Applied and Environmental Microbiology, Dec. 1984, p. 1221–1230
0099-2240/84/121221-10$02.00/0
Copyright © 1984, American Society for Microbiology
Vol. 48, No. 6

# Assessing Phytoplankton and Bacterioplankton Production During Early Spring in Lake Erken, Sweden

RUSSELL T. BELL[1]* AND JORMA KUPARINEN[2]

*Institute of Limnology, Uppsala University, S-751 22 Uppsala, Sweden,[1] and Tvärminne Zoological Station, University of Helsinki, SF-10850 Tvärminne, Finland[2]*

Received 7 May 1984/Accepted 20 August 1984

**The spring development of both phytoplankton and bacterioplankton was investigated between 18 April and 7 May 1983 in mesotrophic Lake Erken, Sweden. By using the lake as a batch culture, our aim was to estimate, via different methods, the production of phytoplankton and bacterioplankton in the lake and to compare these production estimates with the actual increase in phytoplankton and bacterioplankton biomass. The average water temperature was 3.5°C. Of the phytoplankton biomass, >90% was the diatom *Stephanodiscus hantzchii* var. *pusillus*, by the peak of the bloom. The $^{14}C$ and $O_2$ methods of estimating primary production gave equivalent results ($r = 0.999$) with a photosynthetic quotient of 1.63. The theoretical photosynthetic quotient predicted from the $C/NO_3$ N assimilation ratio was 1.57. The total integrated incorporation of [$^{14}C$]bicarbonate into particulate material (>1 μm) was similar to the increase in phytoplankton carbon determined from cell counts. Bacterioplankton increased from $0.5 \times 10^9$ to $1.52 \times 10^9$ cells liter$^{-1}$ (~0.5 μg of C liter$^{-1}$ day$^{-1}$). Estimates of bacterioplankton production from rates of [$^3H$]thymidine incorporation were ca. 1.2 to 1.7 μg of C liter$^{-1}$ day$^{-1}$. Bacterial respiration, measured by a high-precision Winkler technique, was estimated as 4.8 μg of C liter$^{-1}$ day$^{-1}$, indicating a bacterial growth yield of 25%. The bulk of the bacterioplankton production was accounted for by algal extracellular products. Gross bacterioplankton production (production plus respiration) was 20% of gross primary production, per square meter of surface area. We found no indication that bacterioplankton production was underestimated by the [$^3H$]thymidine incorporation method.**

Insights on the structure and function of microbial food webs are now possible due to the development of better methods for the estimation of bacterial growth rates in aquatic systems (2, 68). In particular, methods based on the frequency of dividing cells (25) and rates of [$^3H$]thymidine incorporation (21, 22) have been widely applied, although there is still debate over how results should be interpreted. The two methods often give results of comparable magnitude (50; see Table 10 in reference 3), but direct comparisons of the two methods have not always given similar results (42, 51). However, Hagström (24) found that the frequency of dividing cells and thymidine methods gave similar estimates of bacterial production over a diel cycle and recommended further development of the thymidine method. Further tests of the thymidine method, preferably made in situ, are still needed.

Bacterioplankton production in aquatic ecosystems is often of a magnitude similar to that of primary production (2, 68). For assessing the bacterioplankton rate estimates, the assumption has been that estimates of primary production are realistic. Primary production is usually measured by one method, the radiocarbon ($^{14}C$) method introduced by Steeman Nielsen (57). However, interpretation of the results obtained with the $^{14}C$ method is often equivocal (reviewed in 48). For example, Gieskes et al. (23) claim that current estimates of primary production in oceans are severe underestimates. A model constructed after the addition of [$^{14}C$]bicarbonate to a small lake at the Experimental Lakes Area in Ontario produced results in agreement with the photosynthetic rate measured by an incubator technique (27). The results of Peterson (47), on the other hand, indicate that the $^{14}C$ method could at times be overestimating net primary production.

Improvements in the sensitivity of the Winkler oxygen technique (10, 62, 70) enable an assessment of primary production based on a comparison of the $^{14}C$ and $O_2$ methods (17, 22, 69, 71; J. Kuparinen, Verh. Int. Ver. Limnol., in press). Although these methods were frequently compared during the 1960s (48), only recently has the significance of the photosynthetic quotient (PQ [molar $O_2/CO_2$ flux]) been given attention. A PQ of 1.25 is the expected quotient, but recent evidence indicates that high PQs (>1.5) are expected when nitrate is the dominant assimilated inorganic-nitrogen source (17, 49, 71). The earlier studies did not consider the influence of inorganic nitrogen on the PQ.

The oxygen method, in combination with size fractionation procedures, can also be used to estimate the contribution of various plankton size fractions to total community metabolism (67). By comparing estimates of bacterial respiration and bacterial production, the growth yield of the bacterioplankton can be calculated (31, 68). This growth yield determination provides an additional way to evaluate the estimates of bacterial production.

In this study we assessed the production of both phytoplankton and bacterioplankton during the development of the spring diatom bloom in Lake Erken, Sweden. The special characteristics of the spring bloom are ideal for this purpose: biomass is increasing, species diversity is low, and zooplankton grazing is negligible (46). After the ice melts in Lake Erken, the phytoplankton community is usually dominated by the diatom *Stephanodiscus hantzchii* var. *pusillus*, and nitrate is often depleted during the development of the bloom (6, 7). In short, for the first 2 to 3 weeks after the ice melts, the lake is virtually a batch culture.

We are the first to study bacterioplankton in Lake Erken, but results from other lakes suggest that bacterioplankton may also exhibit a spring increase in response to the increase

* Corresponding author.

in algal activity and the lack of grazing pressure (15, 30). We estimated bacterioplankton production by measuring [$^3$H]thymidine incorporation, [$^3$H]glucose uptake, dark uptake of $^{14}CO_2$, and uptake by bacteria of carbon from algal excretion products, here defined as extracellular organic carbon (EOC). The validity of the production estimates was assessed by comparison with the actual increase in both bacterial numbers and bacterial respiration.

## MATERIALS AND METHODS

**Study site and sampling.** Lake Erken (area, 23 km$^2$; maximum depth, 20.5 m; mean depth, 9 m) is a mesotrophic lake located 60 km north-northeast of Stockholm and 50 km east of Uppsala. (Further information about the lake can be found in references 41 and 46.)

This study was begun on 18 April and was terminated on the morning of 8 May 1983 at the peak of the phytoplankton bloom. The pelagic region of the lake was ice-free by the morning of 22 April. We took samples approximately every other day. Water was collected from four or five stations within the pelagic region ca. 700 m offshore from the limnological laboratory, where the lake is at its maximum depth. A composite sample was taken to minimize variability due to sampling (28). Samples were taken from a depth of 1 to 5 m with a 2-m plexiglas tube sampler. The composite sample (20 to 25 liters) was mixed, and 5 liters was added to a sterile glass bottle. All samples for incubations and biomass determinations were taken from this mixed sample. Flasks used for incubations were autoclaved or heat sterilized between uses. Sampling was normally at 9:30 a.m., and incubations were begun soon after 10:00 a.m. All incubations were at a depth of 1 m. In Lake Erken this is the depth of optimal (light-saturated) photosynthesis (53). Insolation was continuously recorded in Malma Islet (a small island several hundred meters offshore and 150 m from the incubations) with a Moll-Gorczinski solarimeter mounted ca. 2 m above the lake surface. Light penetration was measured by lowering an LI-192S quantum sensor at 0.1- (for the first half meter) to 0.5-m increments.

**Biomass.** A sample for phytoplankton enumeration was fixed with Lugol solution, and phytoplankton were counted with an inverted microscope after the sample had been in a sedimentation chamber for 24 h. The average cell volume was determined and a relationship (phytoplankton carbon equals 0.15 times the average cell volume) was determined by using standard regressions (58). For chlorophyll (Chl) determinations, a 500-ml sample was filtered at low pressure (<100 mmHg [1 mmHg = 133.3 Pa]) through a GF/C filter and stored frozen. A second 500-ml sample was filtered via gravity flow through a 47-mm Nuclepore filter (pore size, 3.0 μm). The samples were extracted in acetone and analyzed spectrophotometrically as described previously (3). When diatoms dominate, extractions in acetone and methanol give equivalent results (61).

Bacterioplankton were enumerated, and cell volumes were estimated by acridine orange staining and epifluorescence microscopy on black Nuclepore filters (pore size, 0.2 μm), as described previously (3). At least 20 fields (>200 cells) were counted. The mean difference between replicate determinations was 6%.

