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# Bacterioplankton Secondary Production Estimates for Coastal Waters of British Columbia, Antarctica, and California

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The principal objective of this study was to quantify the rate of heterotrophic bacterioplankton production. Production was estimated by two approaches: (i) measurement of increasing bacterial abundance with time in filtered (3-μm pore size) seawater and (ii) estimation of bacterial deoxyribonucleic acid synthesis by tritiated thymidine incorporation in unfractionated seawater. The two approaches yielded comparable results when used at the Controlled Ecosystem Population Experiment (Saanich Inlet, British Columbia, Canada), at McMurdo Sound (Antarctica), and off Scripps Pier (La Jolla, Calif.). Estimated bacterioplankton production was lower in Antarctic samples (ranging from ~0 to 2.9 μg of C liter$^{-1}$ day$^{-1}$) than in those from the other two sites (ranging from 0.7 to 71 μg of C liter$^{-1}$ day$^{-1}$). In all three regions studied, it appeared that a significant fraction of the total primary production was utilized by the bacterioplankton and that substantial growth could occur in the absence of large particles. These results support the conclusion that bacterioplankton are a quantitatively important component of coastal marine food webs.

It is generally believed that bacterioplankton are an important metabolic component of the marine biota (8, 29, 30). However, the role of bacterioplankton in the cycling of organic matter has not been fully characterized. Whereas the significance of bacterial catabolic activity has been amply demonstrated, methodological problems have made it difficult to quantify the rates of biosynthesis and growth. Such information is necessary to determine the importance of bacterioplankton in the trophic dynamics of marine food webs.

Other investigators have measured bacterial growth under a variety of experimental conditions. Some workers have measured the growth rates of marine isolates on unsupplemented seawater (6, 18, 37), and Brock (3) has measured the growth of a single epiphytic species on its algal substrate, but extrapolation of these single-species results to natural mixed-species assemblages may not be valid, largely since it is not known whether these species are representative of the bacterioplankton as a whole. Sorokin (32) estimated the bacterial production of intact assemblages by dark uptake of $^{14}CO_2$, but his methods and results have been sharply criticized (2, 28). Sieburth et al. (31) estimated growth rates by measuring adenosine triphosphate (ATP) changes with time in diffusion chambers, but they found a disturbing initial loss of ATP in their chambers; therefore, interpretation of subsequent ATP increases as "natural" growth may be incorrect. Hagström et al. (13) used the frequency of dividing cells to estimate growth rates, but their use of laboratory cultures to calibrate the method may have biased their results. Karl (19) estimated growth rates from rates of stable ribonucleic acid (RNA) synthesis, but this method suffers from a number of drawbacks which are detailed below. The above studies suggest that a sizable fraction of carbon and energy from phytoplankton is channeled into bacterioplankton secondary production. However, the questions about methodology led us to examine the problem by different approaches.

We used two different approaches to estimate bacterioplankton secondary production. One was based on the premise that only growing cells incorporate radioactive thymidine into deoxyribonucleic acid (DNA), a point emphasized by Brock (3, 4) and used by Brock (3) and Tobin and Anthony (35) in aquatic ecological studies. We directly converted net thymidine incorporation (insoluble in cold trichloroacetic acid) to production estimates. Our other approach measured the increase in bacterial abundance with time in filtered (3-μm pore size) seawater which was presumably free of bacteriovores and large particles. Measurements in varied coastal environments not only confirmed the finding that bacterioplankton secondary production is quantitatively important in the marine food web, but

also supported the novel conclusion that net growth of free-living bacteria does occur at a significant rate.

## MATERIALS AND METHODS

**Sampling locations.** Samples were taken from three different locations. The first location was at the Controlled Ecosystem Population Experiment (CEPEX), located in Saanich Inlet, British Columbia, Canada (48° 40′ N, 123° 29′ W). For a description, see reference 25. Samples were taken from inside 1,300-$m^3$ enclosures and from outside water. The second sampling location was at McMurdo Sound, Antarctica. See Fig. 1 for specific locations. Sites were visited by helicopter or surface vehicle, and samples were taken in insulated containers to Eklund Biological Center, McMurdo Station, for further work. The third location was off Scripps Pier, La Jolla, Calif. (32° 53′ N, 117° 15′ W).

**Thymidine incorporation.** Thymidine incorporation in CEPEX and Scripps Pier samples was measured as follows. Replicate samples (≥10 ml) were incubated with various concentrations (depending on the experiment, usually 2 to 20 nM) of [*methyl*-$^3$H] thymidine (40 to 60 Ci/mmol; New England Nuclear Corp.). Incubation times ranged from a few minutes to a few hours, and time courses were performed frequently to check linearity. Incubation in the light did not increase the incorporation rate over incubation in the dark. Subsamples (3 to 10 ml) were chilled in an ice water bath for 1 min, and then an equal volume of ice-cold 10% trichloroacetic acid was added: After 5 min on ice, the mixture was filtered through a 25-mm HA membrane filter (0.45-μm nominal pore size; Millipore Corp.) and rinsed twice with 3 ml of ice-cold 5% trichloroacetic acid. The filter was then placed in a scintillation vial. In experiments after April 1979, 0.5 ml of 0.5 N HCl was added, and the vials were heated in a boiling water bath for 20 min to hydrolyze the DNA to minimize self-absorption. After cooling, 1.0 ml of ethyl acetate was added to dissolve the filter (~10 min required), and 10 ml of Aquasol-2 (New England Nuclear Corp.) was added. The radioactivity was assayed by liquid scintillation spectrometry in a Beckman LS-100C. Radioactivity measurements of intact filters containing nonhydrolyzed DNA in Aquasol-2 from earlier experiments were multiplied by a self-absorption correction factor of 1.54 which was determined during later experiments. Quenching was determined by the external standard ratio method and Beckman quenched standards. Blanks contained 0.1 to 0.5% Formalin or 0.7 μM $HgCl_2$.

