 1, 3, and 5 h, and rates were estimated by calculating the slopes of incorporated radioactivity versus time with standard linear regression techniques.

Efficiency of DNA extraction and recovery. We considered the possibility that the low recovery of ^{3}H radioactivity in the DNA fraction during [^{3}H]thymidine incorporation experiments (see Results) was because the acid-base extraction procedure (9, 23) was not effective with water samples from Lake Oglethorpe. To test this possibility, we added [^{3}H]DNA isolated from *Escherichia coli* (0.02 μCi of New England Nuclear NET-561) to samples (5 ml) from various depths. Concurrently, incorporation of [^{3}H]thymidine was measured. Total DNA from both sets of incubations was extracted by the acid-base procedure outlined above.

Incorporation of [^{3}H]leucine. Bacterial production was estimated from rates of [^{3}H]leucine incorporation into protein (16). Water samples were collected as described for the [^{3}H]thymidine measurements and a 10 nM concentration of [^{3}H]leucine was added. After 30 min uptake was stopped by

adding the sample to 1.45 ml of 50% TCA. The samples were then heated to 85°C for 30 min and filtered after cooling. The filters (Gelman GA-8, 25 mm, 0.22-μm pore size) were rinsed twice with cold 5% TCA and treated with Protosol as described above. Abiotic adsorption of radioactivity was corrected for by killed controls. Possible isotope dilution was examined as described above for thymidine. Extracellular dissolved leucine was measured by high-performance liquid chromatography (20).

Rates of leucine incorporation were converted to rates of bacterial production with the conversion factor 5.4×10^{17} cells per mol (16a). This conversion factor is an average of several experiments, using the Kirchman et al. (15) approach with samples from a salt marsh estuary. The factor is about fivefold higher than the theoretical factor which assumes no isotope dilution (16a).

RESULTS

Lake Oglethorpe is monomictic, with a winter mixing period from November to March followed by development of stratification in spring. Primary production is high, ranging from 100 to 200 g of C m^{-2} $year^{-1}$ (26). Previous studies had demonstrated that during stratification concentrations of particulate matter and chlorophyll are high at the metalimnion (Porter et al., manuscript in preparation). One purpose of our study was to examine whether bacterial abundance and activity are similarly elevated.

Depth profile of bacterial abundance and thymidine incorporation. During stratification bacterial abundance was highest in the metalimnion, the maximum occurring at the depth at which dissolved oxygen dropped to unmeasurable levels (Fig. 1). In July 1984, bacterial abundance was 6×10^9 cells per liter at 3.0 m (Fig. 1). Similar depth profiles were observed in April and August (data not shown). Bacterial abundances in the epilimnion and hypolimnion were similar (Fig. 1).

The maximum rate of [^{3}H]thymidine incorporation into protein coincided with the peak in bacterial abundance. In July 1984, the incorporation rate into protein was about sixfold higher at 3.0 m than in surface waters (Fig. 1). A similar difference was observed in April and August (data not shown). In contrast, [^{3}H]thymidine incorporation into DNA was highest in surface waters and decreased with depth, with low (July; Fig. 1) to unmeasurable rates (August) in the anaerobic hypolimnion. For example, the rate was about 50-fold higher at 1 m than at 7 m in July (Fig. 1). Because incorporation into DNA was highest in the epilimnion whereas incorporation into protein was highest in the metalimnion, total [^{3}H]thymidine incorporation into macromolecules often exhibited two peaks, one in the epilimnion and another in the metalimnion at the depth where oxygen was first unmeasurable (see below).

TABLE 1. [^{3}H]thymidine incorporation into DNA and protein in Lake Oglethorpe[a]

Date	DNA/protein[b]		
	Epilimnion	Metalimnion	Hypolimnion[c]
April	2.3	1.3	0.33
July	2.3	0.22	1.0
August	1.9	0.14	1.0
September	1.9	0.28	1.5
November	2.8	0.49	0.92

[a] All data are from 1984, except for the April experiment in 1985.
[b] Ratio of radioactivity recovered in DNA to that recovered in protein.
[c] Values for epilimnion and hypolimnion are from the middle of each layer. The metalimnion value is the mean of samples from two depths: depth with the maximum thymidine incorporation and the sample depth above the maximum.

