

Microbial Uptake of Dissolved Organic Matter in McMurdo Sound, Antarctica

R. E. Hodson¹, F. Azam², A. F. Carlucci², J. A. Fuhrman², D. M. Karl³ and O. Holm-Hansen²

¹ Department of Microbiology, University of Georgia; Athens, GA 30602, USA

² Institute of Marine Resources, A-018, Scripps Institution of Oceanography, University of California, San Diego; La Jolla, CA 92093, USA

³ Department of Oceanography, University of Hawaii; Honolulu, HI 96822, USA

Abstract

The distribution and activity of bacterioplankton, and the turnover of dissolved organic matter (DOM) were examined in McMurdo Sound, Antarctica. On the eastern side of the Sound, bacteria averaged $6.5 \times 10^8 \text{ l}^{-1}$, and turnover rates of dissolved adenosine triphosphate, D-glucose and l-leucine averaged 16, 116 and 124 h, respectively. These molecules as well as thymidine were taken up maximally from 0° to 5 °C and near-maximally from –1.5° to 0 °C, indicating bacterial adaptation to rapid turnover of dissolved organic matter at the ambient temperature. On the west side of the Sound, bacteria averaged only $0.65 \times 10^8 \text{ l}^{-1}$, and turnover times for adenosine triphosphate, D-glucose and l-leucine averaged 59, 20454, and 3070 h, respectively. Total microbial adenosine triphosphate (an indicator of total microbial biomass) and chlorophyll *a* were also much lower at the western than at the eastern side stations. Moreover, no primary production could be detected at one western side station (New Harbor). Thus, in McMurdo Sound, the western side is highly oligotrophic, but the eastern side has an abundant active bacterioplankton, comparable to that of temperate coastal waters.

ter was supposed to downwell with dense water at the Antarctic convergence, be advected to low latitudes by deep oceanic circulation, eventually be upwelled to warm surface waters where its utilization by bacteria would contribute to the productivity of the tropical waters. Contrary to this hypothesis Gillespie *et al.* (1976) found high microbial heterotrophic potential for the assimilation of several organic substrates from Antarctic seawater at low temperatures. Moreover, the likely dominance of bacterial flora by psychrophiles argues against the preservation of organic matter in Antarctic waters. Thus, Wiebe and Hendricks (1974) found that 77% of the bacterial isolates from south of the Antarctic convergence would not grow above 25 °C.

To elucidate the role of Antarctic bacterioplankton in the food-web and in the cycling of organic matter, we have studied the distribution and activity of bacterioplankton around McMurdo Sound and under the Ross Ice Shelf during three austral summers (1976–77–78). McMurdo Sound is a specially interesting study area because of the dramatic difference between the macro-benthic productivity of the eastern (very high) and western (very low) sides of the sound (Dayton and Oliver, 1977). Dayton and Oliver have also suggested that similar differences exist in the productivity of the overlying water column.

Introduction

Water masses around Antarctica are generally considered too cold for rapid bacterial growth. Persistent sub-zero temperatures are supposed to limit the occurrence (Kriss, 1963; Kriss *et al.*, 1969) and metabolic activity (Pomeroy *et al.*, 1969; Sorokin, 1971) of bacterioplankton. The viable count measurements of Kriss indicated that bacterial population densities in high latitudes tend to be very low. Sorokin hypothesized that the oxidation of DOM in Antarctic waters is slow due to the low rates of microbial activity. The residual reduced organic mat-

Materials and Methods

Sample Collection

Seawater samples were collected in 4-l ethanol-cleaned Niskin[®] bottles at 10 sites in McMurdo Sound (Fig. 1). These sites included locations periodically ice-free during summer breakouts of the annual ice, and locations permanently covered by the Ross Ice Shelf. Samples were transferred to acid-cleaned glass carboys and stored in darkness at 0 °C prior to use. All samples were processed within 4 h of collection.