**$^{14}$C assimilation.** For determination of $^{14}$C uptake, we incubated replicate 100-ml blanks (final concentration, 0.4% Formalin), replicate dark bottles, and replicate light bottles with 10 μCi (370 kBq) of $NaH^{14}CO_3$ for 24 h at a depth of 1 m. Uptake was stopped by the addition of Formalin to a final concentration of 0.4%, and the samples were kept dark and refrigerated until filtration (several hours). We detected no loss of radioactivity due to the fixation process (see also 43).

Total $^{14}$C assimilation (called apparent net production by Niemi et al. [43]) was measured by adding 4 ml of sample directly to glass scintillation vials, acidifying the samples to ~ pH 2 with 1 N HCl, and allowing the open vials to stand for 24 h in a fume hood (43). This procedure is equivalent to what is called the acidification and bubbling technique (55) and measures both $^{14}$C in algal cells and dissolved organic $^{14}$C (plus $^{14}$C in bacteria). The samples were analyzed by liquid scintillation after the addition of 7 ml of PCS (Amersham International, Ltd.) scintillation solution.

Particulate $^{14}$C was determined by filtering 10-ml subsamples via gravity flow through a Nuclepore filter of 3-μm pore size and subsequently through a sequence of Nuclepore filters (pore sizes, 1 and 0.2 μm) at a pressure differential of <200 mmHg. The filters were washed with 5 ml of GF/C-filtered lake water and placed in scintillation vials, and a drop of concentrated HCl was added to ensure removal of any remaining inorganic $^{14}$C. The filters were dissolved in 0.5 ml of TS-1 tissue solubulizer, 15 ml of scintillation solution was added, and the samples were analyzed by liquid scintillation. Quenching was determined by the external-standard-channels-ratio method, and an isotope discrimination factor of 1.06 was used. The concentration of inorganic carbon in the lake water was determined from pH, alkalinity, and conductivity measurements. Dark counts were usually <2% and always <6% of light-bottle counts and were subtracted from light-bottle counts. The mean difference between replicates was 4% for total $^{14}$C assimilation samples, 5% for activity on 3-μm filters (90% of primary production), and 8% for activity on filters of all three pore sizes combined.

**Determination of integrated production per square meter of surface area.** Integral photosynthesis is a function of light-saturated photosynthetic rates as well as the vertical gradient of underwater light and, when phytoplankton are homogeneously distributed, can be approximated by the following equation (59, 60, 63):

$$\text{mg of C m}^{-2}\,d^{-1} = \left[\frac{(\text{mg of C m}^{-3}\,d^{-1})}{e}\right] \times [f(I'_o)] \qquad (1)$$

where mg of C m$^{-3}$ $d^{-1}$ is the $^{14}$C assimilation at otpimal depth, $e$ is the vertical-light attenuation coefficient (ln U m$^{-1}$; 0.645 during this study) and $f(I'_o) = \ln(I_o:I_z)$ where $I_o$ is the quantum flux of photosynthetically available radiation (400 to 700 nm) immediately below the lake surface and $I_z$ is the quantum flux at the depth where half-saturating light levels are encountered. In Lake Erken, $f(I'_o)$ is 2.4 to 2.7 (53). Equation 1 predicts area production to within ±23% (53).

Production by area was also approximated from the actual increase in phytoplankton carbon (cell counts). Since virtually the entire water column is mixed during spring, the phytoplankton obtained from 1 to 5 m is representative of the entire water column. We multiplied phytoplankton carbon as milligrams of C m$^{-3}$ by a factor of 13 to 15 to convert to milligrams of C m$^{-2}$ (based on several years of data from vertical chlorophyll profiles during spring [C. Eriksson and K. Pettersson, unpublished data]).

**Phytoplankton growth rate estimation.** The growth rate was calculated directly from cell counts (assuming exponential growth), but to assess our empirically derived C/Chl *a* ratio, we also derived growth rates from the following equation (47):

$$\mu = \frac{1}{t}\left(\ln \frac{C_o + \Delta C}{C_o}\right) \quad (2)$$

where $\mu = day^{-1}$, $t$ is the incubation period (24 h), $C_o$ is the phytoplankton carbon at the start of the incubation determined from C/Chl *a* ratios, and $\Delta C$ is the incorporation of $^{14}C$ into particulate material for the 24-h period. The maximum potential growth rate ($\mu_{max}$) was calculated by the equation of Eppley (18):

$$\log_{10}\mu = 0.0275T - 0.07 \quad (3)$$

where $T$ is the in situ water temperature in degrees Celsius. Since the latter equation yields a growth rate for a 24-h period (continuous light), several workers (13, 19) have corrected for day length to allow comparison of experimental data. We compared our experimental $\mu$ with both corrected (×0.6) and uncorrected $\mu_{max}$ values. Our hypothesis was that the correct C/Chl *a* value would give a $\mu/\mu_{max}$ closest to unity.

**Oxygen flux measurements.** Incubations for oxygen determination were made in stoppered glass bottles calibrated to a 0.01-ml volume. Two pairs of light-dark replicates were prefiltered (via gravity) through a Nitex net (pore size, 63 μm) and a 47-mm Nuclepore filter (pore size, 3.0 μm), respectively. Two untreated (not filtered) light-dark samples were also incubated, and two zero-time samples were immediately fixed with Winkler reagents (1.0 ml of $MnSO_4$ and 1.0 ml of alkaline iodine solution). By subtraction, the activity in various size fractions could be roughly interpreted as bacterioplankton (<3 μm), phytoplankton (3 to 63 μm), and zooplankton (untreated minus the 63-μm fraction). After 24 h of incubation, Winkler reagents were added, and the samples were kept dark in a water bath. At the same time each day, phosphoric acid was added, and the titration was carried out by using 0.025 N sodium thiosulfate solution. The endpoint was detected electrochemically by using gold-platinum electrodes connected to a galvanometer (62). To obviate the loss of iodine by volatilization, we titrated the whole bottle (10). However, we removed 10.0 ml immediately before titration to allow room for inserting the electrodes. The mean difference between replicates (all samples combined) was 0.098%, within the range of 0.05 to 0.1% obtainable by using a photometric endpoint detector (10, 70). The precision of the method in Lake Erken (the required difference between a sample fixed at time zero and an incubated sample [10]) was 12 μg of $O_2$ liter$^{-1}$. A respiratory quotient of 0.85 was used when converting oxygen to carbon units (stoichiometric ratio × 0.85). Gross production was measured as the difference in light and dark bottles after the incubation period. Respiration was the initial oxygen concentration minus the concentration in dark bottles at the end of the incubation period. The <3-μm size fraction contained both algae and bacteria. The contribution of algae to the total respiration in the <3-μm size fraction was determined by applying the specific respiration rate (micrograms of C per microgram of Chl *a* per day) for the 3- to 63-μm size fraction to the Chl *a* concentration in the <3-μm fraction. Although there are often differences in size-specific rates for different-sized organisms, the literature indicates that there is no simple relationship between size-specific respiration and cell size in phytoplankton (40). Bacterial respiration was thus the difference between total respiration in the <3-μm fraction and the algal respiration in the <3-μm fraction.

**Theoretical photosynthetic quotient.** A theoretical value for the PQ was calculated by the following equation of Davies and Williams (17):

$$PQ = PQ_c + 2/(C/NO_3\ N)_{molar} \quad (4)$$

where $PQ_c$ is the carbon PQ controlled by the relative amount of carbohydrate, protein, and lipid end products of photosynthesis and assumed to be 1.25. $(C/NO_3\ N)_{molar}$ is the molar assimilation ratio of carbon to nitrogen from nitrate.

**[$^3$H]thymidine incorporation.** The approach developed by Fuhrman and Azam (21, 22) was used. Duplicate or triplicate 25-ml samples and duplicate Formalin-killed blanks (final concentration, 2%) were incubated in situ with 10 nM [*methyl*-$^3$H]thymidine (40 to 50 Ci mmol$^{-1}$; Radiochemical Centre, Amersham, England) for 0.5 to 2 h (ca. 11 a.m. to 1 p.m.). Blanks averaged 0.9% of the uptake, and blank uptake was subtracted from the experimental samples. The incubations were terminated by adding 5-ml subsamples directly to screw-top test tubes containing 5 ml of ice-cold 10% trichloroacetic acid (TCA) or by the addition of Formalin to a final concentration of 2% (3). After extraction in ice-cold 5% TCA (final concentration) for about 1 h, the samples were filtered onto Nuclepore polycarbonate filters (pore size, 0.2 μm) and rinsed five times with 2-ml portions of ice-cold 5% TCA. After the filters had dried, 0.5 ml of TS-1 tissue solubulizer was added to dissolve the filters, scintillation solution was added, and the samples were assayed by liquid scintillation. The mean difference between duplicate thymidine incubations was 11%.

