Mem. Natl Inst. Polar Res., Spec. Issue, **40**, 414–422, 1986

ABUNDANCE AND PRODUCTION OF BACTERIOPLANKTON IN THE ANTARCTIC

Kazuhiro KOGURE, Kimio FUKAMI*, Usio SIMIDU and Nobuo TAGA

Ocean Research Institute, University of Tokyo,
15-1, Minamidai 1-chome, Nakano-ku, Tokyo 164

Abstract: The abundance and production rate of bacterioplankton in the Antarctic were measured in order to clarify their role in the food web. The production rate was estimated from the incorporation rate of [*methyl*-^{3}H] thymidine into TCA (trichloroacetic acid) insoluble fraction. In summer, the production in the upper layer ranged 0.45–5.2$\times 10^7$ cells/l/day, which correspond to *e.g.*, 0.068–0.79 μgC/l/day. The apparent doubling time of bacterial population was an order of a few days, which was equal to that observed in an equatorial area. Both abundance and production rate decreased with depth. The isotope dilution method to clarify the size of intracellular dTTP (deoxythymidine triphosphate) pool did not give meaningful data. The present results indicate that the abundance and production rate of bacterioplankton in the Antarctic were as high as those in an equatorial or temperate zone.

1. Introduction

The organic matter produced by phytoplankton is finally degraded by bacterioplankton or grazed by zooplankton in the sea. In order to clarify the contribution of bacteria to mineralization of organic compounds, it is necessary to assess their heterotrophic activity. A number of methods are so far known to measure the activity (KOGURE, 1985; VAN ES and MEYER-REIL, 1982). The kinetic approach for certain chemicals using radio-labeled compounds such as glucose or amino acids has been used for a long time (PARSONS and STRICKLAND, 1962; WRIGHT and HOBBIE, 1965). However, except for the chemical in question, this approach does not give any information on other organic matter present in the seawater. Since the growth is an overall reflection of organic matter consumptions by bacteria, the measurement of bacterial growth rate is much more valuable to understand their role in the marine ecosystem. If we know the bacterial biomass, growth rate, and assimilation efficiency, it is possible to estimate the organic carbon flux through bacterial population in the sea.

There are several techniques to measure bacterial growth rate. Recently, the estimation by the determination of DNA synthesis rate (BROCK, 1967; FUHRMAN and AZAM, 1980; MORIARTY and POLLARD, 1981) has attracted considerable attention. Because DNA is synthesized only in growing cells, the rate of the synthesis is proportional to the growth rate. Usually, the rate of [^{3}H] thymidine incorporation into tri-

* Present address: Department of Fisheries, Faculty of Agriculture, Kyoto University, Kitashirakawa Oiwake-cho, Sakyo-ku, Kyoto 606.

chloroacetic acid (TCA) insoluble fraction is measured (FUHRMAN and AZAM, 1980, 1982). With the average DNA content per one bacterial cell in the sea and also with several other assumptions, the bacterial production rate can be calculated. Since the eucaryotic organisms are known to lack thymidine kinase, which mediates the transport of thymidine into the cell, this method measures only the growth rate of bacterial population. This is also confirmed by the autoradiographic technique (BERN, 1985).

The purpose of the present investigation is to elucidate the role of bacterial population on the cycling of organic carbon in the Antarctic ecosystem. Their production rate in summer was assessed by the method of FUHRMAN and AZAM (1982). On the other hand, in order to estimate the intracellular dTTP (deoxythymidine triphosphate) pool size, which is the immediate precursor of DNA synthesis, the isotope dilution method (MORIARTY and POLLARD, 1981) was applied. We also tried to clarify the significance of low temperature as an important environmental factor in the Antarctic. The result was compared with those obtained in the equatorial area.

2. Materials and Methods

2.1. Sampling

All the samples were collected during the KH-83-4 cruise of R. V. HAKUHO MARU, Ocean Research Institute, University of Tokyo from December 1983 to February 1984. Sampling locations are shown in Fig. 1. At Stns. 3A and 3B, vertical samplings were made by Niskin microbiological samplers (chopstick type, General Oceanics, USA), which were attached to a Rosette sampler system. Samples were also taken in the tropical area (Stns. leg 4–1, 16°S, 114°29′E, and leg 4–2, 5°37′S, 117°E). All samples were treated on board the ship immediately after the collection.