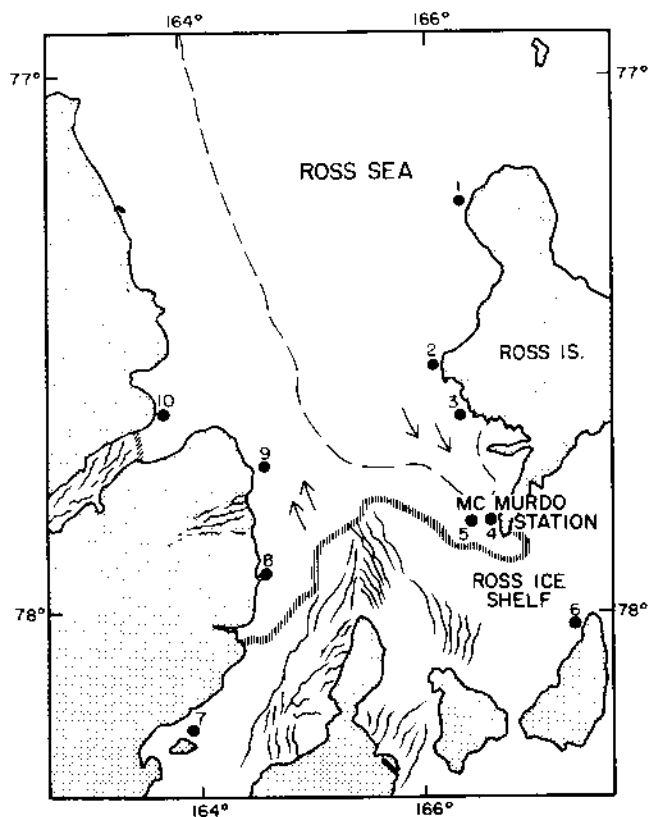


Fig. 1. Sampling locations in McMurdo Sound: (1) Cape Bird; (2) Cape Royds; (3) Cape Evans; (4) McMurdo I; (5) McMurdo II; (6) White Island; (7) Heald Island; (8) Cape Chocolate; (9) Strand Moraines; (10) New Harbor. Arrows indicate direction of surface currents. Dashed line indicates position of January annual ice edge

Microbial Assimilation of ^3H -Labeled Organic Substrates

The rate of assimilation of dissolved adenosine triphosphate (DATP), and rates of turnover of l-leucine, D-glucose and thymidine (since pool size could not be determined) by the total marine microbial populations and the free-living bacterial assemblage were determined over a range of incubation temperatures from 0° to 22°C . Seawater aliquots of 100 ml each were aseptically transferred to 300-ml sterile Whirl Pak[®] disposable sample bags. The bags were then immersed in water baths to adjust the sample temperatures to the desired incubation temperatures. Incubations were initiated by adding $20\ \mu\text{l}$ aqueous labeled substrate to each bag and incubating in darkness for 2 to 5 h at the desired temperature. Uptake rates were constant throughout the incubation periods. All samples were run in quadruplicate, and parallel 5% formalin-killed samples served as adsorption blanks. Incubations were terminated by filtering the samples through 0.2 or $0.6\ \mu\text{m}$ pore-size Nuclepore[®] filters. Filters were then washed twice with filtered seawater to remove residual extracellular radiolabel. The $0.6\ \mu\text{m}$ pore-size filters were used to distinguish between free-living bacteria, most of which pass, and attached

bacteria and large organisms which are retained (Azam and Hodson, 1977a). The filters were transferred to scintillation vials containing 10 ml Aquasol[®] (New England Nuclear) and radioassayed by liquid scintillation spectrometry.

The concentration of DATP in each sample was determined independently (see below) and the rates of microbial assimilation of DATP from seawater were calculated as

$$\frac{f}{t} S_n$$

where f is the fraction of added radioactivity assimilated, S_n is the naturally occurring concentration of DATP in the seawater (expressed as ng l^{-1}), and t is the incubation time in hours. This calculation assumes that the uptake of DATP is not influenced by the presence of structurally-related compounds in seawater (e.g. adenine, adenosine, adenosine monophosphate, adenosine triphosphate). For the other substrates where S_n could not be determined, the turnover time (T_t) of the pool was determined as $\frac{1}{f}$ (Azam and Holm-Hansen, 1973).