Production was calculated as described previously (3). A conversion factor of $1.6 \times 10^9$ to $2.1 \times 10^9$ cells per nmol$^{-1}$ (R. Bell, Arch. Hydrobiol. Beih. Ergeb. Limnol., in press) was derived from measurements of [$^3$H]thymidine incorporation into a DNA fraction (77 ± 9% of the radioactive material in the cold-TCA-insoluble fraction). The assessment of isotope dilution, and the assumption that bacterioplankton have a DNA content of 2.6 fg cell$^{-1}$ (22). The measurement of cells produced was converted to that of carbon production by multiplying the average cell volume (μm$^3$) by the factor $1.21 \times 10^{-13}$ g of C μm$^{-3}$ (64).

**[$^3$H]glucose uptake.** To provide a conservative estimate of production, we calculated the carbon production from the uptake of [$^3$H]glucose. The in situ glucose concentration in Lake Erken has been estimated to be 1 to 20 μg liter$^{-1}$ (73). Duplicate 20-ml samples and a Formalin-killed blank (final concentration, 2%) were incubated with D-[6-$^3$H]glucose (37 Ci mmol$^{-1}$) at a tracer concentration of 0.03 μg liter$^{-1}$ for 4 h in the dark. Incubations were stopped by adding Formalin to a final concentration of 2%. The samples (20 ml) were filtered through a sequence of Nuclepore polycarbonate filters (pore sizes, 1 and 0.2 μm) and washed twice with 10 ml of GF/C-filtered lake water and then were assayed for radioactivity.

**$^{14}CO_2$ dark uptake.** Bacterioplankton heterotrophic production was estimated from the dark uptake of $^{14}C$ (see above) from the assumption (54) that dark $CO_2$ uptake provides 6% of total heterotrophic carbon production. We emphasize that this only gave an approximate value since heterotrophic bacteria are quite variable with respect to their $CO_2$ uptake (45). We calculated production by two methods: (i) 0.2- to 1-μm size fraction corrected for blank uptake and (ii) total dark assimilation (direct acidification method), corrected for blank values, multiplied by the percentage of total dark $^{14}C$ uptake in the <1-μm size fraction (28 ± 5%). If there is negligible excretion of $^{14}C$-labeled dissolved organic carbon by the bacteria, then these calculations should give equivalent results.

TABLE 1. Data on insolation, temperature, and nutrients[a]

| Dates | Insolation[b] (mWh $cm^{-2}$ $day^{-1}$) | Water temp (°C) | Nutrient (mg-at $m^{-3}$) | | |
|---|---|---|---|---|---|
| | | | $NH_4$ N | $NO_3$ N | $PO_4$ P |
| April 18 to 24 | 294 | 2.5 | 0.6 | 8.8 | 0.3 |
| April 25 to May 1 | 144 | 3.5 | 0.6 | 6.0 | 0.1 |
| May 2 to 7 | 476 | 4.6 | 1.8 | 3.0 | 0.03 |

[a] Data for 1 to 5 meters.
[b] Total incoming irradiation.

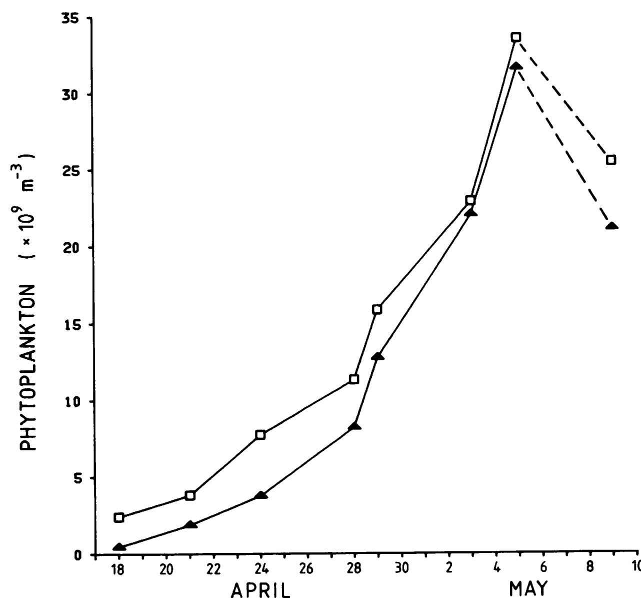


FIG. 1. Phytoplankton abundance during spring 1983 in Lake Erken. Symbols: □, total phytoplankton; ▲, *Stephanodiscus hantzchii* var. *pusillus*.

## RESULTS AND DISCUSSION

**Phytoplankton development and production.** The development of phytoplankton was nearly exponential (Fig. 1) despite low incident light levels during the middle of the study (Table 1). Light intensity at 1 m of depth was always between 40 and 110 microeinsteins $m^{-2}$ $s^{-1}$ during midday; at temperatures <5°C, this would easily saturate photosynthesis (26). By 3 May, *Stephanodiscus hantzchii* var. *pusillus* was 96% of total abundance and 90% of total phytoplankton biomass (Fig. 1). Chl *a* increased to a level of 23 μg $liter^{-1}$ (Table 2), and the C:Chl *a* ratio was 21 ± 6. Although the C/Chl *a* ratios of phytoplankton vary between 10 and 150 (65), low values similar to ours have been reported during diatom blooms (19, 52).

The total increase in phytoplankton carbon for the period 18 April to 5 May, based on direct counts, was 6,100 to 7,100 mg of C $m^{-2}$. The total integrated increase in algal carbon estimated from the incorporation of $^{14}C$ into particulate matter, applying equation 1, was in the range of 6,700 to 7,500 mg of C $m^{-2}$. Considering that both of these estimates have a possible error of at least ±20%, we believe that the agreement is good and indicates that the estimate of phytoplankton carbon from cell counts was realistic. The data also suggest that the incorporation of $^{14}C$ into particulate matter was close to net primary production during this study. Peterson (47) measured $^{14}C$ uptake and the increase in particulate carbon in mixed cultures of lake phytoplankton. He observed that there is a continuum of ratios of particulate carbon yield to $^{14}C$ uptake which depends on growth condi-

TABLE 2. Primary production estimates for Lake Erken, April to May 1983

| Date | Temp (°C) | Chl *a* (μg $liter^{-1}$)[a] | | Oxygen (μg of $O_2$ $liter^{-1}$ $day^{-1}$) from: | | $^{14}C$ (μg of C $liter^{-1}$ $day^{-1}$) | | | PQ | Specific photosynthetic rate[d] (μg of C μg Chl $a^{-1}$ $day^{-1}$) based on: | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | >3 μm | <3 μm | Respiration[b] | Gross production[c] | Total | Particulate | EOC | | Total $^{14}C$ | $^{14}C$ particulate matter |
| April | | | | | | | | | | | |
| 18 | 2.1 | 3.3 | | 0 | 149 | 34 | 26 | 8.5 | 1.64 | 10.4 | 7.8 |
| 21 | 2.3 | 4.2 | 0.7 | 17 | 283 | 69 | 35 | 34 | 1.54 | 14.1 | 7.1 |
| 22 | 2.5 | 5.1 | 1.0 | 37 | 403 | 103 | 64 | 39 | 1.47 | 16.9 | 10.6 |
| 23 | 2.7 | 5.1 | 0.6 | 44 | 353 | 84 | 49 | 35 | 1.58 | 14.7 | 8.6 |
| 24 | 2.9 | 5.5 | 0.5 | 31 | 382 | 82 | 53 | 29 | 1.75 | 13.6 | 8.8 |
| 26 | 3.3 | 6.9 | 0.7 | 110 | 606 | 130 | 76 | 54 | 1.75 | 17.1 | 10.0 |
| 28 | 3.5 | 8.4 | 0.9 | 90 | 625 | 136 | 88 | 47 | 1.72 | 14.6 | 9.5 |
| 29 | 3.6 | 9.4 | 1.0 | 72 | 775 | 181 | 148 | 33 | 1.61 | 17.4 | 14.3 |
| May | | | | | | | | | | | |
| 1 | 3.9 | 11.2 | 1.2 | 47 | 932 | 217 | 190 | 28 | 1.61 | 17.5 | 15.3 |
| 3 | 4.1 | 13.4 | 1.3 | 97 | 1,272 | 295 | 250 | 45 | 1.62 | 20.1 | 17.0 |
| 5 | 4.5 | 14.7 | 1.3 | 68 | 1,230 | 288 | 171 | 117 | 1.60 | 18.0 | 10.7 |
| 7 | 5.7 | 21.2 | 1.4 | 179 | 1,539 | 354 | 288 | 66 | 1.63 | 15.7 | 12.7 |
| Integrated (April 18 to May 7)[e] | | | | 1,120 | 13,830 | 3,188 | 2,317 | 871 | | | |