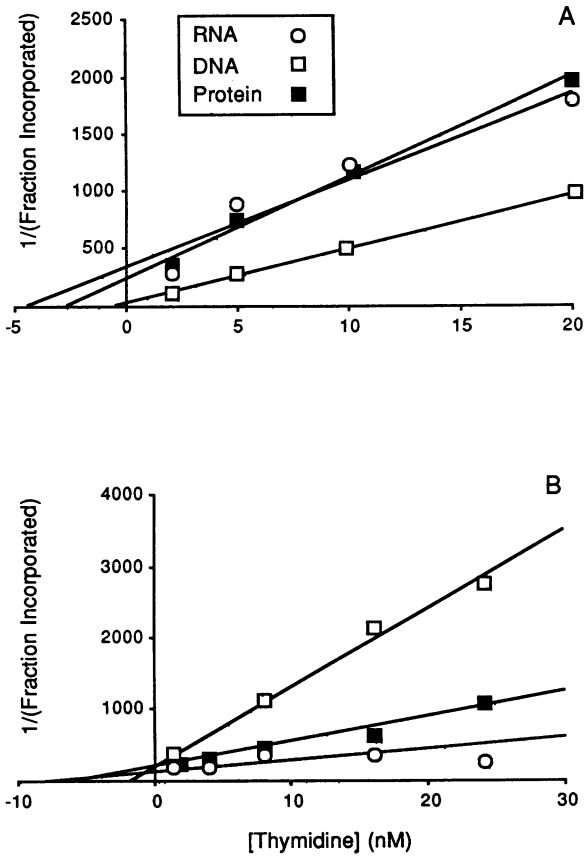

FIG. 2. Incorporation of [^{3}H]thymidine into DNA, RNA, and protein for the epilimnion (A), November 1984, and hypolimnion (B), October 1984.

In the aerobic epilimnion, [^{3}H]thymidine was incorporated mostly into DNA. The ratio of incorporation into DNA versus protein was 2 (Table 1), and approximately 30% of [^{3}H]thymidine incorporation was recovered in the protein fraction (Table 1; Fig. 2). The proportional incorporation into DNA was lowest in the metalimnion where oxygen concentration was low but measurable; the DNA/protein ratio was <0.5, except for one experiment (Table 1). Incorporations into DNA and protein were roughly equal in the anaerobic hypolimnion (Table 1). Incorporation of [^{3}H]thymidine into RNA was 23% in the epilimnion and 46% in the hypolimnion (Fig. 2). These percentages did not vary with incubation time (data not shown) or with concentration (Fig. 2).

The isotope dilution approach (24) was used to determine the apparent pool size of unlabeled thymidine which isotopically diluted the added [^{3}H]thymidine. The apparent pool sizes were 1 and 2 nM in the epilimnion and hypolimnion in July (Fig. 2). Since the apparent pool size (according to the isotope dilution approach) was small compared with the difference between depths in [^{3}H]thymidine incorporation, the data suggest that the incorporation into protein observed in the epilimnion and hypolimnion was not low relative to the metalimnion because of isotope dilution.

We next considered the possibility that incorporation rates of [^{3}H]thymidine into DNA were low in metalimnion and

TABLE 2. Comparison of recovery of added [^{3}H]DNA and incorporation of [^{3}H]thymidine in Lake Oglethorpe

Depth (m)	DNA/protein[a]	
	Recovery of [^{3}H]DNA	[^{3}H]thymidine incorporation
Distilled water[b]	15.7	ND[c]
0	ND	2.3
1	13.3	2.4
2	ND	4.0
3	11.5	0.8
4[d]	ND	0.3
5[d]	11.5	0.9

[a] Ratio of radioactivity recovered in DNA to that recovered in protein.
[b] [^{3}H]DNA was added to distilled water and recovery was measured.
[c] ND, Not determined.
[d] This depth was anaerobic.

hypolimnion samples because of artifacts associated with the DNA extraction procedure. To test this possibility, [^{3}H]DNA was added to different water samples and the macromolecule extraction procedure was followed. Greater than 90% of the added [^{3}H]DNA was recovered in the DNA fraction, regardless of the water sample (Table 2). In contrast, at the same depths the amount of label recovered as DNA from [^{3}H]thymidine incubations was high (70%) in surface waters and low (<50%) in anaerobic waters (Table 2), as we had typically observed (Table 1). These data indicate that the low incorporation of [^{3}H]thymidine into DNA in metalimnion and hypolimnion samples is not an artifact associated with the DNA extraction procedure.