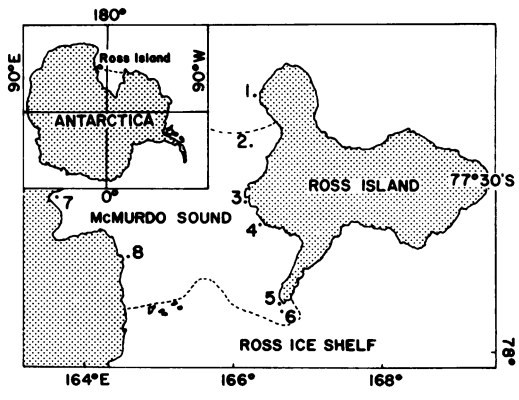


FIG. 1. *Sampling locations in McMurdo Sound. 1, Cape Bird; 2, Ice edge; 3, Cape Royds; 4, Cape Evans; 5, McMurdo Hole no. 2; 6, McMurdo Hole no. 3; 7, New Harbor; 8, Strand Moraines. Dashed line between 1 and 2 indicates the edge of annual ice during our sampling.*

Antarctic samples were obtained in acid-washed (but not sterilized), 5-liter Niskin bottles, except for one replicate sample (24 December 1978) obtained in a homemade polyethylene autoclaved sampler for comparison. All subsequent handling was with sterilized apparatus at 0 ± 1.5°C under simulated in situ light. Replicate 250-ml samples were placed in glass bottles or plastic Whirlpak bags. [*methyl*-$^3$H]thymidine (20 Ci/mmol; New England Nuclear Corp.) was added at 5.5 to 27.5 nM, and the samples were incubated for up to 36 h (see Table 1). Subsamples (50 ml) were removed, filtered through 47-mm PH membrane filters (0.30-μm nominal pore size; Millipore Corp.) in a polycarbonate filtration unit (vacuum, <18 cm of Hg), and rinsed three times with 5 ml of ice-cold filtered seawater. The vacuum hose was disconnected, and 10 ml of ice-cold 5% trichloroacetic acid was added. After 5 min, the vacuum was reapplied, and the filter was then rinsed twice with 5 ml of ice-cold 5% trichloroacetic acid. The funnel was removed, and the edge of the filter was rinsed twice with 3 ml of ice-cold 5% trichloroacetic acid and twice with 3 ml of ice-cold 95% ethanol. We subsequently learned that ethanol is not recommended for these filters, but laboratory tests have shown that few counts are lost by such a rinse. Radioactivity was assayed as described previously.

Addition of "cold" DNA (up to 1 mg $ml^{-1}$) to samples before addition of trichloroacetic acid increased recovery only slightly (less than 5%), whereas it increased filtration time by one order of magnitude. Therefore, we did not use this technique.

**Measurement of increase in bacterial abundance.** All handling was done with sterilized apparatus and filters, except where noted. Millipore filters used on live samples were prewashed to minimize contamination by wetting agents.

Surface water samples were collected in acid-washed, autoclaved flasks and kept at approximate in situ temperatures. Subsamples (100 ml or more) were gravity filtered through 47-mm-diameter, 3-μm-pore size Nucleopore filters in either an all-glass filtration unit or a Millipore Sterifil unit. The sample taken on 9 August 1978 was instead reverse flow filtered in situ. The 3-μm filtration was done to exclude bacteriovores while keeping injury of bacteria to a minimum. In some experiments, another subsample was cleared of most cells by filtration (vacuum, <13 cm of Hg) through an HA Millipore filter. When this was done, a subsample (≥10 ml) of the 3-μm filtrate was diluted 10-fold with the 0.45-μm filtrate. Incubation was at approximate in situ temperature and light. Samples were removed from both the 3-μm filtrate and the 10-fold-diluted filtrate at various times and either preserved in borate-buffered 1% formaldehyde or imme-

diately counted by epifluorescence microscopy of acridine orange-stained cells (14) with a Zeiss microscope with a catalog no. 487709 filter set. All preserved samples were counted within 2 weeks, and most counts were done in duplicate.

**Size fractionation.** Samples were size fractionated after incubation with [*methyl*-$^3$H]thymidine by filtration through 1.0-μm-pore size Nuclepore filters (1). Filterable organisms were collected on HA Millipore filters. Postfiltration was preferred because the incubation would be performed in a less perturbed system than with prefiltration.

**Biomass determination.** Size estimates of bacteria for biomass determination were made in three ways. In method 1, scanning electron microscopy of 1% glutaraldehyde- or 1% formaldehyde-preserved samples after critical-point drying in Freon-13 was carried out as described previously (7). Bacteria on 0.2-μm-pore size Nuclepore filters were photographed by scanning electron microscopy (Cambridge Stereoscan S4), and the volumes of rods and cocci were computed as though they were cylinders and spheres, respectively. In method 2, photographs of acridine orange-stained cells taken through the epifluorescence microscope (Leica eyepiece camera) were used for calculations similar to those used with the scanning electron photographs. In method 3, visual estimates of cell sizes were made using epifluorescence microscopy by dividing the cells into three size classes. Volumes were approximated by treating the three classes as 0.4-, 0.6-, and 0.8-μm-diameter spheres These sizes gave results most comparable to the scanning electron microscopy estimates when tested together. Volume was converted to biomass by multiplying by $1.21 \times 10^{-13}$ g of C $\mu m^{-3}$ (36).