[a] In particle sizes >3 μm or <3 μm.
[b] Average of total and <63-μm size fraction.
[c] Production in the <63-μm size fraction was 92% of total gross primary production.
[d] Rates based on total $^{14}C$ assimilation and $^{14}C$ assimilation into particulate matter.
[e] Units are micrograms $liter^{-1}$ for the entire study period.

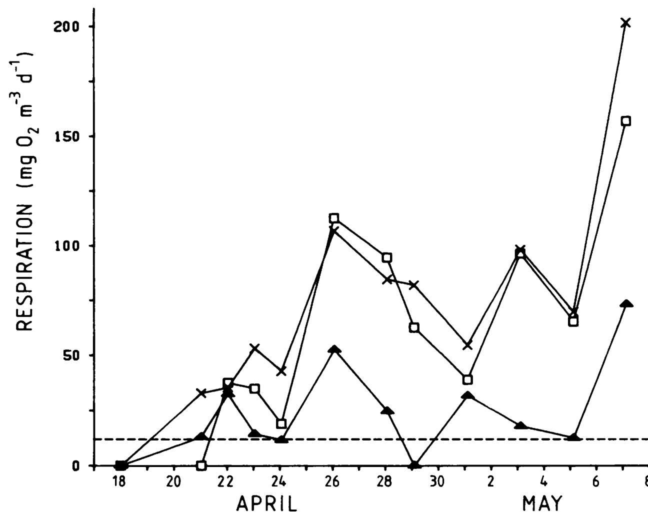


FIG. 2. Size-fractionated respiration measurements. Symbols: □, total (unfiltered); ×, <63-μm fraction; ▲, <3-μm fraction. The dotted line is the significance level.

tions. In his experiments, $^{14}C$ uptake agreed with estimates of particulate carbon increase when growth rates were 0.3 to 0.6 $day^{-1}$ at 20°C ($\mu/\mu_{max}$, ~ 0.3 [from equation 3]). We calculated growth rates for Lake Erken phytoplankton by using a variety of C/Chl *a* ratios (Table 3). The growth rate based on incubations at 1 m of depth should be close to maximal since light is saturating photosynthesis at this depth. The growth rate is near maximal when a C/Chl *a* ratio of 20 is assumed. This was our experimentally derived C/Chl *a* ratio. The relationship $\mu/\mu_{max}$ calculated from phytoplankton counts is only one-third of the maximum because the mixed layer is much deeper than the euphotic zone (1% light level; ca. 6- to 7-m depth).

**Size-fractionated respiration.** Respiration was about 9% of gross primary production (Table 2). There was no significant difference between total respiration and <63-μm respiration (Fig. 2; $P > 0.05$ [Wilcoxson signed-rank test]); thus, zooplankton respiration was negligible. About 75% of the respiration in the <3-μm fraction was bacterial; therefore, the overall ratio of algal photosynthesis/respiration was 15:1. If we assume that respiration was similar during the dark and light periods (a reasonable assumption [26]), then the relationship between maximum specific photosynthesis (see Table 4) and specific respiration was 25:1. Harris (26), in reviewing production/respiration ratios, stated that the most commonly encountered value was 10. Obviously this ratio will vary depending on the physiological state and species composition of the phytoplankton community. In general, diatoms dominate when the mixed layer in the lake is greater than the euphotic zone, and during such situations high production/respiration ratios are common (26). The $^{14}C$ method is usually considered to measure something between net and gross primary production (26, 47, 48) because of uncertainties in measuring the respiratory loss of $CO_2$ from natural phytoplankton. The close agreement between $^{14}C$ incorporation and the actual increase in phytoplankton biomass described above indicates that respiration was minimal, and this was confirmed by the oxygen method. However, during most of the year it is impossible to use our size fractionation technique to separate phytoplankton and zooplankton respiration.

**Photosynthetic quotient.** The correlation between total $^{14}C$ assimilation and gross primary production (oxygen method) was exceptionally good ($r$ = 0.999). The photosynthetic quotient was 1.63 ± 0.08 (Table 2). Our experimental PQ can be compared to a theoretical PQ (see above). From 21 April through 7 May, 1,000 mg-atoms (mg-at) of C $m^{-2}$ was assimilated and 160 mg-at $m^{-2}$ $NO_3$ N was consumed, a C/$NO_3$ N assimilation ratio (atomic basis) of 6.25:1. This gives a calculated PQ of 1.57, in good agreement with our experimentally derived PQ. $NH_4$ remained constant at about 0.6 mg-at $m^{-3}$ (Table 1) until increasing to over 2 mg-at $m^{-3}$ after 5 May. This does not necessarily imply that no ammonium was assimilated since ammonium can be regenerated via excretion and grazing. However, if reliable $NH_4$ regeneration rates (35) are applied to a potential zooplankton biomass of 0.1 mg (dry weight) $liter^{-1}$, at best 2 mg-at of $NH_4$ N was regenerated during the bloom. If all the regenerated $NH_4$ N was assimilated by the phytoplankton, $NH_4$ would have constituted about 20% of the total assimilated inorganic nitrogen. In general, $NH_4$ is the preferred N source for phytoplankton (1, 5), but Berman et al. (5) found that at times during the *Peridinium* bloom in Lake Kinneret, when ambient $NO_3$ N is high and ambient $NH_4$ N is low (<1 mg-at $m^{-3}$), the bulk of assimilated N may be derived from nitrate. Our results support the findings of Williams and co-workers (17, 69, 71) and emphasize the importance of evaluating the effect of nitrate on the PQ, when comparing the $^{14}C$ and $O_2$ methods.

**2-h versus 24-h $^{14}C$ incubations.** We compared a 24-h incubation with a sequence of 2-h incubations on 3 and 4 May (Table 4). Since the 2-h incubations were with 20-ml samples drawn from a 5-liter sample incubating at a 1-m depth, this was also an indirect test of bottle volume on $^{14}C$ uptake. We found no evidence of any discrepancy. It is apparent from Table 4, however, that dark $^{14}C$ uptake in 2-h incubations was nearly equal to the total dark uptake in the 24-h incubation. This is because most of the algal dark $^{14}C$ fixation occurs during the first 30 min of incubation (34). Even if we did not subtract the dark uptake from the 2-h incubations, the sum of the $^{14}C$ light assimilation in the sequence of 2-h incubations was still only 8% greater than

TABLE 3. Phytoplankton growth rate estimates[a]

| Method (C/Chl *a*) | Growth measured in: | | |
|---|---|---|---|
| | $\mu$[b] | $k$[c] | $\mu/\mu_{max}$[d] |
| $^{14}C$ at optimal depth[e] | | | |
| 50 | 0.20 | 0.29 | 0.27–0.45 |
| 30 | 0.34 | 0.49 | 0.46–0.78 |
| 20 | 0.48 | 0.69 | 0.65–1.08 |
| Total phytoplankton abundance[f] | 0.15 | 0.22 | 0.22–0.35 |
| *Stephanodiscus hantzchii* var. *pusillus*[f] | 0.25 | 0.36 | 0.33–0.56 |

[a] Average for the entire study period.
[b] $\mu$ = $day^{-1}$.
[c] $k = \mu/0.693$; $k$ = doublings $day^{-1}$.
[d] Data used to derive equation 3 (18) are in doublings $day^{-1}$; in our comparison $\mu/\mu_{max}$ is actually ($k/\mu_{max}$).
[e] Equation 2.
[f] Growth rate for the mixed layer (water column).