Effect of inhibitors on thymidine incorporation. The high incorporation of [^{3}H]thymidine into protein and RNA was unexpected because previous studies had shown that most (>80%) of the assimilated [^{3}H]thymidine is incorporated into DNA (9). The possible uptake of thymidine by eucaryotes was examined by measuring the incorporation of [^{3}H]thymidine in the presence of cycloheximide. Cycloheximide inhibited incorporation of [^{3}H]thymidine radioactivity into protein more so than incorporation into DNA in samples from the epilimnion (Table 3). Thymidine incorporation into DNA decreased by an average of 33% in the epilimnion, 33% in the metalimnion, and 22% in the hypolimnion (Table 3). In contrast, cycloheximide inhibited incorporation of thymidine into protein by 55% in the epilimnion, 51% in the metalimnion, and 15% in the hypolimnion.

The lack of an inhibitory effect by cycloheximide in the hypolimnion suggested that [^{3}H]thymidine incorporation into protein was due solely to procaryotic activity. To test this possibility further, we examined the effect of chloramphenicol on [^{3}H]thymidine incorporation into DNA and protein in samples from the hypolimnion. Chloramphenicol inhibited thymidine incorporation into DNA by 40% and incorporation into protein by 55% (Table 3). The lack of complete inhibition by either inhibitor indicates that they were not totally effective in anaerobic waters (see also reference 35).

To examine whether or not the thymidine incorporation we observed in anaerobic samples was by strict anaerobic bacteria, we aerated anaerobic samples and compared the rate of thymidine incorporation into DNA and protein with unaltered control samples. Aeration, which increased oxygen concentrations from unmeasurable levels to 7 mg/liter, inhibited thymidine incorporation into DNA by 66% and into protein by 42% (Table 3). These results suggest that some anaerobic bacteria in the hypolimnion of Lake Oglethorpe can assimilate thymidine.

Depth profile of [^{3}H]leucine incorporation. Depth profiles of [^{3}H]leucine incorporation into protein (Fig. 3) were similar to those of incorporation of [^{3}H]thymidine radioactivity into protein (Fig. 1). [^{3}H]leucine incorporation was three- to ninefold higher in the metalimnion compared with the epilimnion or hypolimnion (Fig. 3). In addition to the results presented in Fig. 3, similar depth profiles of [^{3}H]leucine incorporation were observed on three other dates when Lake Oglethorpe was stratified. In August, concentrations of dissolved free leucine were 4 nM in the epilimnion, 6 nM in the metalimnion, and 1 nM in the hypolimnion (L. Proctor, unpublished data). These data indicate that the relatively low rates of leucine incorporation in the epilimnion and hypolimnion were not due to isotope dilution by extracellular leucine.

When the lake became destratified, there was no difference in bacterial abundance and [^{3}H]thymidine incorporation with depth. In addition, at all depths [^{3}H]thymidine was mostly incorporated into DNA (DNA/protein = 4.0) (Fig. 4).

Comparison of different measures of bacterial production. Bacterial production was estimated from the FDC and thymidine incorporation into both protein and DNA. These measures indicated that bacterial production was highest in the metalimnion (Fig. 5). The depth profile of actual FDC values was similar to that of bacterial abundance. Both FDC and thymidine incorporation rates per cell were highest in the metalimnion (data not shown). The FDC and the thymidine-based measure gave roughly similar estimates of bacterial production in the epilimnion, but in the metalimnion and hypolimnion the FDC-based estimates were two- to threefold higher than the thymidine-based estimates (Fig. 5). Bacterial production was roughly 10^8 cells liter^{-1} h^{-1} in the epilimnion and hypolimnion, based on both techniques; in the metalimnion, bacterial production was 2.5×10^8 cells liter^{-1} h^{-1} according to the thymidine approach and 7.0×10^8 cells liter^{-1} h^{-1} according to the FDC method (Fig. 5). The depth profiles of both measures of bacterial production were similar in other months when the lake was stratified (Fig. 1 and 6; data from May not shown for FDC).