**Bottle effect experiment.** All glassware was baked (2 h at 400°C) to eliminate organic contamination, and handling was aseptic. Water was collected off Scripps Pier (6 June 1979) and filtered through a 47-mm-diameter, 3.0-μm-pore size Nuclepore filter (vacuum, <2 cm of Hg). Filtrate was distributed as follows: 100 ml into each of three 150-ml beakers, 15 ml into each of four scintillation vials, and 500 ml into a 600-ml beaker. Lengths (3 cm each) of glass tubing (6-mm inside diameter and 8-mm outside diameter), which acted as removable surfaces, were added to the containers as follows: 10 to each 150-ml beaker, 3 to each scintillation vial, and 5 to the 600-ml beaker. Incubation was in the dark at 18°C. Cell biomass in the water was determined by epifluorescence cell counts of 5-ml samples or by ATP determinations (17, 20) of 25-ml samples, using HA Millipore filters to collect the cells, and 20 μg of purified luciferin was added per ml. Biomass on the surfaces was determined by a boiling tris(hydroxymethyl)aminomethane-ATP extraction of one tubing section at a time and assaying as above. Samples for both water and surface biomass were taken periodically for 30 h. Each scintillation vial was used only once. Total biomass on all surfaces was computed by assuming that the tubing surfaces were colonized to the same extent per unit area as the container walls. Surface/volume ratios changed somewhat during the course of the experiment due to removal of tubing sections and water and were 0.63 to 0.72 $cm^{-1}$ for the 600-ml beaker, 1.4 to 2.3 $cm^{-1}$ for the 150-ml beakers, and 3.78 $cm^{-1}$ for the scintillation vials.

**Primary production.** Primary production was measured by $^{14}CO_2$ uptake into particulate material (34). At CEPEX, incubations were made in situ from 1000 to 1400 local time; HA Millipore filters were used. The average uptake of two 125-ml samples minus the dark uptake was multiplied by 2 to get an estimate of daily production. McMurdo Sound measurements were from ~24-h incubations of 250-ml samples in situ (2 December 1978) or under simulated in situ conditions (29 December 1978 through 7 January 1979), and Gelman GF/C glass fiber filters were used. At a station ~10 km north of Scripps Pier, production was measured on 12 cruises (February 1975 through March 1979) of the Southern California Bight study by the Food Chain Research Group. Incubations of prefiltered (183-μm mesh), 250-ml samples were for 24 h, and glass fiber filters were used (10).

## RESULTS

**Growth rate estimates from thymidine incorporation.** Thymidine incorporation results are shown in Table 1. Biomass estimates necessary for derivation of production in terms of carbon are shown in Table 2.

At the one Antarctic station where replicate samples were collected in sterilized and nonsterilized samplers, no significant difference was found in thymidine incorporation rates for the two samples (Fig. 2; Wilcoxon signed rank test, $P > 0.50$). This result, combined with the near-zero incorporation rate in western McMurdo Sound samples, leads us to believe that our Antarctic samples collected with the acid-washed Niskin bottles were uncontaminated.

Size fractionation of thymidine uptake at CEPEX (25 July 1978), McMurdo Sound (3 January 1979), and Scripps Pier (14 August 1979) showed that 97, 91, and 93%, respectively, of the total uptake occurred in particles which passed through a 1.0-μm-pore size Nuclepore filter. Organisms filterable through a 1.0-μm-pore size Nuclepore filter contained 95% of the total trichloroacetic acid-insoluble radioactive material in the Scripps Pier sample.

Isotope dilution appeared insignificant when examined by measuring incorporation at various thymidine concentrations. The rates were only slightly increased by 5- or 10-fold increases in concentration over that used in other experiments (Table 3).

**Growth rate estimates from increasing bacterial abundance measurements.** The time courses of cell count increases in diluted and undiluted 3-μm filtrates are shown in Fig. 3. All samples from CEPEX and Scripps Pier had significant increases with time (*t*-test of correlation coefficient, $P < 0.05$), except the CEPEX undiluted sample taken on 18 May, which could