TABLE 4. Total $^{14}C$ assimilation: comparison of 2-h and 24-h incubations, 3 to 4 May[a]

| Incubation period | $^{14}C$ assimilated (μg of C liter$^{-1}$ time$^{-1}$) | | |
|---|---|---|---|
| | Total | Dark | Blank |
| 10 a.m.–12 noon | 56.8 | 2.6 | |
| 12 noon–2 p.m. | 60.9[b] | 2.6 | |
| 2 p.m.– 4 p.m. | 44.8 | 2.7 | |
| 4 p.m.–6 p.m. | 40.2 | 2.3 | |
| 6 p.m.–8 p.m. | 29.3 | 2.7 | |
| 8 p.m.–10 p.m. | 5.1 | 2.2 | |
| 10 p.m.–12 a.m. | | 2.5 | 2.1 |
| 12 a.m.–6 a.m. | | 2.7 | 2.0 |
| 6 a.m.–8 a.m. | 41.1 | 2.5 | |
| 8 a.m.–10 a.m. | 36.8 | 2.4 | |
| Σ (light-dark)[c] | 295.0 | | |
| 24-h incubation ($n$ = 2)[c] | 297.8 | 3.0 | 2.1 |
| 24-h (light-dark)[c,d] | 294.8 | | |

[a] A 5-liter mixed sample collected at 9:30 a.m. on 3 May was incubated at a depth of 1 m. At 2-h intervals, samples (20 ml) were withdrawn and incubated with 2 μCi of $NaH^{14}CO_3$.
[b] The maximum light-saturated rate of specific photosynthesis equals 58.3 μg of C liter$^{-1}$ 2 h$^{-1}$/14.7 μg of Chl *a* liter$^{-1}$ (see Table 2) equals 2 μg of C μg of Chl *a*$^{-1}$ h$^{-1}$. The specific rate on a per-hour basis can also be estimated by dividing the daily rate by a factor of 10. For example, from Table 2 on 3 May, 20.1 μg of C μg Chl *a*$^{-1}$ day$^{-1}$/10 = 2 μg of C μg of Chl *a*$^{-1}$ h$^{-1}$.
[c] Sum of the 2-h incubations. As micrograms of carbon per liter per day.
[d] See Table 2.

TABLE 5. Bacterioplankton production estimates for Lake Erken

| Date | Bacterioplankton (μg of C liter$^{-1}$ day$^{-1}$) measured by: | | | |
|---|---|---|---|---|
| | [3H]thymidine incorporation[a] | $^{14}CO_2$ dark uptake for: Fraction[b] | $^{14}CO_2$ dark uptake for: Total[c] | EOC uptake[d] |
| 18.4 | 0.4–0.5 | 1.7 | 3.9 | 0.24 |
| 21.4 | [e] | 0.3 | 1.6 | 1.85 |
| 22.4 | | —[f] | 1.9 | 1.8 |
| 23.4 | 0.5–0.7 | — | — | — |
| 24.4 | 0.6–0.8 | — | 0.5 | 1.25 |
| 26.4 | 1.2–1.6 | — | — | 1.9 |
| 28.4 | 1.0–1.3 | 1.6 | 1.3 | 1.1 |
| 29.4 | 1.6–2.1 | — | 6.9 | 1.3 |
| 1.5 | 1.4–1.9 | 4.4 | 1.2 | 1.7 |
| 3.5 | 1.4–1.9 | 4.1 | 4.4 | 2.3 |
| 5.5 | 1.6–2.1 | 3.1 | 4.3 | 2.3 |
| 6.5 | 1.4–1.9 | | | |
| 7.5 | 4.8–7.6 | 7.4 | 11.4 | 2.7 |

[a] Range due to conversion factor of $1.6 \times 10^9$ to $2.1 \times 10^9$ cells nmol$^{-1}$.
[b] Calculated from 0.2- to 1-μm size fractions (see text).
[c] Calculated as total dark $^{14}CO_2$ uptake × 0.28, where 0.28 is the fraction of total dark uptake <1 μm. Calculated under the assumption that $CO_2$ dark uptake was 6% of total carbon uptake.
[d] Fraction (0.2 to 1 μm) (corrected for dark uptake) from $^{14}C$ light bottle incubations.
[e] Blank space, No measurements were made.
[f] —, Calculations were not possible due to overlapping blank and sample values.

$^{14}C$ uptake during the 24-h period. The correct procedure would be to estimate only the bacterial and inactive algal dark $^{14}C$ incorporation (34). Li and Harrison (36), in experiments conducted during a 24-h photoperiod in the Canadian Arctic, found a close correspondence between $^{14}C$ incorporation into protein in 32 h of incubation and in a sequence of short (2-h) incubations. Since growth and division are more closely dependent on protein synthesis than on synthesis of storage products, we reasoned that this is the best explanation for the close agreement between short and long incubations in the spring bloom situation that we investigated.

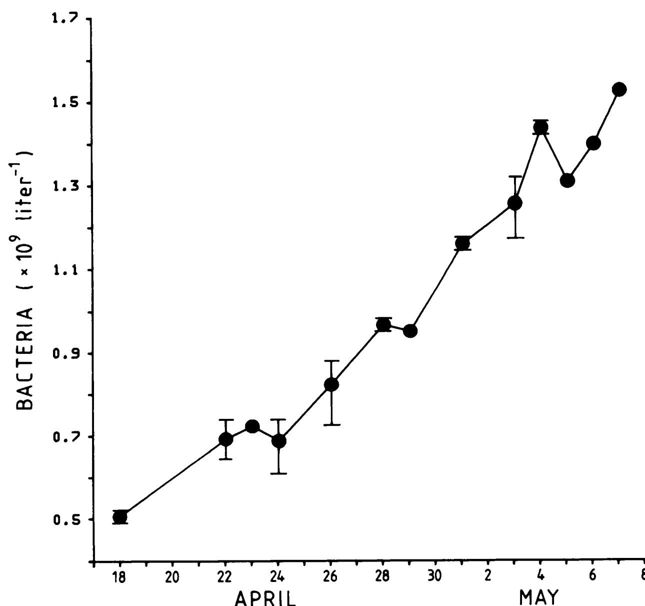


FIG. 3. Development of bacterioplankton during the spring bloom. Vertical bars are the ranges of duplicate or triplicate determinations.

**Bacterioplankton development and production.** The total increase in bacteria was $10^9$ liter$^{-1}$ (Fig. 3). In contrast to the increase in phytoplankton, this increase was linear instead of exponential. The average cell volume was 0.083 ± 0.017 μm$^3$. A day-to-day comparison of several production estimates is shown in Table 5. The daily averages of the various production estimates encompassed a range of 0.5 to 3 μg of C liter$^{-1}$ day$^{-1}$ (Table 6). The estimate of bacterial respiration was in the same order of magnitude. All of the production estimates were at least as great as the net increase in bacterial abundance as determined by acridine orange direct counts (AODC). There was a very significant relationship between AODC and Chl *a* as described by the following relationship: log AODC = 5.391 + 0.576 log Chl *a* ($r^2$ = 0.99; $n$ = 11).

Interestingly, bacterial uptake of EOC was equal to the bacterial production estimate by the [$^3$H]thymidine incorporation method (Tables 5 and 6). The calculation of bacterial

TABLE 6. Average bacterioplankton production estimates for Lake Erken

| Estimation method | Estimate (μg of C liter$^{-1}$ day$^{-1}$)[a] |
|---|---|
| [$^3$H]thymidine incorporation[b] | 1.2–1.7 |
| Bacterial uptake of EOC (0.2–1-μm fraction)[b] | 1.7 |
| Dark uptake of $^{14}CO_2$ (0.2–1-μm fraction)[b] | 2.4–3.0 |
| [$^3$H]glucose uptake[c] | 0.02–0.5 |
| Biomass increase (change in AODC) | 0.5 |
| Bacterial respiration[d] | 4.8 |
| Estimated bacterial growth yield[e] | 20–27 |

[a] Integrated average for the period 18 April to 7 May.
[b] See footnotes to Table 5.
[c] Assumes an ambient glucose concentration of 1 to 20 μg liter$^{-1}$ (73).
[d] <3-μm fraction corrected for algal respiration (see text).
[e] Growth yield = [production/(production plus respiration)] × 100; production is based on the [$^3$H]thymidine estimate. Data for this determination are given in percent.

production from the uptake of algal EOC, by using size fractionation data, required several assumptions. The first problem was achieving a good separation of algae and bacteria. Several studies (e.g., 33) have shown that autotrophic picoplankton pass through Nuclepore filters with pore sizes of 3 μm. We observed no cells smaller than 2 μm in Lake Erken, and since we filtered in sequence, it is unlikely that algae would pass a second filter of 1-μm pore size. Still, this uncertainty must be kept in mind when evaluating the results.