In July estimates of bacterial production based on thymidine incorporation into protein and DNA were compared

TABLE 3. Inhibition of [^{3}H]thymidine incorporation into DNA and protein by cycloheximide (0.1 mg/ml), chloramphenicol (0.1 mg/ml), and aeration of anaerobic water

Date	Inhibitor	% Inhibition[a]	
		DNA	Protein
Epilimnion			
13 Sept.	Cycloheximide	26	55
27 Sept.	Cycloheximide	40	55
Metalimnion			
13 Sept.	Cycloheximide	33	51
Hypolimnion			
13 Sept.	Cycloheximide	22	18
27 Sept.	Cycloheximide	22	13
19 Sept.	Chloramphenicol	40	55
27 Sept.	Aeration[b]	66	42

[a] Inhibition was estimated by comparing incorporation of [^{3}H]thymidine with and without the addition of an inhibitor in subsamples taken at 0, 1, 3, and 5 h.
[b] Aeration of hypolimnion water increased O_2 concentration from unmeasurable to 7 mg/liter.

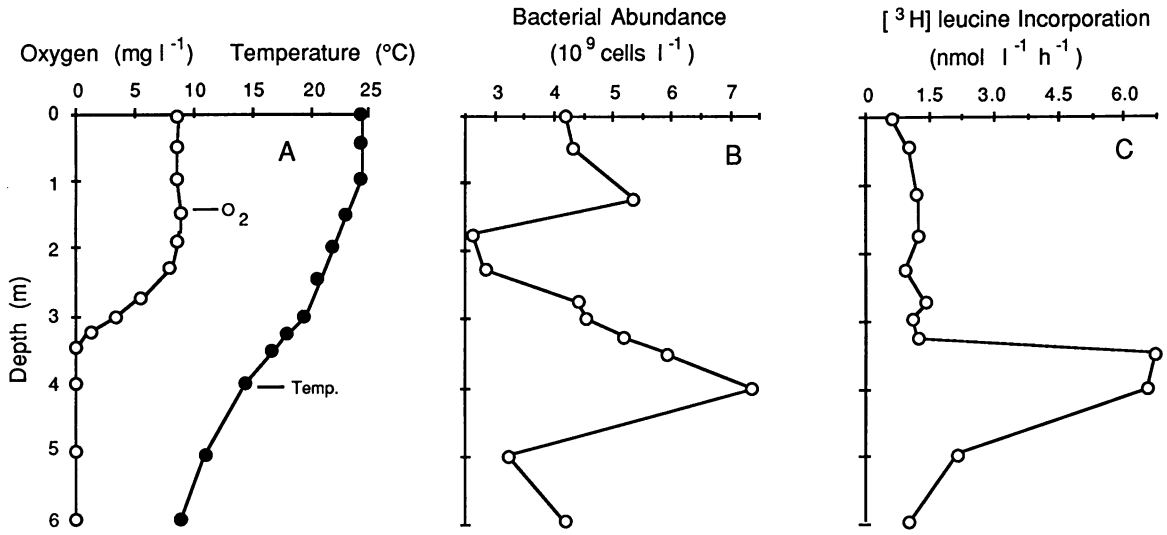

FIG. 3. Depth profile of oxygen and temperature (A), abundance (B), and [^{3}H]leucine incorporation into protein (C), May 1985.

with those based on the incorporation of leucine into protein (Fig. 6). As in April, both measures indicated that bacterial production was highest in the metalimnion, although the metalimnion peaks were 3 and 4 m for thymidine- and leucine-based measures, respectively. Except for the low leucine incorporation rates at 3 m, the leucine-based measure was 1.6- to 7.2-fold higher than the thymidine-based measure in July. The leucine measure was higher than the thymidine measure by about the same factor in both epilimnion and hypolimnion samples in July (Fig. 6). However, in August the ratio of the leucine-based measure/thymidine-based measure of bacterial production increased 2- to 10-fold in anaerobic waters relative to aerobic waters (Fig. 7).