TABLE 1. *Production estimates derived from thymidine incorporation rates*

| Location[a] | Date | Incubation time (h) | Concn (nM) of thymidine added | Cells liter$^{-1}$ ($\times 10^9$) | Production | |
|---|---|---|---|---|---|---|
| | | | | | Cells liter$^{-1}$ day$^{-1}$[b] | $\mu$g of C liter$^{-1}$ day$^{-1}$[c] |
| CEPEX | | | | | | |
| Outside | 6/29/78 | 1.1 | 1.76 | | $0.56–3.6 \times 10^9$ | 7.2–47 |
| CEE3 | 8/1/78 | 1.0 | 1.76 | 1.4 | $0.68–4.3 \times 10^9$ | 8.9–57 |
| CEE3 | 8/3/78 | 0.5 | 1.76 | 2.5 | $0.51–3.2 \times 10^9$ | 6.6–42 |
| CEE2 | 8/4/78 | 0.5 | 17.6 | 2.5 | $0.85–5.4 \times 10^9$ | 11–71 |
| Antarctic | | | | | | |
| MCM2 | 12/18/78 | 22 | 5.5 | 0.25 | $1.7–11 \times 10^6$ | 0.013–.089 |
| MCM2 | 12/20/78 | 23 | 5.5 | 0.19 | $0.20–1.3 \times 10^7$ | 0.018–0.11 |
| MCM2 | 12/24/78 | 14 | 5.5 | 0.30 | $0.60–3.9 \times 10^7$ | 0.050–0.32 |
| MCM3 | 12/27/78 | 6 | 11 | 0.65 | $1.6–9.8 \times 10^7$ | 0.13–0.83 |
| CB | 12/29/78 | 23 | 5.5 | 0.30 | $1.0–6.5 \times 10^7$ | 0.086–0.56 |
| CR | 12/29/78 | 23 | 5.5 | 0.62 | $0.43–2.8 \times 10^8$ | 0.38–2.4 |
| CE | 12/29/78 | 23 | 5.5 | 0.73 | $0.40–2.6 \times 10^8$ | 0.33–2.1 |
| SM | 1/1/79 | 36 | 27.5 | 0.07 | $0.73–4.8 \times 10^5$ | 0.00061–0.0039 |
| NH | 1/1/79 | 36 | 27.5 | 0.06 | $1.1–7.4 \times 10^5$ | 0.00042–0.0061 |
| MCM2 | 1/3/79 | 3.4 | 11 | 0.95 | $0.28–1.8 \times 10^8$ | 0.23–1.4 |
| CB | 1/5/79 | 2.3 | 11 | 1.0 | $0.42–2.7 \times 10^8$ | 0.38–2.4 |
| IE | 1/5/79 | 2.3 | 11 | 0.93 | $0.54–3.4 \times 10^8$ | 0.44–2.9 |
| MCM2 | 1/7/79 | 5.9 | 11 | 0.96 | $0.36–2.3 \times 10^8$ | 0.26–1.7 |
| Scripps Pier | 3/6/79 | 1 | 4 | | $0.98–6.2 \times 10^8$ | 2.0–13 |
| | 3/21/79 | 2.5 | 4 | | $0.36–2.3 \times 10^8$ | 0.7–4.7 |
| | 5/11/79 | 1 | 4 | 1.2 | $0.41–2.7 \times 10^9$ | 8.3–53 |
| | 5/16/79 | 1 | 20 | 2.9 | $0.46–3.0 \times 10^9$ | 2.2–13 |
| | 5/18/79 | 1 | 8.7 | 1.6 | $0.13–8.8 \times 10^8$ | 2.2–13 |
| | 8/1/79 | 0.5 | 4.4 | 0.66 | $0.13–8.8 \times 10^8$ | 2.2–13 |

[a] CEE3 and CEE2, Controlled ecosystem enclosures 3 and 2, respectively; MCM2, McMurdo Hole no. 2; MCM3, McMurdo Hole no. 3; CB, Cape Bird; CR, Cape Royds; CE, Cape Evans; SM, Strand Moraines; NH, New Harbor; IE, ice edge.

[b] Moles of thymidine incorporated liter$^{-1}$ day$^{-1}$ were converted to cells liter$^{-1}$ day$^{-1}$ by multiplying by $0.2 \times 10^{18}$ to $1.3 \times 10^{18}$. This range reflects uncertainty in DNA per cell (see text).

[c] Estimates were computed by multiplying cells liter$^{-1}$ day$^{-1}$ by average per cell biomass. Regional averages were used when no specific measurement was made.

not be statistically tested. Not shown are two Antarctic samples (from 28 December 1978 and 3 January 1979) which had no significant abundance increases in undiluted samples (the sample taken on 28 December increased 1% in 11 h, and the sample taken on 3 January decreased 15% in 14 h).

Bacterial secondary production estimates were made from the data in Fig. 3 by computing the increase in cell number (i.e., the measured increase in 3-$\mu$m filtrates or increases in diluted samples extrapolated to the original bacterial abundance) and multiplying by the average biomass per cell for that sample. Since the average range of all 23 pairs of duplicate counts was only 13% of the mean between the duplicates, we concluded that the nonduplicated counts were acceptable for use in our calculations. The results are tabulated in Table 4.

In five instances, thymidine incorporation was measured in subsamples taken from the cell count growth experiments. Production estimates from thymidine incorporation for undiluted samples agreed well with cell counts, but those for diluted samples were low (Fig. 4).

While counting cells with the microscope, we found that cells did not decrease in size during the incubation as starved cells may do (27). Also, the cells (including those in unfiltered samples) appeared singly or occasionally in small colonies, and few, if any, were visibly attached to detrital particles.

In the bottle effect experiment, the cell counts from the three different surface/volume ratio containers were within 5% of each other and paralleled the ATP measurements from the 600-ml beaker (Fig. 5). ATP on the walls was negligible up to 15 h, rose to 3 to 5% of the total ATP by 22 h, and was 8 to 14% of the total by 30 h.

**Primary production.** Primary production by CEPEX was 40 to 240 $\mu$g of C liter$^{-1}$ day$^{-1}$ during the time that we sampled. McMurdo

TABLE 2. *Estimates of average biomass per cell*

| Location[a] | Date | Method[b] | Biomass (fg of C)[c] |
|---|---|---|---|
| CEPEX | | | |
| CEE2 | 7/29/78 | V | 16 |
| CEE2 | 8/4/78 | V | 13 |
| CEE2 | 8/9/78 | V | 17 |
| CEE2 | 8/21/78 | S | 9.7 ± 3.3 |
| CEE2 | 9/15/78 | V | 12 |
| outside | 9/20/78 | V | 10 |
| Avg | | | 13 |
| Antarctic | | | |
| CR | 12/28/78 | S | 8.7 ± 1.2 |
| CB | 1/5/79 | S | 9.1 ± 0.9 |
| MCM2 | 1/7/79 | S | 7.0 ± 0.6 |
| Avg | | | 8.3 |
| Scripps Pier | 5/16/79 | S | 4.6 ± 0.6 |
| | 5/18/79 | P | 16 |
| | 5/29/79 | S | 39 ± 8 |
| Avg | | | 20 |

[a] CEE2, Controlled ecosystem enclosure no. 2.; CR, Cape Royds; CB, Cape Bird; MCM2, McMurdo Hole no. 2.