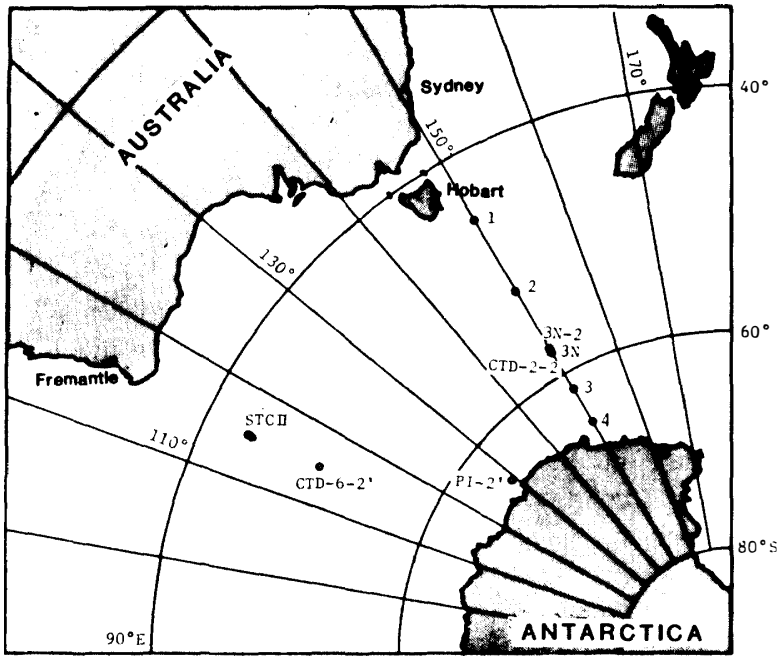

Fig. 1. Sampling location.

2.2. Bacterial numbers

The acridine orange direct count method was applied (HOBBIE *et al.*, 1977). The countings were performed on board during the cruise, using the Olympus epifluorescent microscope (BH) with an ultrahigh pressure mercury lamp. The dichroic splitter (DM-500+O-515), exciting filter (EY-455), and barrier filter (O-530) were used.

2.3. Measurement of bacterial production rate

Bacterial production rate was measured from the incorporation rate of [*methyl*-^{3}H] thymidine into cold TCA insoluble fraction (FUHRMAN and AZAM, 1982). In brief, duplicate of 20 m*l* of sample seawaters were incubated with 5 nM [*methyl*-^{3}H] thymidine (40–60 Ci/mM, Amersham) in 50 m*l* glass bottles with rubber plugs. The temperature was kept at 0°C in the Antarctic, and at the ambient for other stations. Incubation was stopped by adding cold TCA (final concentration 5%). After at least 5 min, the sample was filtrated through Millipore GS filter (pore size, 0.22 μm), and was then transferred to a glass vial. The radioactivity was measured by LKB Wallack RackBeta 1215 scintillation counter on board. Aquasol 2 scintillation cocktail (New England Nuclear) was used. The formalin-fixed duplicate seawater samples were treated in parallel as control. Numbers of cells produced were calculated by multiplying the moles thymidine incorporated with 2.4×10^{18} (FUHRMAN and AZAM, 1982).

2.4. Isotope dilution method

A thymidine isotope dilution method (MORIARTY and POLLARD, 1981) was applied to clarify the size of intracellular dTTP pool in the surface seawaters at Stns. 2, 3 and 4. To sample seawater, [*methyl*-^{3}H] thymidine and unlabeled thymidine were added and incubated as described above. During the cruise, however, DNA fraction was not extracted. The fraction in cold TCA insoluble material was assumed to be the same in all samples.

3. Results

The time course of thymidine uptake by natural bacterial assemblage is shown in Fig. 2. From the result, 6 h incubation was chosen for the present investigation. Figure 3 shows the vertical profiles of temperature and chlorophyll *a* at Stn. 3B. The temperature profile was a typical one in the Antarctic in summer. In the euphotic zone, a relatively broad peak of chlorophyll *a* was observed. The vertical profiles of bacterial total count, production rate, and the apparent doubling time, which was calculated from the bacterial numbers and production rates at this station, are shown in Fig. 4. Total counts slightly decreased with depth. There was a broad peak of production rate in the euphotic zone and also a small subsurface one at 100 m. Under the present experimental condition, no bacterial growth was detected below 150 m. The apparent doubling time of bacteria in the upper euphotic zone was about 10 days, whereas 10 to 30 days below the zone. Table 1 shows the bacterial numbers and production rates at other stations. Except for Stn. 1 and deeper layers at Stns. 3A and 3B, the cell production rates were quite constant, regardless of water temperature. In the surface layer at Stn. 3A, the doubling time was only 2.3 days, which indicates that