A second problem was the kinetics of extracellular release. If an endpoint incubation is terminated before steady state is achieved, the true release rate will be underestimated. Time series experiments often show dissolved $^{14}C$ accumulation to a constant value (steady state) after 2 to 4 h (14, 32, 39, 66) assumed to represent (i) attainment of isotopic equilibrium or (ii) the balanced release of EOC and heterotrophic utilization of EOC, or both. Theoretical studies, however, indicate that at least 24 h may be required before isotopic equilibrium is attained (29). Despite these uncertainties, a reasonable conclusion is that the bulk of substrate for bacterial production was derived from algal excretory products of photosynthesis. Brock and Clyne (9) found instances during April and May when algal EOC in Lake Mendota supported 67 to 100% of total heterotrophic bacterial production.

The quantity of EOC transferred to bacteria (i.e., the percentage of released EOC that is recovered in the bacterial size fraction) is often greater than 50% (14, 52). In the present study, although 44 μg of C liter$^{-1}$ day$^{-1}$ of EOC was excreted (27% of total $^{14}C$ assimilation), only 1.7 μg of C liter$^{-1}$ day$^{-1}$ appeared in the bacterial size fraction. Similarly low rates of EOC utilization (2 μg of C liter$^{-1}$ day$^{-1}$) occur in a Norwegian fjord (4). A combination of low temperature (14) and high-molecular-weight (i.e., recalcitrant) extracellular products (11, 12) could have contributed to the low utilization of EOC by the bacterioplankton in Lake Erken.

The sudden increase in bacterial activity by 7 May was shown by several different methods (Fig. 2; Tables 5 and 7). An increase in the rate of [$^3$H]thymidine incorporation was observed during the evening of 6 May (Table 7), and the incorporation rate was still high on the morning of 7 May. The increase was not due to a bottle artifact since the thymidine incorporation rate was equally high in a water sample collected on the morning of 7 May. No increase in EOC uptake by the bacteria was evident on 7 May (Table 5), suggesting that the increase in bacterial production was not due to a qualitative change in the composition of the EOC.

TABLE 7. Diurnal variation in [$^3$H]thymidine incorporation rates, 6 to 7 May[a]

| Day and time | Rate ($10^{-12}$ mol liter$^{-1}$ h$^{-1}$) |
|---|---|
| 6 May | |
| 12 noon | 2.7 |
| 6 p.m. | 3.9 |
| 10 p.m. | 12.1 |
| 7 May | |
| 12 noon | 12.7 |
| 12 noon (new sample)[b] | 12.5 |

[a] Sample collected on 6 May was incubated in a 5-liter flask at a depth of 1 m; samples for incubation with [$^3$H]thymidine were withdrawn from this flask.
[b] Water collected from the pelagic region on 7 May.

The increase in bacterial activity was possibly a response to the increased availability of ammonium (Table 1).

**Bacterial growth yield.** The bacterial growth yield was 20 to 27% (Table 6). Estimates of growth yield based on measuring $^{14}CO_2$ released during incubations with $^{14}C$-labeled organic compounds (e.g., glucose, acetate, glutamate, etc.) are often ≥50% (% respiration, <50%), but a wide range of estimates for growth yield have been reported (16 to 98%; references summarized in reference 31). An assessment of growth yield is important since high growth yields imply that bacteria are inefficient remineralizers of organic matter. Current theoretical discussions on the trophic role of bacterioplankton assume that bacterioplankton have a growth efficiency of 50% (2, 68). However, if different substrates are respired to widely varying degrees by the same bacterial populations (16, 44), then it may be precarious to make presumptions about growth yields of bacterioplankton from measurements of $^{14}CO_2$ release unless many compounds are tested. Simultaneous assessments of bacterial production and respiration are needed since it seems plausible that growth yield and growth rate are correlated.

Lower growth efficiencies (10 to 40%) have been estimated by methods other than measuring the $^{14}CO_2$ release from low-molecular-weight organic compounds (31). In this context we see no a priori reason to assume that our estimate of growth yield is too low, but our method for the estimation of respiration also has uncertainties. Prefractionation of plankton samples is mainly used to (i) monitor the increase in bacterioplankton in the absence of grazers as an estimator of bacterial productivity (72) and (ii) assess the respiratory activity of different size fractions of the plankton (67, 68). At the same time, interactions between the different size fractions are disrupted by the fraction procedure. Grazers, for example, are also a source of regenerated nutrients (35). Likewise, depending on whether bacteria mainly utilize allochthonous or autochthonous carbon sources, the separation of bacteria from phytoplankton can be a serious artifact. We acknowledge the potential artificiality of measuring bacterial respiration in a situation where particles of >3 μm have been removed; nonetheless, the magnitude of the respiration estimate does lend credibility to the estimates of bacterioplankton production.

**Assessing the [$^3$H]thymidine method.** The rate of incorporation of [$^3$H]thymidine into cold-TCA-insoluble material averaged $0.7 \times 10^{-10}$ mol liter$^{-1}$ day$^{-1}$, equivalent to ~$2.6 \times 10^9$ cells produced per liter during the entire study period. This result implies that >50% of the cells were eliminated (compare Fig. 3). At least four factors could contribute to the difference between the actual (net) increase in AODC and the increase predicted from the rate of [$^3$H]thymidine incorporation. (i) Uncertainty in the conversion factor relating thymidine incorporation to cells produced cannot be discounted. Still, the factor we used was within the range reported by Fuhrman and Azam (22) and considered by these workers to provide a conservative estimate of production. Also, the factors used to convert cell volumes to cell carbon encompass a range of $0.94 \times 10^{-13}$ g of C μm$^{-3}$ to $1.43 \times 10^{-13}$ g of C μm$^{-3}$ (24). The factor we used (64) is in the middle of this range. (ii) The assumption that [$^3$H]thymidine incorporation rates measured during midday can be converted to daily rates may be false. On two occasions (excluding 6 to 7 May) when we measured rates of thymidine incorporation at 8 p.m., the rates were about 25% higher than during midday (data not shown), but we made no incubations during the early morning. In one intensive study, chemoheterotrophic activity showed a late-evening peak and

an early-morning minimum, with midday incubations close to the daily average (based on 84 diel measurements [38]). (iii) Cell lysis may be a loss mechanism, but experimental evidence is lacking. (iv) Grazing by microflagellates may crop the bacterioplankton. We can show, theoretically at least, that 50% of the bacteria could have been grazed. The average density of microflagellates (3 to 5 μm) in Lake Erken was $2.1 \times 10^3 \pm 1 \times 10^3$ ml$^{-1}$ (an underestimate since epifluorescence was not used to enumerate flagellates). Unfortunately, heterotrophic microflagellates were not specifically enumerated, but if 15 to 20% of the flagellates were phagotrophic (37) and all elimination is assumed to be caused by grazing, then 10 bacteria flagellate$^{-1}$ h$^{-1}$ ($10^{-5}$ ml flagellate$^{-1}$ h$^{-1}$) were consumed. This rate is within the range of values in the literature (20, 37, 56). We conclude as did Fuhrman and Azam (22) that the estimates of bacterioplankton production from [$^3$H]thymidine incorporation rates are within a factor of two of the actual production rates. If we assume that bacterioplankton production was similar throughout the water column, then gross bacterial production (production plus respiration) was 20% of gross primary production, as milligrams of C per square meter. As temperatures increase and the spring diatom bloom collapses, bacterioplankton production may increase to an even greater percentage of primary production.

**Conclusions.** The spring bloom provided an ideal opportunity both to compare the various techniques commonly used to estimate primary production and bacterial production and to use the pre-size fractionation procedure (67). The agreement between our experimentally derived PQ and the theoretical PQ based on the C/$NO_3$ N assimilation ratio provides evidence from a freshwater environment in support of the studies of Williams and co-workers (17, 49, 69, 71) on the importance of considering the nitrogen source when comparing the $^{14}$C and $O_2$ methods. We point out that the agreement does not mean that these techniques are not without problems (26, 47, 48, 71). However, the reasonably good agreement between the integrated $^{14}$C production and the actual increase in phytoplankton biomass does suggest that, in the present investigation, the methods gave an accurate estimate of organic production and were not just a relative index of activity. One may argue that this agreement between methods would only occur during a short spring phytoplankton outburst when zooplankton respiration is minimal and the ratio of photosynthesis to algal respiration is high, but some studies have shown that the $^{14}$C method is reliable even when respiration is substantial and net primary productivity is negative (8, 69).