[b] V, Visual estimate; P, photograph by epifluorescence; S, scanning electron microscopy.

[c] Estimates determined by scanning electron microscopy have 95% confidence limits based on the *t* distribution.

Sound productivity was increasing while we were there and was unmeasurable on the west side and also unmeasurable near McMurdo Hole no. 2 on 2 December 1978 (O. Holm-Hansen, personal communication), but ranged from 1.7 μg of C liter$^{-1}$ day$^{-1}$ at McMurdo Hole no. 2 on 3 January 1979 to 26 μg of C liter$^{-1}$ day$^{-1}$ at Cape Bird on 5 January 1979 (S. G. Horrigan, personal communication). Twelve measurements of productivity made near Scripps Pier ranged from 8 to 288 μg of C liter$^{-1}$ day$^{-1}$, with a mean of 57 μg of C liter$^{-1}$ day$^{-1}$.

## DISCUSSION

Thymidine incorporation, defined here as the amount of all cold trichloroacetic acid-insoluble material produced from the added radioactive thymidine, is not necessarily a direct measurement of DNA synthesis, let alone production. Several assumptions or measurements are required to convert thymidine incorporation into a production estimate. Our assumptions are as follows.

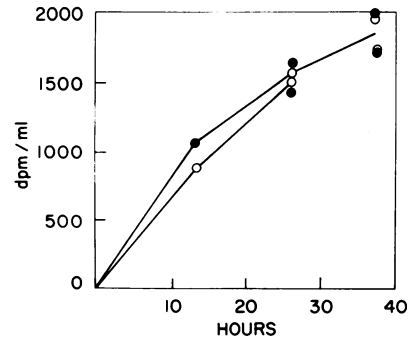


FIG. 2. *Thymidine incorporation rates for water from sterilized (●) and nonsterilized (○) samplers, 24 December 1978.*

TABLE 3. *Relative thymidine incorporation rates at different thymidine concentrations*

| Location[a] | Date | Concn (nM) of thymidine added | Relative incorporation rate[a] |
|---|---|---|---|
| CEPEX CEE2 | 8/4/79 | 0.176 | 0.262 |
| | | 1.76[b] | 0.986 |
| | | 17.6 | 1.00 |
| Antarctic MCM2 | 1/3/79 | 2.2 | 0.773 |
| | | 11[b] | 0.963 |
| | | 55 | 1.00 |
| Scripps Pier | 5/16/79 | 1 | 0.539 |
| | | 4[b] | 0.875 |
| | | 20 | 1.00 |

[a] Relative rate is computed from slopes of two- or three-point time courses of thymidine incorporation. CEE2, Controlled ecosystem enclosure 2; MCM2, McMurdo Hole no. 2.

[b] Most experiments in this region used approximately this concentration.

(i) Only bacteria can utilize thymidine added at the low concentrations used (usually a few nanomolar). This was supported by size fractionation experiments, which demonstrated that >90% of the total thymidine uptake was in <1.0-μm-size particles.

(ii) All bacteria can utilize exogenous thymidine during growth. This would be impossible to verify, but it is the most conservative assumption. The existence of significant numbers of bacteria unable to utilize thymidine would make our estimates too low.

(iii) The amount of incorporated label in DNA is 80% of the total cold trichloroacetic acid-insoluble label. We have found that a 5 to 20% fraction (15; and unpublished data) of the total trichloroacetic acid-insoluble label is resistant to treatment (5% trichloroacetic acid, 100°C, 30

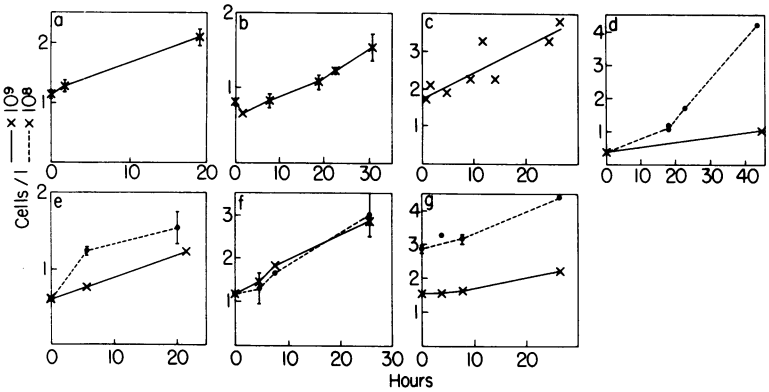


FIG. 3. *Increase in bacterial abundance with time in 3-μm filtrates (×) and 3-μm filtrates diluted approximately 10-fold into filtered (0.45-μm pore size) seawater (●). (a to e) CEPEX on 11 July 1978, 29 July 1978, 9 August 1978, 15 August 1978, and 20 September 1978, respectively; (f) and (g) are from Scripps Pier on 11 May 1979 and 18 May 1979, respectively. Error bars indicate the range of duplicates.*

TABLE 4. *Production estimates derived from cell counts of 3-μm filtrates*

| Location[a] | Date | Cells liter$^{-1}$ × $10^9$ | Cells liter$^{-1}$ day$^{-1}$ × $10^9$ | μg of C liter$^{-1}$ day$^{-1}$ [a] |
|---|---|---|---|---|
| CEPEX | | | | |
| CEE3 | 7/11/78 | 1.1 | 1.2 | 15 |
| CEE3 | 7/29/78 | 0.8 | 0.8 | 13 |
| CEE2 | 8/9/78 | 1.8 | 1.7 | 29 |
| CEE2 | 9/15/78 | 0.38 | 0.4 | 4.8 |
| | | | 1.0[b] | 12 |
| Outside | 9/28/78 | 0.59 | 0.8 | 8 |
| | | | 1.1[b] | 11 |
| Scripps Pier | 5/11/79 | 1.2 | 1.6 | 32 |
| | | | 1.7[b] | 34 |
| | 5/18/79 | 1.5 | 0.6 | 10 |
| | | | 0.8[b] | 13 |
| | 5/23/79 | 1.0 | 1.2 | 24 |
| | 6/6/79[c] | 1.3 | 1.3 | 26 |

[a] Derived from cells liter$^{-1}$ day$^{-1}$ multiplied by the average cell biomass. CEE2 and CEE3, Controlled ecosystem enclosures 2 and 3, respectively.