Incorporation of [^{3}H]leucine radioactivity was at least 40-fold higher than incorporation of [^{3}H]thymidine radioactivity at all depths (Fig. 7). Some, but not all, of the difference was because twice as much [^{3}H]leucine was added as [^{3}H]thymidine. Incorporation of leucine and thymidine gave the same order of magnitude estimate of bacterial production because of the difference in conversion factors. Nevertheless, these data indicate that the leucine approach is potentially more sensitive than the thymidine approach. The high incorporation of [^{3}H]leucine radioactivity may enable low production rates to be measured in smaller incubation volumes and shorter incubation times than possible with the thymidine approach.

DISCUSSION

The rate of bacterial production is probably the best available indicator of the relative importance of heterotrophic bacteria in aquatic systems. The amount of bacterial biomass potentially available to grazers and higher trophic levels can be directly estimated from rates of bacterial production. Furthermore, coupled with information about the bacterial growth efficiency, rates of bacterial production

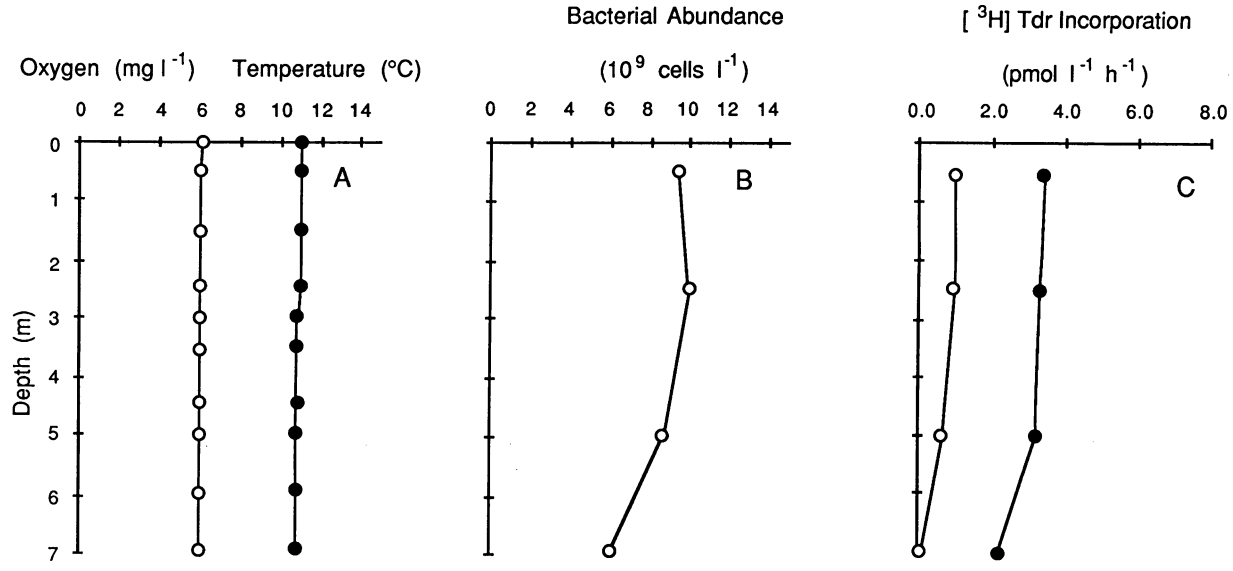

FIG. 4. Depth profile of oxygen and temperature (A), bacterial abundance (B), and [^{3}H]thymidine (Tdr) incorporation into DNA and protein (C), November 1984.