Algal excretory products appeared to provide the main source of energy for the bacterioplankton, a result not unexpected during a spring bloom. The bacterial growth yield of ~25% is lower than the presently accepted value of 50% (2, 31, 68). However, growth yields of 50% are perhaps more realistic when the bacterioplankton have generation times of 1 to 2 days. The bacterioplankton obviously grow slower, albeit steadily, during the early spring in Lake Erken. Our estimate of the growth yield is thus realistic if a low growth yield is correlated with a slow growth rate. Finally, we found no indication that the [$^3$H]thymidine method is underestimating bacterioplankton production (42, 51). Based on circumstantial evidence, microflagellate predation on the bacterioplankton was substantial, and we concur with the conclusion of Hagström (24) that finding alternative ways to measure consumption of bacteria by predators such as the flagellates may thus prove to yield the true growth rates of bacteria.


## ACKNOWLEDGMENTS

We thank Tuija Talsi for field assistance, Cecilia Eriksson and Kurt Pettersson for hospitality at the Lake Erken laboratory and for providing the nutrient data, Mats Nebaeus for counting the phytoplankton, and Jan Johansson for excellent technical assistance.

This work was supported by grant B-BU 3083-105 from the Swedish Natural Science Research Council and grants from the Malmén Foundation of Uppsala University (to R.B.) and the Walter and Andrée de Nottbeck Foundation (to J.K.).


## LITERATURE CITED


1. **Axler, R. P., R. M. Gersberg, and C. R. Goldman.** 1982. Inorganic nitrogen assimilation in a subalpine lake. Limnol. Oceanogr. **27**:53–65.
2. **Azam, F., T. Fenchel, J. G. Field, J. G. Gray, L. A. Meyer-Riel, and F. Thigstad.** 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. **10**:257–263.
3. **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.
4. **Bell, W., and E. Sakshaug.** 1980. Bacterial utilization of algal extracellular products. 2. A kinetic study of natural populations. Limnol. Oceanogr. **25**:1021–1033.
5. **Berman, T., B. F. Sherr, E. Sherr, D. Wynne, and J. J. McCarthy.** 1984. The characteristics of ammonium and nitrate uptake by phytoplankton in Lake Kinneret. Limnol. Oceanogr. **29**:287–297.
6. **Boström, B.** 1981. Factors controlling the seasonal variation of nitrate in Lake Erken. Int. Rev. Gesamten Hydrobiol. **66**:821–836.
7. **Boström, B., and K. Pettersson.** 1977. The spring development of phytoplankton in Lake Erken. Freshwater Biol. **7**:327–335.
8. **Bower, P., and D. McCorkle.** 1980. Gas exchange, photosynthetic uptake, and carbon budget for a radiocarbon addition to a small enclosure in a stratified lake. Can. J. Fish. Aquat. Sci. **37**:464–471.
9. **Brock, T. D., and J. Clyne.** 1984. Significance of algal excretory products for growth of epilimnetic bacteria. Appl. Environ. Microbiol. **47**:731–734.
10. **Bryan, J. R., J. P. Riley, and P. J. B. Williams.** 1976. A Winkler procedure for making precise measurements of oxygen concentration for productivity and related studies. J. Exp. Mar. Biol. Ecol. **21**:191–197.
11. **Chrost, R. H., and M. A. Faust.** 1983. Organic carbon release by phytoplankton: its composition and utilization by bacterioplankton. J. Plankton Res. **5**:477–493.
12. **Cole, J. J., W. H. McDowell, and G. E. Likens.** 1984. Sources and molecular weight of dissolved organic carbon in an oligotrophic lake. Oikos **42**:1–9.
13. **Coté, B., and T. Platt.** 1983. Day-to-day variations in the spring-summer photosynthetic parameters of coastal marine phytoplankton. Limnol. Oceanogr. **28**:320–344.
14. **Coveney, M. F.** 1982. Bacterial uptake of photosynthetic carbon from freshwater phytoplankton. Oikos **38**:8–20.
15. **Coveney, M. F., G. Cronberg, M. Enell, K. Larsson, and L. Olofsson.** 1977. Phytoplankton, zooplankton, and bacteria-standing crop and production relationships in a eutrophic lake. Oikos **29**:5–21.
16. **Cuehl, R. L., H. W. Jannasch, C. D. Taylor, and D. R. S. Lean.** 1983. Microbial growth and macromolecular synthesis in the northwestern Atlantic Ocean. Limnol. Oceanogr. **28**:1–18.
17. **Davies, J. M., and P. J. le B. Williams.** 1984. Verification of $^{14}$C and $O_2$ derived primary organic production measurements using an enclosed ecosystem. J. Plankton Res. **6**:457–474.
18. **Eppley, R. W.** 1972. Temperature and phytoplankton growth in the sea. Fish. Bull. **70**:1063–1084.
19. **Eppley, R. W., W. G. Harrison, S. W. Chisholm, and E. Stewart.** 1977. Particulate organic matter in surface waters off Southern California and its relation to phytoplankton. J. Mar. Res. **35**:671–696.
20. **Fenchel, T.** 1982. Ecology of heterotrophic microflagellates. IV.

Quantitative occurrence and importance as bacterial consumers. Mar. Ecol. Prog. Serv. **9**:35–42.