[b] Extrapolated from a sample diluted approximately 10-fold into filtered (0.45-μm pore size) water.

[c] Bottle effect experiment, incubated in the dark.

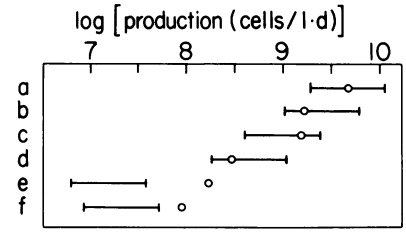


FIG. 4. *Production estimates from thymidine incorporation (—) versus increase in bacterial abundance (○) in filtered (3-μm pore size) seawater. (a and c to f) Scripps Pier on 24 May 1979, 11 May 1979, 18 May 1979, 11 May 1979, and 18 May 1979, respectively; (b) CEPEX on 8/9/78; (e) and (f) were diluted approximately 10-fold into filtered (0.45-μm pore size) seawater. The sample taken on 24 May 1979 had been in a bottle for 20 h before starting this experiment, so the results were used only to compare the two methods and not to estimate in situ rates (as in Tables 1 and 4).*

min) that should hydrolyze DNA (22). This material is probably protein produced from thymidine breakdown products entering biosynthetic pathways. Very little label appears in RNA (15), probably because conversion of thymidine to urindine or cytidine involves loss of the tritium label.

(iv) The specific activity of thymidine is unaffected by natural thymidine. This assumption cannot be absolutely correct, but we found that the amount of isotope dilution in our samples appears to be small (Table 3). Because of the possible intracellular production of thymidine, it would be best to know the specific activity of the immediate precursor of DNA, namely, thymidine triphosphate. Currently, there is no way to completely separate the algal thymidine triphosphate from the bacterial thymidine triphosphate, a step which would be essential for this measurement. Again, we have used the most conservative assumption; correcting for unlabeled thymidine and its phosphorylated derivatives would increase our production estimates.

(v) The total bacterial DNA is considered to contain 25 mol% thymidylic acid residues. This is the mean of the range of 14 to 35% for the bacteria studied (24).

(vi) The amount of DNA per cell ranges from $7.47 \times 10^{-16}$ to $4.82 \times 10^{-15}$ g. This is the range of genome size reported for all non-photosyn-

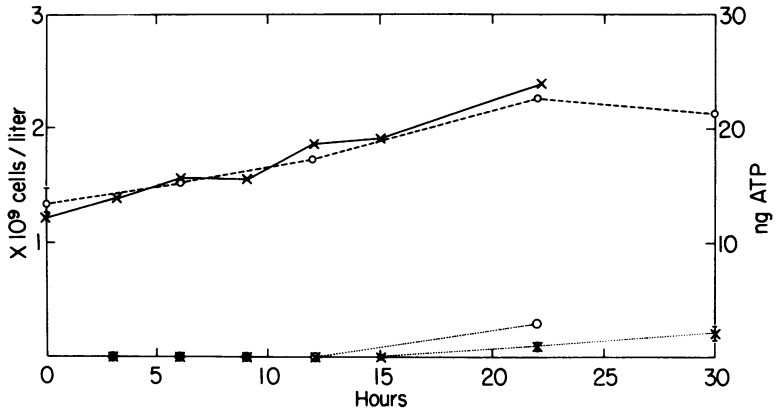


FIG. 5. *Bottle effect experiment; growth of bacteria in water and on surfaces in filtered (3-μm pore size) seawater in three different-sized containers. Symbols:* (—) *bacterial abundance in water in 150-ml beaker;* (---) *ATP per 100 ml of water in 600-ml beaker;* (···) *total ATP on surfaces in* (×) *150-ml beaker,* (○) *600-ml beaker, and* (●) *scintillation vials (always <0.01 ng ml$^{-1}$ up to 15 h). Error bars indicate the range of triplicates.*

thetic procaryotes studied to date (33) and is used since the average value for naturally occurring marine bacteria is not yet known. We believe that use of this range leads to a conservative production estimate since this amount of DNA would make up a substantial fraction of the total dry weight of a typical marine bacterium. For example, a 0.05-μm$^3$ bacterium would be 6 to 40% DNA, using the above DNA-per-cell range, and a small but common 0.02-μm$^3$ bacterium would be 15 to 100% DNA. Adjusting this assumption by decreasing the DNA per cell would increase our production estimates.

The combination of these assumptions results in a conversion factor ranging from $2.0 \times 10^{17}$ to $1.3 \times 10^{18}$ cells produced per mol of thymidine incorporated. Our estimates of production in cells liter$^{-1}$ day$^{-1}$ were derived by multiplying moles of thymidine incorporated liter$^{-1}$ day$^{-1}$ by these conversion factors to provide a range estimate (Table 1).