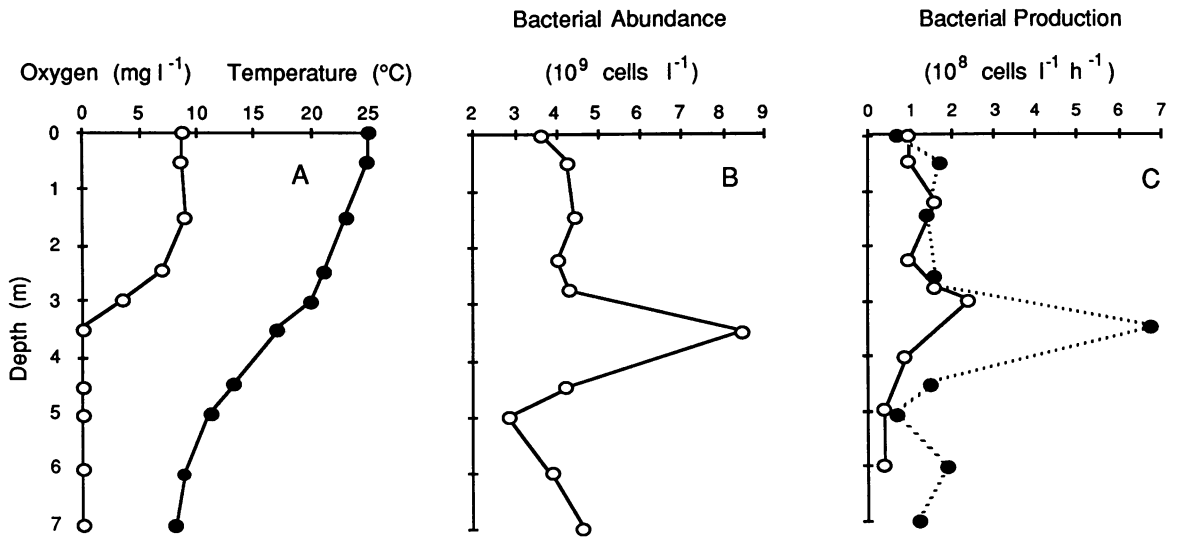

FIG. 5. Depth profile of oxygen and temperature (A), bacterial abundance (B), and estimates of bacterial production based on FDC (●) and thymidine incorporation (○) (C), April 1985.

can be used to estimate the flux of dissolved organic matter. The direct approach of measuring the concentration and turnover of all compounds possibly used by bacteria is not feasible, even if all of the compounds could be identified and their concentrations could be measured. Recent reports of high rates of bacterial production, compared with primary production, suggest that bacteria can account for a substantial portion of the carbon flow in aquatic trophic dynamics and eventual mineralization of primary production (ca. 50% of primary production; see review in reference 4).

The [^{3}H]thymidine approach (8) has emerged as the method of choice for estimating bacterial production (28). Although many studies have examined thymidine uptake in aerobic marine and freshwater ecosystems, our study is among the first to examine thymidine incorporation by microorganisms in anaerobic waters. We did not expect to see high thymidine incorporation in anaerobic waters because we thought the usually strict nutritional habits of anaerobic bacteria would preclude uptake of thymidine. However, since the rate of [^{3}H]thymidine incorporation in anaerobic samples was substantially inhibited by aeration, apparently some obligate anaerobic bacteria in Lake Oglethorpe are capable of transporting thymidine. Pollard and Moriarty (32) used the [^{3}H]thymidine approach to measure accurately the production of a mixed culture of anaerobic bacteria.

Even though the anaerobic bacteria in Lake Oglethorpe assimilated thymidine, incorporation into DNA was usually very low in samples from the metalimnion and anaerobic hypolimnion and high amounts of radioactivity were recovered in the protein fraction. Furthermore, [^{3}H]thymidine incorporation into protein was high (on the average 30% of DNA plus protein incorporation) even in aerobic epilimnion samples. It is possible that some of the apparent incorporation of [^{3}H]thymidine radioactivity into protein is due to ingestion of bacterial DNA by eucaryotic grazers, since we observed that appearance of radioactivity in protein was partially inhibited by cycloheximide. However, the effect of cycloheximide on both bacterial and eucaryotic metabolism in samples from Lake Oglethorpe is complex and poorly understood (35). It is also important to point out that the fraction of total [^{3}H]thymidine incorporation recovered in protein varied greatly from sample to sample. One explanation for the high incorporation of thymidine into total macromolecules and low incorporation into DNA is that many bacteria in Lake Oglethorpe are able to transport thymidine but do not have thymidine kinase. This enzyme is responsible for phosphorylating thymidine ultimately to thymidine triphosphate, which is polymerized with other nucleotides to form DNA.