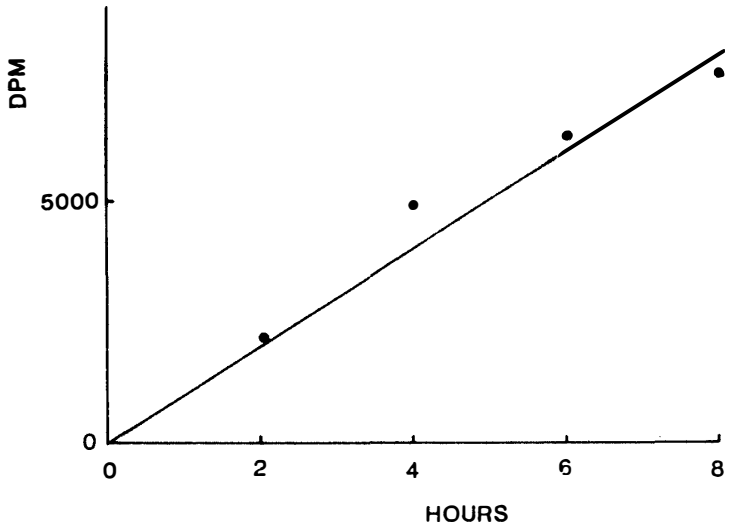

Fig. 2. Time course of [methyl-^3H] *thymidine incorporation into TCA insoluble fraction.*

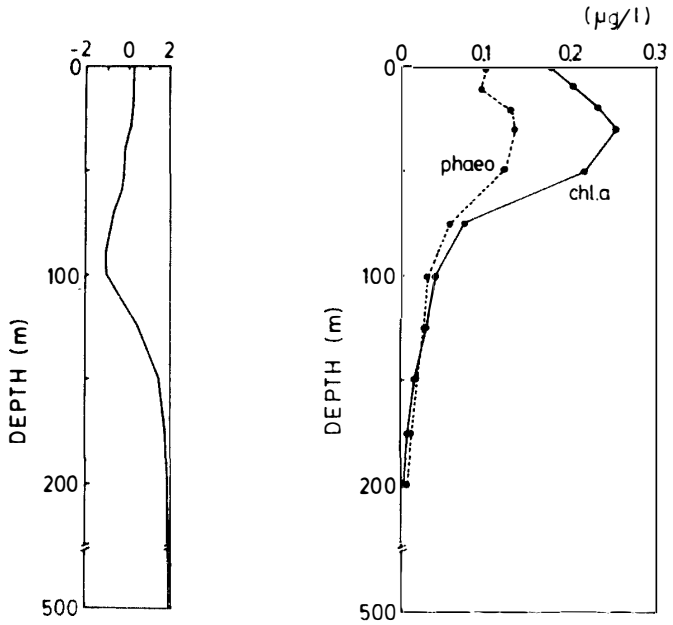

Fig. 3. Vertiacl profile of temperature and chlorophyll a at Stn. 3B (from the Preliminary Date Report).

bacteria were actively metabolizing organic matter in the layer. In the equatorial area, the doubling time was even longer than in the Antarctic.

The result of the isotope dilution method are shown in Figs. 5 and 6. For each sample, a straight line was obtained at lower concentrations of thymidine. From these results, it looks that the isotope dilution due to an intracellular pool or *de novo* synthesis of dTTP was negligible. However, at higher concentrations, data were not consistent, especially for the sample at Stn. 3. With more than 10 nM thymidine, the lower slope was obtained.