21. **Fuhrman, J. A., and F. Azam.** 1980. Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. Appl. Environ. Microbiol. **39**:1085–1095.
22. **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.
23. **Gieskes, W. W. C., G. W. Kraay, and M. A. Baars.** 1979. Current $^{14}C$ methods for measuring primary production: gross underestimates in oceanic waters. Neth. J. Sea Res. **13**:58–78.
24. **Hagström, Å.** 1984. Aquatic bacteria: measurements and significance of growth, p. 495–501. *In* M. J. Klug and C. A. Reddy (ed.), Current perspectives in microbial ecology. American Society for Microbiology, Washington, D.C.
25. **Hagström, Å., U. Larsson, P. Hörstedt, and S. Normark.** 1979. Frequency of dividing cells, a new approach to the determination of bacterial growth rates in aquatic environments. Appl. Environ. Microbiol. **37**:805–812.
26. **Harris, G. P.** 1978. Photosynthesis, productivity and growth: the physiological ecology of phytoplankton. Arch. Hydrobiol. Beih. Ergeb. Limnol. **10**:1–171.
27. **Hesslein, R. H., W. S. Broecker, P. D. Quay, and D. W. Schindler.** 1980. Whole-lake radiocarbon experiment in an oligotrophic lake at the experimental lakes area, Northwest Ontario. Can. J. Fish. Aquat. Sci. **37**:454–463.
28. **Heyman, U., G. Ekbohm, P. Blomqvist, and R. Grundström.** 1982. The precision of abundance estimates of plankton from composite samples. Water Res. **16**:1367–1370.
29. **Hobson, L. A., W. J. Morris, and K. T. Pirguet.** 1976. Theoretical and experimental analysis of the $^{14}C$ technique and its use in studies of primary production. J. Fish. Res. Board Can. **33**:1715–1721.
30. **Johansson, J. Å.** 1983. Seasonal development of bacterioplankton in two forest lakes in central Sweden. Hydrobiologia **101**:71–88.
31. **Joint, I. R., and R. J. Morris.** 1982. The role of bacteria in the turnover of organic matter in the sea. Oceanogr. Mar. Biol. Annu. Rev. **20**:65–118.
32. **Larsson, U., and Å. Hagström.** 1979. Phytoplankton extracellular release as an energy source for bacterial growth in a pelagic ecosystem. Mar. Biol. **52**:199–206.
33. **Larsson, U., and Å. Hagström.** 1982. Fractionated phytoplankton primary production, exudate release, and bacterial production in a Baltic eutrophication gradient. Mar. Biol. **67**:57–70.
34. **Legendre, L., S. Demers, C. M. Yentsch, and C. S. Yentsch.** 1983. The $^{14}C$ method: patterns of dark $CO_2$ fixation and DCMU correction to replace the dark bottle. Limnol. Oceanogr. **28**:996–1003.
35. **Lehman, J.** 1980. Nutrient recycling as an interface between algae and grazers in freshwater communities, p. 251–263. *In* W. C. Kerfoot (ed.), Evolution and ecology of zooplankton communities. University Press of New England, Hanover, N.H.
36. **Li, W. K. W., and W. G. Harrison.** 1982. Carbon flow into the endproducts of photosynthesis in short and long incubations of a natural phytoplankton population. Mar. Biol. **72**:175–182.
37. **Linley, E. A. S., R. C. Newell, and M. I. Lucas.** 1983. Quantitative relationships between phytoplankton, bacteria and heterotrophic microflagellates in shelf waters. Mar. Ecol. Prog. Ser. **12**:77–89.
38. **McKinley, K. R., and R. G. Wetzel.** 1979. Photolithotrophy, photoheterotrophy and chemoheterotrophy: patterns of resource utilization of an annual and a diurnal basis within a pelagic microbial community. Microb. Ecol. **5**:1–15.
39. **Mague, T. H., E. Friberg, D. L. Hughes, and I. Morris.** 1980. Extracellular release of carbon in marine phytoplankton: a physiological approach. Limnol. Oceanogr. **25**:262–279.
40. **Malone, T. C.** 1980. Algal size, p. 433–463. *In* I. Morris (ed.), Physiological ecology of phytoplankton. Blackwell Scientific Publishers, Ltd., Oxford.
41. **Nauwerck, A.** 1963. Die beziehungen zwischen Zooplankton und Phytoplankton im See Erken. Symb. Bot. Ups. **17**:1-163.
42. **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 measurements of thymidine incorporation. Microb. Ecol. **8**:33–46.
43. **Niemi, M., J. Kuparinen, A. Uusi-Rauva, and K. Korhonen.** 1983. Preparation of $^{14}C$ labelled algal samples for liquid scintillation counting. Hydrobiologia **106**:149–156.
44. **Novitsky, J. A.** 1983. Heterotrophic activity throughout a vertical profile of seawater and sediment in Halifax Harbor, Canada. Appl. Environ. Microbiol. **45**:1753–1760.
45. **Overbeck, J.** 1979. Dark $CO_2$ uptake-biochemical background and its relevance to in situ bacterial production. Arch. Hydrobiol. Beih. Ergeb. Limnol. **12**:38–47.
46. **Pechlaner, R.** 1970. The phytoplankton spring outburst and its conditions in Lake Erken (Sweden). Limnol. Oceanogr. **15**:113–130.
47. **Peterson, B.** 1978. Radiocarbon uptake: its relation to net particulate carbon production. Limnol. Oceanogr. **23**:179–184.
48. **Peterson, B.** 1980. Aquatic primary productivity and the $^{14}CO_2$ method: a history of the productivity problem. Annu. Rev. Ecol. Syst. **11**:359–385.
49. **Raine, R. C. T.** 1983. The effect of nitrogen supply on the photosynthetic quotient of natural phytoplankton assemblages. Bot. Mar. **26**:417–423.
50. **Riemann, B.** 1983. Biomass and production of phyto- and bacterioplankton in eutrophic Lake Tystrup, Denmark. Freshwater Biol. **13**:358–367.
51. **Riemann, B., and M. Søndergaard.** 1984. Measurements of diel rates of bacterial secondary production in aquatic environments. Appl. Environ. Microbiol. **47**:632–638.
52. **Riemann, B., M. Søndergaard, H.-H. Schierup. S. Bosselmann, G. Christensen, J. Hansen, and B. Nielsen.** 1982. Carbon metabolism during a spring diatom bloom in eutrophic Lake Mossø. Int. Rev. Gesamten Hydrobiol. **67**:145–185.
53. **Rodhe, W., R. A. Vollenweider, and A. Nauwerck.** 1958. The primary production and standing crop of phytoplankton, p. 299–325. *In* A. A. Buzzati-Traverso (ed.), Perspectives in marine biology. University of California Press, Berkeley.
54. **Romanenko, V. I., J. Overbeck, and Y. I. Sorokin.** 1972. Estimation of production of heterotrophic bacteria using $^{14}C$, p. 82–85. *In* Y. I. Sorokin and H. Kadota (ed.), Techniques for the assessment of microbial production and decomposition in freshwater. International Biological Programme handbook no. 23. Blackwell Scientific Publishers, Ltd., Oxford.
55. **Schindler, D. W., R. V. Schmidt, and R. A. Reich.** 1972. Acidification and bubbling as an alternative to filtration in determining phytoplankton production by the $^{14}C$ method. J. Fish Res. Board Can. **29**:1627–1631.
56. **Sherr, B. F., and E. B. Sherr.** 1984. Role of heterotrophic protozoa in carbon and energy flow in aquatic ecosystems, p. 412–423. *In* M. J. Klug and C. A. Reddy (ed.), Current perspectives in microbial ecology. American Society for Microbiology, Washington, D.C.
57. **Steeman Nielsen, E.** 1952. The use of radioactive carbon ($^{14}C$) for measuring organic production in the sea. J. Cons. Int. Expl. Mar. **18**:117–140.
58. **Strathmann, R. R.** 1967. Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. Limnol. Oceanogr. **12**:411–418.
59. **Talling, J. F.** 1957. The phytoplankton population as a compound photosynthetic system. New Phytol. **56**:133–149.
60. **Talling, J. F.** 1971. The underwater light climate as a controlling factor in production ecology of freshwater phytoplankton. Mitt. Int. Ver. Theor. Angew. Limnol. **19**:214–243.
61. **Tolstoy, A.** 1980. Information on the standardization of pigment methodology in Nordic countries. Arch. Hydrobiol. Beih. Ergeb. Limnol. **14**:81–87.
62. **Väätänen, P.** 1979. Microbial activity in brackish water determined as oxygen consumption. Arch. Hydrobiol. Beih. Ergeb. Limnol. **12**:32–37.
63. **Vollenweider, R. A.** 1965. Calculation models of photosynthesis-

depth curves and some implications regarding day rate estimates in primary production measurements. Mem. Ist. Ital. Idrobiol. **18**(Suppl.):425–457.

64. **Watson, S. W., T. J. Novitsky, H. L. Quinby, and F. W. Valois.** 1977. Determination of bacterial number and biomass in the marine environment. Appl. Environ. Microbiol. **33**:940–954.
65. **Welschmeyer, N. A., and C. J. Lorenzen.** 1984. Carbon-14 labelling of phytoplankton carbon and chlorophyll *a* carbon: determination of specific growth rates. Limnol. Oceanogr. **29**:135–145.
66. **Wiebe, W. J., and D. F. Smith.** 1977. Direct measurements of dissolved organic carbon release by phytoplankton and incorporation by microheterotrophs. Mar. Biol. **42**:213–223.
67. **Williams, P. J.** 1979. Microbial contribution to overall marine plankton metabolism: direct measurements of respiration. Oceanol. Acta **4**:359–364.
68. **Williams, P. J.** 1981. Incorporation of microheterotrophic processes into the classical paradigm of the planktonic food web. Kiel. Meeresforsch. **5**:1–28.
69. **Williams, P. J. B., K. R. Heinemann, J. Marra, and D. A. Purdie.** 1983. Comparison of $^{14}C$ and $O_2$ measurements of phytoplankton production in oligotrophic waters. Nature (London) **305**:49–50.
70. **Williams, P. J. B., and N. W. Jenkinson.** 1982. A transportable microprocessor-controlled precise Winkler titration suitable for field station and shipboard use. Limnol. Oceanogr. **27**:576–584.
71. **Williams, P. J. B., R. C. T. Raine, and J. R. Bryan.** 1979. Agreement between the $^{14}C$ and oxygen methods of measuring phytoplankton production: reassessment of the photosynthetic quotient. Oceanol. Acta **2**:441–416.
72. **Wright, R. T., and R. B. Coffin.** 1984. Factors affecting bacterioplankton density and productivity in salt marsh estuaries, p. 485–494. *In* M. J. Klug and C. A. Reddy (ed.), Current perspectives in microbial ecology. American Society for Microbiology, Washington, D.C.
73. **Wright, R. T., and J. E. Hobbie.** 1965. Use of glucose and acetate by bacteria and algae in aquatic ecosystems. Ecology **47**:447–464.