Dissolved ATP Concentration

The concentrations of DATP in seawater were measured and used to calculate *in situ* rates of DATP uptake from turnover data (Azam and Hodson, 1977b). A 100-ml sample of seawater was filtered through a $0.2\ \mu\text{m}$ Nuclepore[®] filter directly into 10 ml of $6.5\ \text{N H}_2\text{SO}_4$. A trace amount of $\gamma\text{-}^{32}\text{P-ATP}$ (less than $1\ \text{nCi sample}^{-1}$; $700\ \text{Ci mmol}^{-1}$) was added as an internal standard and the sample was passed through a column of activated charcoal (Hodson *et al.*, 1976) to adsorb the ATP quantitatively. The columns were then washed with 10 ml of $5 \times 10^{-4}\ \text{M HCl}$ to remove salts that interfere with ATP assay. Adsorbed ATP was eluted with 5 ml of ammoniacal ethanol (ethanol:water:ammonium hydroxide 2:2:1 v/v), dried *in vacuo* at 40°C , and dissolved in 1 ml of Tris (tris [hydroxymethyl] aminomethane) buffer (20 mM, pH 7.7). The percentage recovery of ATP from the column was determined by radioassaying the sample for emission of Cerenkov Radiation without addition of a scintillation fluor, so that the same sample could be used for determination of ATP. Elution recovery averaged about 50%. ATP was then determined by the firefly luminescence method of Holm-Hansen and Karl (1978). The specific activity of added $^{32}\text{P-ATP}$ remained the same before and after column adsorption and elution, indicating that no appreciable ATP was degraded by column treatment.

Microbial ATP Concentrations

The concentrations of ATP associated with the total microbial population and with the free-living bacterial fraction of the microbiota were measured by a procedure

Table 1. DATP concentration and assimilation rates in McMurdo Sound. Station locations are shown in Fig. 1

Location	Date	Depth (m)	DATP (ng l ⁻¹)	DATP assimilation (T _t ; h)	V _{ATP} (ng l ⁻¹ h ⁻¹)	% by 0.6 μm filterable organisms
East Sound						
4	1/14/77	4	487	13	37.0	71
5	12/22/76	5	519	3.9	133	—
South Sound						
6	1/23/77	15	112	19	6.0	79
6	1/23/77	50	90	27	3.3	80
West Sound						
10	1/24/77	5	307	32	9.6	84
10	1/24/77	23	256	39	6.6	85
8	1/22/77	15	89	41	2.2	82
8	1/22/77	50	90	82	1.1	82
7	1/20/77	15	135	71	1.9	84
7	1/20/77	25	169	66	2.6	88
7	1/20/77	75	222	80	2.8	86

Table 2. Bacterial concentrations and heterotrophic assimilation rates in McMurdo Sound

Location	Date	Bacteria (x 10 ⁸ l ⁻¹)	l-Leucine (T _t ; h)	D-Glucose (T _t ; h)
East Sound				
4	1/7/79	9.60	366	53
5	12/27/78	6.49	63	179
1	12/29/78	2.99	130	235
2	12/29/78	6.24	39	52
3	12/29/78	7.20	24	60
Average		6.5	124	116
West Sound				
9	1/1/79	0.69	4065	24242
10	1/1/79	0.61	2075	16667
Average		0.65	3070	20454

identical to the one described above for DATP except for the following modifications: seawater samples for measurement of total particulate ATP were acidified to 0.6 N by pouring 100 ml of sample into Erlenmeyer flasks containing 10 ml of 6.5 N H₂SO₄, swirling momentarily, and letting them stand at room temperature for 20 min. The extracts were then processed in a manner identical to that for DATP. The ATP associated with free-living marine bacteria was determined by passing 100-ml samples of seawater through 0.6 μm pore-size Nuclepore® filters directly into 10 ml of 6.5 N H₂SO₄, allowing the acidified samples to extract at room temperature for 20 min, then processing the samples as described above for dissolved and total particulate ATP. We have shown previously that the fraction of microbial ATP which is filterable through 0.6 μm Nuclepore filters is associated with free-living bacteria (Azam and Hodson, 1977a).

Bacterial abundance was measured from samples preserved in 10% buffered formaldehyde, stained with

acridine orange and observed with a Zeiss microscope with a catalog no. 487709 filter set (Hobbie *et al.*, 1977).