Each of our two approaches for estimating bacterioplankton secondary production has its strong and weak points. The short incubation time required by the thymidine incorporation approach (even when growth is slow) is a great advantage since the effects of containment increase with time. Incorporation has been found to be linear from 5 to 70 min (15), and we have found linearity in up to 6-h incubations in Antarctica. However, in all six long-term (>24-h) incubations in Antarctica, the incorporation rates decreased slightly with time (~20% decline in 24 h), presumably due to depletion of growth substrates. Another advantage of this approach is that no prefiltration is required, and the samples are therefore minimally perturbed. The major drawback is the large number of assumptions required to convert the data into production figures. We are currently making measurements of DNA content, adenine plus thymine/guanine plus cytosine ratios in the DNA, and other factors which would allow us to put more precision and confidence in these production estimates.

The cell count approach is based on the premise that bacterial growth occurs at the expense of dissolved or <3-μm particulate organic matter and that predation by organisms >3 μm in size is the major cause of mortality. Dilution of the sample is used to minimize substrate depletion during the course of the experiment and therefore is most important at high cell densities and growth rates. This approach does not work well where growth is slow (e.g., McMurdo Sound) because the increase in cells must be measurable within 1 day (preferably within hours). Growth which only occurs after this time is suspect, due to the possibility of serious bottle effects.

ZoBell and Anderson (38) found that plate counts of bacteria increased drastically in seawater samples stored for longer than 1 day in bottles, a phenomenon that they attributed to interactions with the bottle surfaces. We believe that our results were not significantly influenced by surface growth since growth was always apparent fairly early in our incubations, and the bottle effect experiment suggested that little growth occurs on surfaces during day 1. Additional evidence comes from the similarity of results from three different surface/volume ratio containers in that experiment, because if surface effects are important, then we would expect different results when different surface/volume ratio containers are used. However, day-long

containment in bottles may have other stimulatory or inhibitory effects which we could not assess.

The prefiltration used in the cell count increase method may present a problem. Although we used very gentle filtration, namely, gravity or reverse-flow filtration with less than a 5-cm hydrostatic head, this treatment may have injured some delicate cells, possibly releasing nutrients into the water. However, when even relatively harsh pressure filtration was used, we found that thymidine incorporation rates in 3-μm filtrates were only 12 to 20% higher than the rates in unfiltered samples (range of four samples). Therefore, the enhancement of growth rate by prefiltration is likely to be small.

One remaining problem with both approaches is that an average cell volume is used to convert "cells" to "carbon," yet it is possible that either larger- or smaller-than-average cells may realize a disproportionate amount of growth.

A direct comparison of production estimates by the two different approaches (Fig. 4) shows a general agreement, but thymidine incorporation gives a lower estimate for the diluted samples. A likely explanation is that our conversion factor from thymidine incorporation to production is overly conservative for these diluted samples and is possibly due to a change in the concentration of dissolved organic matter in 0.45-μm filtrates.

Another comparison can be made by looking at the general results of both approaches (Tables 1 and 4). It can be seen that the production estimates for CEPEX or Scripps Pier are similar with either approach. We believe that such agreement between two independent approaches strengthens our estimates.

We have chosen to compare our results with primary production since the latter is the source of reduced carbon for bacterial growth. Our results show that CEPEX and Scripps Pier bacterial secondary production was on the order of 10% of the magnitude of local primary production, with both regions showing sizable fluctuations. In eastern McMurdo Sound, bacterial secondary production usually was only a few percent of the magnitude of primary production, but the percentage there was comparable to that at CEPEX and Scripps Pier for at least a portion of the season. Western McMurdo Sound had negligible primary and secondary production.

The marked difference between the eastern and western sides of McMurdo Sound has been observed by other investigators (9), and Holm-Hansen et al. (16) have found that heterotrophic uptake and ATP biomass follow this same pattern. Bunt and Lee (5) observed that primary production in the east is concentrated in a short seasonal bloom. Our sampling covered only the early part of this bloom, and we did not witness its peak. Therefore, we may have missed the highest bacterial activity since this could occur during the senescent stage of the bloom. Still, our results indicate that bacterioplankton secondary production can be more than 10% of the magnitude of primary production.

It follows from these results that bacteria can grow fast enough in coastal zones to utilize a significant fraction of the available fixed carbon. Taking bacterial respiration into account, it is likely that typically 20 to 25% of the total production passes through bacterioplankton.

Significant bacterial growth in the absence of >3-μm particles as we measured it would seem to refute the commonly held belief that most growth occurs on large detrital particles. Although we cannot completely rule out the possibility of growth on small particles, the fact that virtually all of the bacteria in the 3-μm filtrates that we observed microscopically were not visibly attached to particles suggests that most of the observed growth was at the expense of dissolved organic matter. Additional evidence for this kind of growth comes from Sieburth et al. (31), who found even higher growth rates on material which passed through a 0.2-μm filter.

It has recently been suggested by Karl (19) that bacterial growth rates in the ocean can be estimated from rates of synthesis of stable RNA. The author emphasized the importance of measuring the specific activity of precursor pools. We believe that methodological problems currently prohibit the accurate assessment of nucleic acid precursors in natural marine bacteria. Furthermore, we believe that differences in the regulation of RNA and DNA syntheses make the latter more suitable as a basis for growth rate measurements.