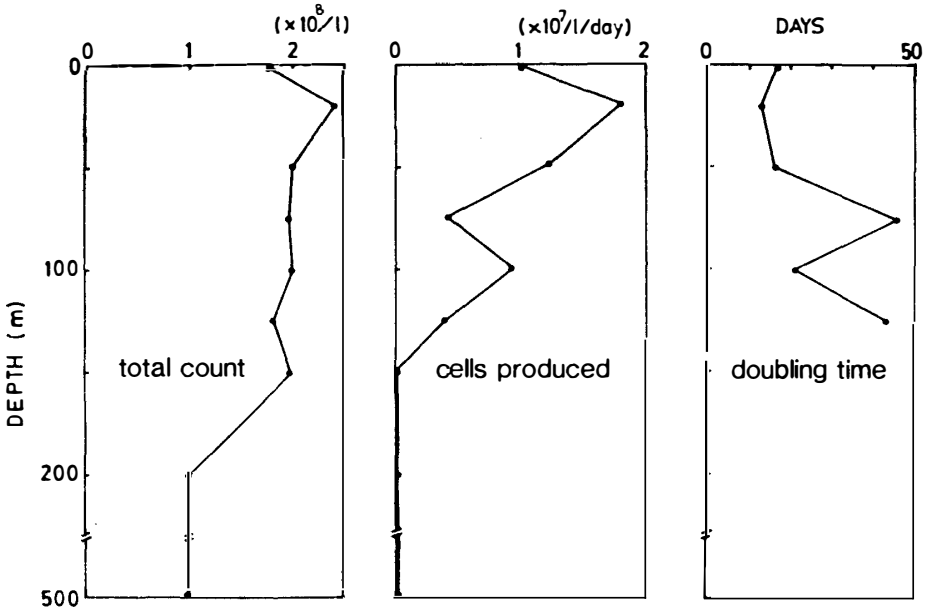

Fig. 4. Vertical profile of bacterial number, production rate, and doubling time at Stn. 5B.

Table 1. Bacterial number and production rate in the Antarctic and the equatorial area.

Station	Depth (m)	Temp. (°C)	Total count ($\times 10^7$ cells/l)	Cells produced ($\times 10^7$ cells/l/day)	Doubling time (day)
1	0	13	220	52	3.2
2	0	8	54	3.6	10
CTD-2-2	0	3	26	5.2	3.8
3A	0	0	9.5	3.3	2.3
	100	0	9.3	3.7	2.1
	200	0	7.4	0.72	7.3
	300	0	7.1	0.16	35
3N	0	3	43	3.9	7.3
3N-2	0	3	52	5.3	7.3
PI-2′	0	0	27	2.2	9.0
CTD-6-2′	0	6.5	68	5.7	9.0
STC II-S	0	11.5	120	8.5	10
STC II-C	0	12.5	76	(3.5)	—
STC II-N	0	12.5	86	6.1	10
leg 4-1	0	30	76	2.2	24
leg 4-2	0	26.5	74	2.7	18

4. Discussion

The thymidine uptake method is now widely used for the measurement of bacterial production rate in the aquatic ecosystem. The method is rather simple and its validity for natural samples has been checked by many workers recently (BELL *et al.*, 1983; FUHRMAN and AZAM, 1982; HANSON and LOWERY, 1983; LOVELL and KONOPKA, 1985; NEWELL and FALLON, 1982; RIEMANN *et al.*, 1982). One of the arguments with this technique is the application of the isotope dilution method, which was mainly tested and

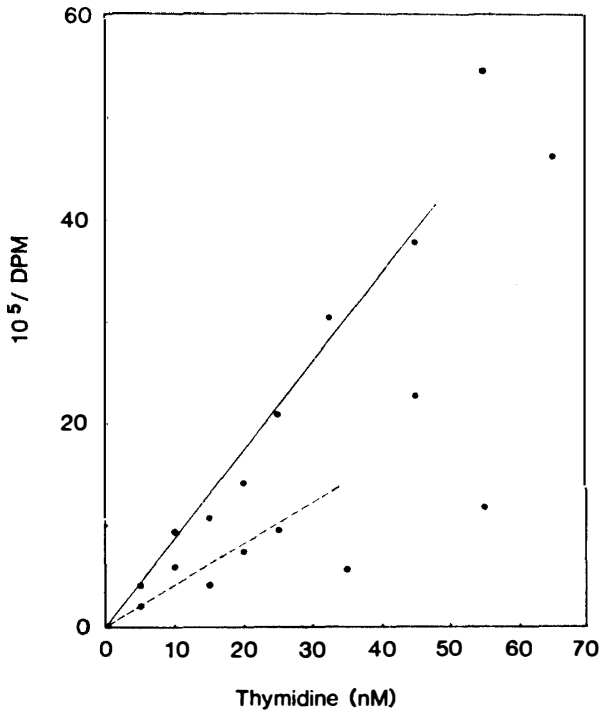

Fig. 5. Plots of reciprocal of [methyl-^3H] *thymidine incorporation into TCA insoluble fraction. Closed circle: Stn. 2; open circle: Stn. 4.*