Results

DATP Concentration

The locations of the stations sampled in McMurdo Sound during January 1977 and the concentrations of DATP in each sample are given in Table 1. DATP concentration ranged from a low of 89 ng l⁻¹ in the western sound at Cape Chocolate, to a high of 519 ng l⁻¹ at McMurdo Sound Site Number 2. Over the narrow range of depths sampled, the values did not change appreciably with depth except at the Heald Island Station which is 40 km south of the northern edge of the Ross Ice Shelf. At this location the concentration of DATP increased with depth from 135 ng l⁻¹ at 15 m to 222 ng l⁻¹ at 75 m.

Heterotrophic Microbial Activity and Bacterial Abundance

The intensity of heterotrophic activity in surface waters differed markedly between the eastern and western sides of the sound (Tables 1 and 2). The rates of ATP assimilation by the total microbial populations (1976–77) were highest at sites near McMurdo Station in the eastern sound, averaging 37 and 133 ng l⁻¹ h⁻¹, respectively at McMurdo-1 and McMurdo-2. These 2 stations are under relatively thin (less than 3 m) annual ice cover during much of the year, but are in open water at certain times during the austral summer (Dayton and Oliver, 1977). In the southeastern sound, beneath the permanent ice cover of the Ross Ice Shelf, the rates were much lower, averaging only 6.0 ng l⁻¹ h⁻¹ at the northern end of White Island, which is 22 km south of the northern edge of the Ice Shelf. Low activity was detected beneath

Table 3. Particulate chlorophyll *a* and adenosine triphosphate in McMurdo Sound

Station	Date	Depth	Chl <i>a</i> ($\mu\text{g l}^{-1}$)	Total microbial ATP ($\mu\text{g l}^{-1}$)	
East Sound 4	January 1977	5	-	4.6	
		10	0.165	-	
		50	0.160	0.7	
		100	0.175	-	
		200	0.185	0.8	
5	January 1977	5	-	1.6	
South Sound 6	January 1977	15	0.033	0.4	
		50	0.036	0.4	
West Sound 7	January 1977	15	0.01	0.1	
		25	0.009	0.1	
		50	0.009	0.1	
		75	0.007	0.2	
		8	January 1977	15	0.026
	8	January 1977	25	0.024	0.2
			50	0.010	0.1
			10	January 1977	5
	10	January 1977	23	0.042	0.3

the permanent ice shelf at Heald Island, where the assimilation rate was $1.9 \text{ ng l}^{-1} \text{ h}^{-1}$, and showed a northerly increase to $2.1 \text{ ng l}^{-1} \text{ h}^{-1}$ at Cape Chocolate, and $9.6 \text{ ng l}^{-1} \text{ h}^{-1}$ at New Harbor.

To see if turnover of substrates other than DATP was also (1) rapid, and (2) different on eastern and western sides of McMurdo Sound, D-glucose and l-leucine were used for activity measurement during 1978–79. Table 2 gives the rates of turnover of D-glucose [$6\text{-}^3\text{H}$] and l-leucine [$3, 4\text{-}^3\text{H}$] at various stations on the eastern and the western sides of McMurdo Sound. The differences in bacterial heterotrophic activity between eastern and western sides were even more dramatic in 1978–79 than found previously for DATP. Average turnover times for glucose were 116 and 20454 h for the eastern and western sound, respectively; corresponding values for leucine were 124 and 3070 h. The fraction of the natural microbial population which is retained by $0.6 \mu\text{m}$ filters includes both larger eukaryotic organisms, bacteria aggregated or attached to large particles, and some large bacteria. In every case, ATP assimilation by this larger size-fraction of the population amounted to only a small percentage ($18.4 \pm 4.7\%$) of the overall rate supported by the total microbial population. Percent assimilation by free living bacteria in samples from the eastern side averaged $77 \pm 4\%$, whereas the corresponding value for the western side was $84 \pm 2\%$. Also, we observed no relationship between depth in the water column and percentage activity due to free-living bacteria.

Acridine orange direct counts of bacterial abundance averaged $6.5 \times 10^8 \text{ l}^{-1}$ for all eastern sound stations

(Table 2). An order of magnitude lower bacterial count was obtained for the western side stations (average $0.65 \times 10^8 \text{ l}^{-1}$).