It is obvious that precursor specific activity must be known for an exact determination of growth rate with tracer techniques. Karl (19) pointed out that the problem was especially critical for RNA since the rapid turnover of messenger RNA is certain to dilute the added label with unlabeled precursors. An obstacle to measuring specific activity of bacterial nucleic acid precursors in natural samples is that bacteria cannot be completely isolated from the other organisms present in seawater. Since these other organisms can contain large amounts of precursors, such as ATP, failure to exclude them from precursor pool measurements would yield totally erroneous specific activity estimates. In surface waters, this problem could result in underestimates of specific activity of one to two orders of magnitude, and hence overestimates of growth rates, since bacteria make up a small

fraction of the microplankton (0.4 to 17% [J. A. Fuhrman, J. Ammerman, and F. Azam, manuscript in preparation]; 4 to 25% [11]). Data are not available for deeper waters, but there is no a priori reason for assuming that the bacteria there make up a larger biomass than do protozoa, flagellates, and sinking phytoplankton. Karl (19) apparently made no attempt to isolate bacterial ATP (the precursor that he used), nor did he address this important consideration. This problem can be overcome only partially by size fractionation because the smallest filters which allow the large majority of bacteria to pass do not completely retain all eucaryotic material (1). A second problem of Karl's proposed method is the glass fiber filters (Reeve Angel 984H) that were used to collect microorganisms after incubation with tritiated adenine. We have found that these filters allow roughly 50% of the label associated with bacteria to pass; the remaining labeled bacteria can be collected on an HA Millipore filter (unpublished data). The effect of this problem on specific activity estimates cannot be assessed. A third problem is that the precursor of interest, ATP, is partially converted to adenosine diphosphate or adenosine monophosphate due to filtration stress. Karl and Holm-Hansen (21) showed that this problem could cause estimates of ATP per liter to vary by 50%, depending simply upon sample volume. Unless the response to filtration stress is identical for all bacteria in the sample, the effect on specific activity estimates cannot be assessed. These problems demonstrate that current attempts to measure specific activity could lead to large errors. Moreover, it is not known whether the errors result in underestimates or overestimates, and the direction of error could vary from sample to sample. Therefore, the results of such attempts are of little use for calculating bacterial growth rates in nature.

Whereas messenger RNA turnover makes specific activity measurements essential for determination of RNA synthesis (19), there is no analogous problem with DNA. We chose to assume that the specific activity of thymidine triphosphate was the same as that of the added thymidine. Our results suggested that this was not likely to lead to large error. More importantly, this choice could not result in overestimates of growth rates, and it was made because it was useful in this study to know that the bacteria were growing at least as fast as was estimated.

Estimates of growth from rates of nucleic acid synthesis cannot be made merely by knowing the specific activity and rate of incorporation of a tracer. It is of equal importance to know the amount of nucleic acid present in the bacteria. This information is essential for the conversion of synthesis rates to growth rates. Given the current lack of methodology for measuring the bacterial DNA or RNA in environmental samples, a general advantage of using DNA synthesis in preference to RNA synthesis is the relative accuracy in estimating DNA content. This is largely because bacterial DNA content varies much less than RNA content as a function of growth rate. For example, in *Salmonella typhimurium*, at doubling times ranging from 0.5 to 5 h, DNA content ranges from 3 to 4% of dry weight, whereas RNA content ranges from 31 to 12% of dry weight (23). Therefore, even if the average RNA content of marine bacteria were known (which is not the case), one must know the growth rate itself to calculate growth rates by using RNA synthesis. DNA content varies by one order of magnitude less than RNA, so the problem for DNA is relatively minor. Karl's proposed method (19) for measuring growth rates did not include a measurement of bacterial RNA, but at one point used "assumed and reported growth rates of marine bacteria" to estimate RNA content. A range of 5 to 15% of cellular dry weight for stable RNA was apparently arbitrarily chosen. This is only one-fifth of the range of literature values reported by the author for ribosomal RNA alone (19). Our estimates of growth from DNA synthesis used the entire range of genome sizes for all non-photosynthetic procaryotes studied to date (33).

In the context of this discussion, estimates of RNA or DNA synthesis rates are useful only if they can be interpreted in terms of growth. A basic difference between the regulation of RNA and DNA syntheses is that the DNA synthesis rate always closely follows growth rate, whereas the RNA synthesis rate is near the growth rate only under the special condition known as balanced growth. This is a consequence of the fact that the cellular RNA content increases dramatically, whereas the DNA content decreases slightly with increasing growth rate. Therefore, when the growth rate is increasing (shift up), the cells must synthesize RNA much faster than other cellular components to end up with more RNA per cell (23). When the growth rate is declining (shift down), RNA synthesis slows or stops entirely, whereas other cellular components continue to increase, resulting in lowered RNA per cell, and total cessation of stable RNA synthesis could proceed for more than a generation (23). On the other hand, the DNA synthesis rate during shift up or shift down is always between the old and new growth rates and generally follows the "current" rate (23). Therefore, DNA synthesis rates can always be interpreted in terms of growth, but even perfect measure-

ments of RNA synthesis rates are uninterpretable unless growth rates are unchanging (balanced growth). Sustained balanced growth of natural marine bacteria seems unlikely, given the spatial and temporal variability of many marine environments. Recent studies of short-term variability of marine microbial activity and biomass (12, 26) support this view. This conceptual problem involving interpretation of RNA synthesis rates was not considered by Karl (19).

Other investigators (3, 6, 13, 18, 19, 31, 37) have reported a wide range of bacterial growth rates in natural waters, with doubling times ranging from a few to hundreds of hours. Our results lie within this broad range. We do not know to what extent the differences in reported growth rates are due to differences in methodology and the extent to which the differences are due to real differences in growth rates in the variety of environments sampled. Therefore, a direct comparison between our results and those of others is neither intended nor possible in this study.

In conclusion, we have shown that quantitatively important secondary production occurs in bacterioplankton, and we have evidence that suggests that this growth is largely at the expense of dissolved organic matter. If this is the case, then bacterioplankton can act as a vital link in the food web, converting dissolved material into nutritional particles available to other trophic levels.

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