Several studies of both marine and freshwater systems have also observed relatively high amounts of [^{3}H]thymidine incorporation into protein (5, 11, 13, 21). At the very least this finding indicates that the incorporation into DNA needs to be measured and its variability needs to be determined. The standard acid-base hydrolysis procedure for isolating DNA may not be ideal for samples from natural aquatic ecosystems (34), but our experiment showing high recovery of added [^{3}H]DNA indicates that this procedure is not grossly in error. By using the same procedure, other studies of thymidine incorporation in aquatic systems have found high incorporation into DNA and little incorporation into protein (9, 21, 34), suggesting that the incorporation of [^{3}H]thymidine into the different macromolecules varies with environmental conditions and probably the species composition of bacterial assemblages.

The concentration of oxygen is an important but probably not the only factor determining the incorporation of [^{3}H]thymidine radioactivity into DNA versus protein. If oxygen were the only factor, then we would have expected the ratio of incorporation into DNA/protein to be lowest in the hypolimnion. In fact, the ratio of DNA/protein incorporation was lowest in the metalimnion, which had low but measurable oxygen concentrations. Since rates of [^{3}H]thymidine and [^{3}H]leucine incorporation into protein were highest in the metalimnion, high rates of protein synthesis compared with DNA synthesis may cause high incorporation of the [^{3}H]methyl moiety (the tritiated site of [^{3}H]thymidine) into amino acids and eventually into proteins. Protein synthesis may be high relative to DNA synthesis when cells are growing in size but not dividing, but this imbalance could not occur indefinitely.

If many bacteria do indeed take up thymidine but do not incorporate it into DNA, interpretation of thymidine incor-

poration needs to be reexamined. One of the basic premises of the thymidine approach, which is that thymidine is incorporated into DNA and is a measure of DNA synthesis, would no longer be true. However, even if large amounts of radioactivity are recovered in protein, thymidine incorporation may still be closely coupled to new cell production because synthesis of protein is often synchronized with new cell production (16a). Thus, even if thymidine incorporation no longer reflected DNA synthesis, the thymidine approach could still give reasonable estimates of bacterial cell production, as long as the thymidine approach is calibrated against changes in bacterial abundance (8, 9, 15). Theoretical factors based on the amount of thymidine in DNA and DNA content per bacterium would no longer be relevant.

The thymidine approach for estimating bacterial production would not be a good measure of bacterial production if many bacteria were incapable of assimilating thymidine. In August, although not in July, we observed that the ratio of [^{3}H]leucine/[^{3}H]thymidine incorporation into protein was higher in anaerobic waters, one explanation being that more bacteria in anaerobic waters were capable of assimilating leucine than thymidine. Even in aerobic waters, microautoradiography studies have shown that the number of bacteria labeled with [^{3}H]thymidine can be substantially lower than the number labeled with ^{3}H-amino acids (3, 38). Note that a large number of dividing bacteria incapable of incorporating thymidine would result in high, but presumably correct, empirical conversion factors (15).

In spite of problems with the thymidine-, leucine-, and FDC-based measures of bacterial production, these approaches usually would lead to similar qualitative conclusions about the depth distribution of bacterial production in Lake Oglethorpe. FDC values, the standard approach of measuring thymidine incorporation into the cold TCA-insoluble cell fraction (28), and leucine incorporation into protein all indicate that bacterial production is high at the metalimnion. Data on thymidine incorporation into DNA give a different qualitative profile (maximal rates of bacterial production in the epilimnion) than observed with the other measures.

The method of converting incorporation rates or FDC to rates of bacterial production is just as important in estimating bacterial production as the raw data on incorporation rates or FDC. Our data indicate that the commonly used conversion factor for thymidine incorporation (2.0×10^{18} cells per mol) is too low for Lake Oglethorpe. Both the

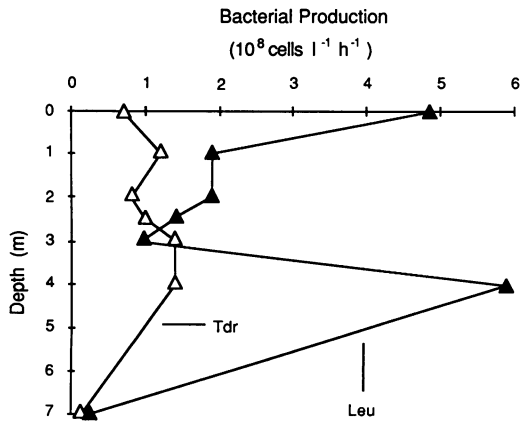

FIG. 6. Estimates of bacterial production based on thymidine (Tdr) and leucine (Leu) incorporation, July 1984.