Microbial ATP and Chlorophyll

Determinations of particulate ATP showed that the eastern and western sound stations differed in total microbial biomass (as indicated by total microbial ATP); chlorophyll *a* concentrations also differed between these stations (Table 3). The highest total biomass values were found in the eastern sound under annual ice cover at McMurdo Site 1 and McMurdo Site 2 where the concentrations of total particulate ATP in the shallowest depths sampled averaged 4.6 and $1.6 \mu\text{g l}^{-1}$, respectively in surface waters. The station at White Island under permanent Ross Ice Shelf cover had a much lower total microbial biomass of $0.4 \mu\text{g ATP l}^{-1}$. The ATP biomass values at stations in the western sound were generally much lower than those for stations in the eastern sound. The total particulate ATP concentration was lowest at Heald Island where the values averaged $0.1 \mu\text{g ATP l}^{-1}$ and increased slightly in a northerly direction to $0.2 \mu\text{g ATP l}^{-1}$ at Cape Chocolate and $0.4 \mu\text{g ATP l}^{-1}$ at New Harbor. The pattern of distribution of chlorophyll *a* was similar to that of total microbial ATP: high on the eastern side and very low on the western side.

The free-living bacterial-size fraction accounted for roughly 10% of the total microbial ATP in surface waters.

Temperature Dependence

Fig. 2A illustrates the dependency of the rate of microbial assimilation of $^3\text{H-ATP}$ on water temperature from 0° to 22°C . The data plotted are from the shallowest sample taken at each station. At all the stations with the exception of Cape Chocolate, the rate of uptake of dissolved ATP (expressed as percentage of the maximum) was highest at the lowest temperature of incubation (0° or 2°C). The samples collected at Cape Chocolate showed slightly lower assimilation rates at 2°C than at 7° to 12°C . At all other stations, incubation temperatures above 10°C resulted in decreased rates of assimilation. At 21°C , the assimilation rates were no more than 25% of the maximum at all stations, and, at Cape Chocolate and Heald Island, uptake was inhibited entirely at this temperature. Similar results were obtained during 1977/79 using glucose, leucine and thymidine as substrates and samples from McMurdo 1 (Fig. 2B).

Several samples from Cape Chocolate (January 1977) were preincubated for 1 h at 21°C , then cooled to 2°C and incubated with $^3\text{H-ATP}$. Even after 4 h at the lower temperature, no ATP uptake could be detected (data not shown), suggesting that most of the microheterotrophs are killed by an exposure to 21°C even for a relatively short time.

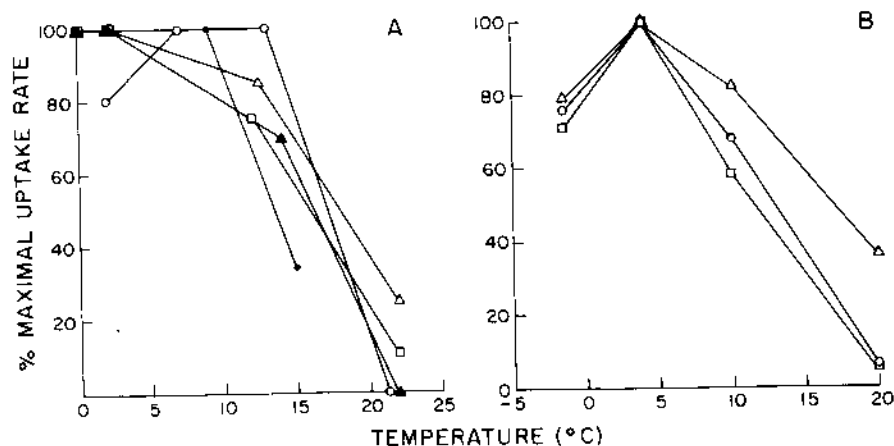


Fig. 2. (A) Temperature dependence of ^3H -(2,8)-ATP assimilation by natural microbial assemblages (Expressed as percentage of maximal rate) in samples of surface seawater collected in January 1977 from McMurdo I (closed circles), White Island (open squares), Heald Island (closed triangles), Cape Chocolate (open circles), and New Harbor (open triangles). (B) Temperature dependence of ^3H -l-leucine (open circles), ^3H -thymidine (open triangles), and ^3H -D-glucose (open squares) assimilation by natural microbial assemblages (expressed as percent of maximal rates) in surface seawater samples collected in January 1979 from McMurdo Station Site No. 1

Discussion

High rates of bacterial heterotrophic activity were found at stations on the eastern side of McMurdo Sound. Values for DATP assimilation and turnover times for D-glucose and l-leucine were similar to values reported previously for samples from eutrophic temperate waters such as the Southern California Bight (Azam and Hodson, 1977a, b). Values for bacterial abundance were also typical of those from the Southern California Bight (Fuhrman *et al.* 1980). In contrast, the bacterial abundance and activity at stations in the western sound were much lower and similar to those from the open ocean (Azam and Hodson, 1977a).