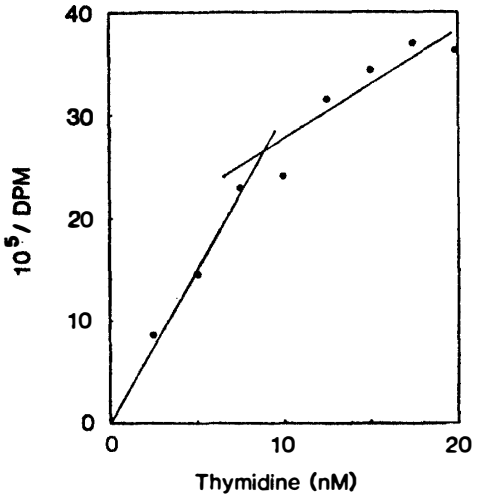

Fig. 6. Plots of reciprocal of [methyl-^3H] *thymidine incorporation into TCA insoluble fraction at Stn. 3B.*

used by MORIARTY and his colleagues (MORIARTY and POLLARD, 1981, 1982; MORIARTY *et al.*, 1985; POLLARD and MORIARTY, 1984). The main purpose of this kinetic approach is to measure the specific activity of dTTP, which is the immediate precursor of DNA synthesis in the cell (MORIARTY, 1985). The specific activity is not always equal to that of added [*methyl*-^{3}H] thymidine, because of *de novo* synthesis of thymidine in the cell and conversion of dUMP (deoxyuridine monophosphate) to dTMP (deoxythymidine monophosphate). The deviation leads to the miscalculation of growth rate. Since there is no good way to separate only bacterial population from other organisms

in the seawater, it is very difficult to extract bacterial dTTP fraction for the measurement of the specific activity. Even if it is possible, rapid turnover and functional versatility of this compound in the cell make the interpretation very complicated.

The isotope dilution method, however, did not always yield the meaningful data. LOVEL and KONOPKA (1985) and HANSON and LOWERY (1983) could not obtain a single straight line, which was predicted from the theory. Our results also did not fit the straight line when the higher concentration of thymidine was added. In the recent paper, POLLARD and MORIARTY (1984) stated that there is a critical concentration of added thymidine above which *de novo* synthesis of dTMP would be inhibited. They also said that with the low concentration of thymidine, the activity of thymidine kinase, which mediates the transport of thymidine into the cell, might be suppressed by dTTP originating from intracellular *de novo* synthesis. Both cases will result in the deviation from the predicted straight line with different ways. However, it is not clear under what condition one of them will happen. They showed an example of biphasic plots, which is quite similar to Fig. 6 in this paper. They obtained the sample from the sediment (MORIARTY and POLLARD, 1981), and interpreted that most of thymidine added was adsorbed to the sediment when the concentration was low, thereby leading to the suppression of thymidine kinase. They thought this situation is reversed by the addition of a larger amount of thymidine. However, our sample did not contain sediment, and the data shown in Fig. 6 was inconsistent with other two cases shown in Fig. 5. If their explanation is true for this case, as much as 16 nM intracellular thymidine pool should be present in the surface seawater at Stn. 3, whereas the sizes were negligible at other two stations. Considering the low-nutrient environment in the Antarctic, and other reports (MORIARTY and POLLARD, 1982; FUHRMAN and AZAM, 1982), it is difficult to accept the presence of such a large pool in the natural bacterial population at only Stn. 3. It seems more likely that the biphasic plots in Fig. 6 are the result of multiphasic uptake kinetics described by AZAM and HODSON (1981). As was stated by RIEMANN *et al.* (1982), it is doubtful whether the isotope dilution method truely measures the dTTP pool itself in the cell. Therefore, it is difficult to interpret the data obtained during this cruise. However, there remains some possibility that this is because the DNA fraction was not extracted for the present investigation.

For the calculation of bacterial doubling time, it is generally postulated that all the bacteria observed under the epifluorescent microscope behave equally. However, the active fraction among them is usually from a few to *ca.* 60% (KOGURE *et al.*, 1980; VAN ES and MEYER-REIL, 1982). In the off-shore area like the Antarctic, the fraction may be less than 10% of total bacteria. The true growth rate should have been calculated for such active fraction, which is actually responsible for the growth and turnover of organic matter in the sea. However, because of the limitation of time, we could not measure the active fraction during the cruise. The growth of these bacteria is expected to be much faster than the 'apparent' one shown in Fig. 4.