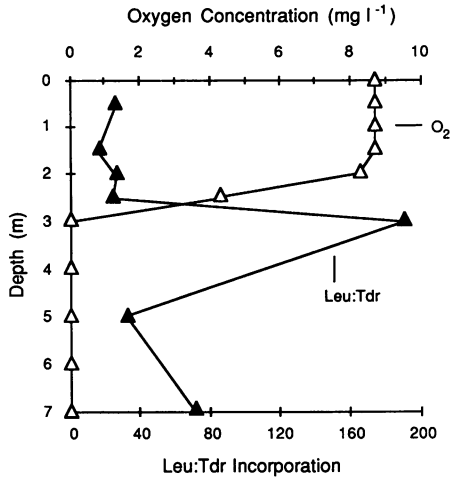

FIG. 7. Depth profile of oxygen concentration and ratio of leucine (Leu) to total thymidine (Tdr) incorporation (both rates expressed as disintegrations per minute per liter per hour), August 1984.

leucine and FDC approaches gave higher estimates of bacterial production than those based on the thymidine approach. The thymidine-based estimate of bacterial production was about 10^8 cells liter^{-1} h^{-1}, whereas the leucine-based estimate was about 2×10^8 cells liter^{-1} h^{-1} in the epilimnion in July. Independent measures of grazing on the bacteria suggest that bacterial production is about 2×10^8 cells liter^{-1} h^{-1} for these waters in summer (35). We have estimated the conversion factor for the thymidine approach by the method of Kirchman et al. (15) and found it to be fivefold higher than the literature value we used here. When the high conversion factor is used, thymidine-based estimates of bacterial production are in balance with the estimated grazing rates (35). Scavia et al. (36) also measured high conversion factors by the Kirchman et al. (15) approach. They found that the rates of bacterial production based on these conversion factors were similar to grazing estimates, whereas production rates based on the theoretical conversion factor were too low to balance the measured grazing rate on bacteria.

The good agreement between grazing and the bacterial production rates based on leucine incorporation and FDC is in part fortuitous. Both the conversion equation for the FDC method (2) and the conversion factor for leucine incorporation (16a) were measured with samples from the salt marsh estuary at Sapelo Island, Ga. In general, bacterial production is estimated with the greatest accuracy when conversion factors are measured specifically for the aquatic system being examined. Based on the appropriate conversion factors, it appears that the thymidine, leucine, and FDC methods all give roughly similar information about rates of bacterial production in Lake Oglethorpe.

Several questions concerning the source of carbon supporting high bacterial production at the metalimnion and the fate of that production remain unanswered. The relative importance of phytoplankton (22) versus the detrital pools of dissolved and particulate organic matter in supporting the high rates of bacterial production in these waters is not clear. Since rates of bacterial production are much higher than the in situ change in bacterial abundance, grazing must roughly balance bacterial production. The identity of grazers in the anaerobic hypolimnion is unknown. Ciliates are abundant in

the hypolimnion of Lake Oglethorpe (27), as are heterotrophic microflagellates (R. W. Sanders, personal observation). Protozoa are often important grazers of bacteria in aerobic waters (6), and selected species that are either facultative or obligate anaerobes have been isolated from anaerobic hypolimnion waters and sediments (1, 7, 37).

We observed in Lake Oglethorpe that bacterial biomass, production, and growth rates (indicated by FDC and incorporation rates per cell) were greatest in the metalimnion where oxygen first approached unmeasurable levels. More detailed sampling in the metalimnion is needed to determine the relationship between bacterial production and oxygen concentration and other parameters that covary with oxygen. Even on the coarse spatial scale that we sampled, several measures of microbial biomass and growth apparently are maximal at the interface between the aerobic epilimnion and the anaerobic hypolimnion. More information about bacterial metabolism at this interface and others may be useful for understanding the flux of materials between water masses and the factors that regulate bacterial abundance and production.

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