An average of 84% of the turnover of DATP at our sampling sites in McMurdo Sound was attributable to the fraction of the microbial population which passes through 0.6 μm pore-size Nuclepore filters. As reported previously (Azam and Hodson, 1977a), this fraction appears to consist primarily of unattached bacterial cells. Considering that in the waters of McMurdo Sound this fraction comprises only about 10% of the total microbial biomass (based on particulate ATP determinations), it follows that, per unit biomass, free-living bacteria are approximately 50- to 100-fold more active than larger organisms and/or attached bacteria in the turnover of DATP in these waters.

Dayton and Oliver (1977) studied the distribution of biomass of infaunal macrobenthos in McMurdo Sound and reported patterns of distribution strikingly similar to those reported here for planktonic microbial biomass and heterotrophic activity. The biomass and diversity of the benthos of the eastern sound were among the highest reported anywhere in the world ocean. In contrast, the values from sites in the western sound were much lower and comparable to those for deep-sea benthos of the open ocean. The observed patterns of biomass distribution were attributed to local current structure. Along the eastern side of the sound, the surface currents flow south bringing with them phytoplankton populations from open waters to the north. Along the western sound, on the other hand, currents at the sur-

face flow north, bringing with them water that has been under the Ross Ice Shelf for an undetermined period of time and consequently contains a much lower density of phytoplankton which could serve as a source of food for the macrobenthos. The further north one goes in the sound, the thinner becomes the average thickness of annual ice; this may possibly increase the rate of phytoplankton photosynthesis in the northwestern sound relative to the southwestern sound. Fuhrman and Azam (1980) reported that microbial incorporation of tritiated thymidine, an indicator of growth rates, was much higher in the eastern than western sides of the sound, supporting the proposal of Dayton and Oliver (1977). In the present, more extensive study, the distribution of chlorophyll and total microbial biomass in the water column (Table 3) also clearly supports this proposal. Both chlorophyll and microbial biomass were highest in the eastern sound at McMurdo Station, lowest under the Ross Ice Shelf at Heald Island in the southwestern sound and increased slightly in a northerly direction to New Harbor in the northwestern sound.

Chlorophyll *a* concentration and microbial biomass in the western side of McMurdo Sound are usually very low in comparison with those values reported for waters south of the Antarctic Convergence (Holm-Hansen *et al.* 1977b). There are live phytoplankton populations in the water column under the ice on the western side as shown by Bunt (1964) and by Holm-Hansen during 1976-77 by exposing water samples to sunlight attenuated by filters to about 20% of the incident intensity. No light-activated radiocarbon fixation by phytoplankton could be detected when using *in situ* techniques, however, either on the western side in January or on the eastern side in late November (O. Holm-Hansen, unpublished data); this is most likely due to the ice cover which reduces the light intensity to the water column to values less than the compensation light intensity. As the ice cover begins to thin and disappear in mid-December in the eastern portions of the sound, there is a dramatic increase in phytoplankton biomass and activity. In the summer, both the biomass and production of phytoplankton in the eastern side of McMurdo Sound are

similar to those in the Ross Sea and waters south of the Polar Front (O. Holm-Hansen, unpublished data from cruise on the "USCGC *Glacier*", 1977-78) and thus may be considered as fairly typical of Antarctic waters. As the ice cover persists on the western portion of McMurdo Sound, there is relatively little increase in the phytoplankton biomass during the summer (Bunt, 1964), and hence this area of the sound remains a uniquely oligotrophic environment in terms of biomass.