The vertical profiles of chlorophyll *a*, bacterial abundance, and bacterial production rate at Stn. 3 indicate that most of organic matter turnover proceeded in the upper layer ($<$100 m) of the water column. It is now possible to estimate the bacterial contribution to turnover of organic matter in the upper layer of the Antarctic zone, which was defined as south of the Polar Front (NAKAI *et al.*, 1985). In Table 1, the zone includes

Stns. CTD-2-2, 3A and PI-2'. Station 3B (Figs. 3 and 4) is also in the zone. Assuming that the conversion factor from bacterial biovolume to carbon content is 0.22 (BRATBAK and DUNDAS, 1984), and that an average bacterial biovolume in the Antarctic is 0.0686 μm^3 (FUHRMAN and AZAM, 1980), the bacterial production rate in the Antarctic zone in summer is 0.068–0.79 $\mu gC/l/day$. If we postulate that the bacterial assimilation efficiency is 50%, the organic carbon which passes through bacterial population constitutes up to *ca.* 22% of primary production. This estimation, however, is a conservative one, because, first, the recent paper (BRATBAK, 1985) suggested that the conversion factor from cellular volume to carbon content is even higher than 0.22. It is noteworthy that 0.121 (WATSON *et al.*, 1977) is currently most widely used. Second, the size of actively growing bacteria are usually larger than other 'resting' ones. The calculation should have been based on the size of the active fraction.

The result of the present investigation showed an accordance with those by other workers (FUHRMAN and AZAM, 1980; GILLESPIE *et al.*, 1976; HODSON *et al.*, 1981). The production rate in the Antarctic was comparable to tropical or other off-shore areas. This means that aquatic bacteria in the polar region have adapted themselves to the low-temperature environment. Although the work has not yet been completed, it is shown that a considerable part of bacterial strains isolated during the cruise are psychrophilic ones (MORITA, 1975; SIMIDU *et al.*, 1986). The temperature was not a limiting factor of bacterial activity in the Antarctic. This observation does not support the idea by SOROKIN (1971) that the bacterial metabolic activity was suppressed by the low temperature in the Antarctic.

Acknowledgments

The authors are grateful to Dr. M. TERAZAKI, Ocean Research Institute, University of Tokyo, for his kind help in the radioisotope experiments during the cruise. We also wish to thank the captain, officers and crew of the R. V. HAKUHO MARU, Ocean Research Institute, University of Tokyo, for their assistance.

References

AZAM, F. and HODSON, R. E. (1981): Multiphasic kinetics for D-glucose uptake by assemblages of natural marine bacteria. Mar. Ecol. Prog. Ser., **6**, 213–222.

BELL, R. T., AHLGREN, G. M. and AHLGREN, I. (1983): Estimating bacterioplankton production by measuring [^{3}H] thymidine incorporation in a eutrophic Swedish lake. Appl. Environ. Microbiol., **45**, 1709–1721.

BERN, L. (1985): Autoradiographic studies of [*methyl*-^{3}H] thymidine incorporation in a cyanobacterium (*Mycrocystis wesenbergii*)-bacterium association and in selected algae and bacteria. Appl. Environ. Microbiol., **49**, 232–233.

BRATBAK, G. (1985): Bacterial biovolume and biomass estimations. Appl. Environ. Microbiol., **49**, 1488–1493.

BRATBAK, G. and DUNDAS, I. (1984): Bacterial dry matter content and biomass estimations. Appl. Environ. Microbiol., **48**, 755–757.

BROCK, T. D. (1967): Bacterial growth rate in the sea; Direct analysis by thymidine autoradiography. Science, **155**, 81–83.

FUHRMAN, J. A. and AZAM, F. (1980): Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica and California. Appl. Environ. Microbiol., **39**, 1085–1095.

FUHRMAN, J. A. and AZAM, F. (1982): Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters; Evaluation and field results. Mar. Biol., **66**, 109–120.

GILLESPIE, P. A., MORITA, R. Y. and JONES, L. P. (1976): The heterotrophic activity for amino acids, glucose and acetate in Antarctic waters. J. Oceanogr. Soc. Jpn., **32**, 74–82.