Our results do not support the hypothesis of Sorokin (1971) that DOM in Antarctic waters is preserved due to a low rate of its microbial utilization. On the contrary, our data suggest that: (1) the bacterial assemblages have temperature optima for assimilation of organic substrates near the ambient seawater temperatures; (2) the abundance of bacteria in the seawater is not atypically low, but rather is comparable to that of temperate regions; and (3) the rate of bacterial turnover of DOM is rapid (turnover times on the order of 100 h, Tables 2 and 3). Thus utilizable DOM would not be expected to persist for long periods of time. The applicability of these observations to conditions in the Southern Ocean in general merits further investigation.

Acknowledgements. This work was supported by National Science Foundation Grant DPP76-22134. The authors thank W. J. Wiebe for his helpful comments on the manuscript.

Literature Cited

- Azam, F. and R. E. Hodson: Size distribution and activity of marine microheterotrophs. *Limnol. Oceanogr.* 22, 492-501 (1977a)
- Azam, F. and R. E. Hodson: Dissolved ATP in the sea and its utilization by marine bacteria. *Nature. Lond.* 267, 696-698 (1977b)
- Azam, F. and O. Holm-Hansen: Use of tritiated substrates in the study of heterotrophy in seawater. *Mar. Biol.* 23, 191-196 (1973)
- Bunt, J. S.: Primary productivity under sea ice in Antarctic waters. I. Concentrations and photosynthetic activities of microalgae in the waters of McMurdo Sound, Antarctica. *In*: Antarctic research series 1. biology of the antarctic seas, pp 13-26. Ed. by Lee. Washington, D.C.: Amer. Geophysical Union 1964
- Dayton, P. K. and J. S. Oliver: Antarctic soft-bottom benthos in oligotrophic and eutrophic environments. *Science, N.Y.* 197, 55-58 (1977)
- Fuhrman, J. A., J. W. Ammerman and F. Azam: Bacterioplankton in the coastal euphotic zone: Distribution, activity and possible relationships with phytoplankton. *Mar. Biol.* (In press, 1980)
- Fuhrman, J. A. and F. Azam: Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. *Appl. Environ. Microbiol.* 39, 1085-1095 (1980)
- Gillespie, P. A., R. Y. Morita and L. P. Jones: The heterotrophic activity for amino acids, glucose and acetate in Antarctic waters. *J. Oceanogr. Soc. Japan* 32, 74-82 (1976)
- Hobbie, J. E., R. J. Daley and S. Jaspers: Use of nuclepore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.* 33, 1225-1228 (1977)
- Hodson, R. E., O. Holm-Hansen and F. Azam: Improved methodology for ATP determination in marine environments. *Mar. Biol.* 34, 143-149 (1976)
- Holm-Hansen, O., S. U. El-Sayed, G. A. Franceschini and R. L. Cuhel: Primary production and the factors controlling phytoplankton growth in the Southern Ocean. *In*: Adaptations within Antarctic ecosystems, pp 11-50. Ed. by Llano. Texas: Gulf Publishing Co. 1977b
- Holm-Hansen, O. and D. M. Karl: Biomass and adenylate energy charge determination in microbial cell extracts and environmental samples. *In*: Methods in enzymology, v. LVII, pp 73-85. Ed. by M. M. DeLuca. New York: Academic Press 1978
- Kriss, A. E.: Marine microbiology (deep sea). 536 pp. (Trans. by J. M. Shewan and Z. Kabata.) London: Oliver and Boyd 1963
- Kriss, A. E., I. E. Mishustina and M. N. Lebedeva: Bacterial population densities (heterotrophs) in the water column of the Southern and Indian Oceans. *Mikrobiologiya* 38, 511-517 (1969)
- Pomeroy, L. R., W. J. Wiebe, D. Frankenberg, C. Hendricks and W. L. Layton: Metabolism of total water column. *Ant. J. U.S.* 5, 149-150 (1969)
- Sorokin, Yu. I.: On the role of bacteria in the productivity of tropical oceanic waters. *Int. Rev. ges. Hydrobiol.* 56, 1-48 (1971)
- Wiebe, W. J. and C. W. Hendricks: Distribution of heterotrophic bacteria in a transect of the Antarctic Ocean. *In*: Effect of the ocean environment on microbial activities, pp 524-535. Ed. by Colwell and Morita. Baltimore: University Park Press 1974

Date of final manuscript acceptance: October 27, 1980.

Communicated by N. Holland, La Jolla