HANSON, R. B. and LOWERY, H. K. (1983): Nucleic acid synthesis in oceanic microplankton from the Drake Passage, Antarctica; Evaluation of steady state growth. Mar. Biol., **73**, 79–89.

HOBBIE, J. E., DALEY, R. J. and JASPERS, S. (1977): Use of nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol., **33**, 1225–1228.

HODSON, R. E., AZAM, F., CARLUCCI, A. F., FUHRMAN, J. A., KARL, D. M. and HOLM-HANSEN, O. (1981): Microbial uptake of dissolved organic matter in McMurdo Sound, Antarctica. Mar. Biol., **61**, 89–94.

KOGURE, K. (1985): Mizu no seitaikei (Aquatic ecosystem). Biseibutsu Seitaigaku II (Microbial Ecology II), ed. by U. SIMIDU. Tokyo, Kyoritsu Shuppan, 40–90.

KOGURE, K., SIMIDU, U. and TAGA, N. (1980): Distribution of viable marine bacteria in neritic seawater around Japan. Can. J. Microbiol., **26**, 318–323.

LOVELL, C. R. and KONOPKA, A. (1985): Primary and bacterial production in two dimictic Indiana Lakes. Appl. Environ. Microbiol., **49**, 485–491.

MORIARTY, D. J. W. (1985): Measurement of bacterial growth rates in aquatic systems using rates of nucleic acid synthesis. Adv. Aquat. Microbiol., **3** (in press).

MORIARTY, D. J. W. and POLLARD, P. C. (1981): DNA synthesis as a measure of bacterial productivity in seagrass sediments. Mar. Ecol. Prog. Ser., **5**, 151–156.

MORIARTY, D. J. W. and POLLARD, P. C. (1982): Diel variation of bacterial productivity in seagrass (*Zostera capricorni*) beds measured by rate of thymidine incorporation into DNA. Mar. Biol., **72**, 165–173.

MORIARTY, D. J. W., POLLARD, P. C. and HUNT, W. G. (1985): Temporal and spatial variation in bacterial production in the water column over a coral reef. Mar. Biol., **85**, 285–292.

MORITA, R. Y. (1975): Psychrophilic bacteria. Bacteriol. Rev., **39**, 144–167.

NAKAI, T., HASUMOTO, H. and NEMOTO, T. (1985): Hydrographic characteristics. Prelim. Rep. HAKUHO MARU Cruise, **KH-83-4**, 3–7.

NEWELL, S. Y. and FALLON, D. (1982): Bacterial productivity in the water column and sediments of the Georgia (USA) coastal zone; Estimates via direct counting and parallel measurement of thymidine incorporation. Microb. Ecol., **8**, 33–46.

PARSONS, T. R. and STRICKLAND, J. D. H. (1962): On the production of particulate organic carbon by heterotrophic processes in sea water. Deep-Sea Res., **8**, 211–222.

POLLARD, P. C. and MORIARTY, D. J. W. (1984): Validity of the tritiated thymidine method for estimating bacterial growth rates; Measurement of isotope dilution during DNA synthesis. Appl. Environ. Microbiol., **48**, 1076–1083.

RIEMANN, B., FUHRMAN, J. A. and AZAM, F. (1982): Bacterial secondary production in freshwater measured by ^{3}H-thymidine incorporation method. Microb. Ecol., **8**, 101–114.

SIMIDU, U., KOGURE, K., FUKAMI, K. and IMADA, C. (1986): Heterotrophic bacterial flora of the Antarctic Ocean. Mem. Natl Inst. Polar Res., Spec. Issue, **40**, 405–412.

SOROKIN, YU. (1971): On the role of bacteria in the productivity of tropical oceanic waters. Int. Rev. Gesamten Hydrobiol., **56**, 1–48.

VAN ES, F. B. and MEYER-REIL, L.-A. (1982): Biomass and metabolic activity of heterotrophic marine bacteria. Adv. Microb. Ecol., **6**, 111–170.

WATSON, S. W., NOVITSKY, J. A., QUINBY, H. L. and VALOIS, F. W. (1977): Determination of bacterial number and biomass in the marine environment. Appl. Environ. Microbiol., **33**, 940–946.

WRIGHT, R. T. and HOBBIE, J. E. (1965): The uptake of organic solutes in lake water. Limnol. Oceanogr., **10**, 22–28.

(*Received June 25, 1985; Revised manuscript received August 13, 1985*)