APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1984, p. 788–795
0099-2240/84/040788-08$02.00/0

Vol. 47, No. 4

Sea Ice Microbial Communities: Distribution, Abundance, and Diversity of Ice Bacteria in McMurdo Sound, Antarctica, in 1980

CORNELIUS W. SULLIVAN* AND ANNA C. PALMISANO

Marine Biology Research Section, Department of Biological Sciences, University of Southern California, Los Angeles, California 90089-0371

Received 12 September 1983/Accepted 23 January 1984

An abundant and diverse bacterial community was found within brine channels of annual sea ice and at the ice-seawater interface in McMurdo Sound, Antarctica, in 1980. The mean bacterial standing crop was 1.4×10^{11} cells m^{-2} (9.8 mg of C m^{-2}); bacterial concentrations as high as 1.02×10^{12} cells m^{-3} were observed in ice core melt water. Vertical profiles of ice cores 1.3 to 2.5 m long showed that 47% of the bacterial numbers and 93% of the bacterial biomass were located in the bottom 20 cm of sea ice. Ice bacterial biomass concentration was more than 10 times higher than bacterioplankton from the water column. Scanning electron micrographs showed a variety of morphologically distinct cell types, including coccoid, rod, fusiform, filamentous, and prosthecate forms; dividing cells were commonly observed. Approximately 70% of the ice bacteria were free-living, whereas 30% were attached to either living algal cells or detritus. Interactions between ice bacteria and microalgae were suggested by a positive correlation between bacterial numbers and chlorophyll *a* content of the ice. Scanning and transmission electron microscopy revealed a close physical association between epibacteria and a dominant ice alga of the genus *Amphiprora*. We propose that sea ice microbial communities are not only sources of primary production but also sources of secondary microbial production in polar ecosystems. Furthermore, we propose that a detrital food web may be associated with polar sea ice.

Polar oceans are unique because they are covered with annual sea ice for most of the year. During the austral winter, an estimated 17×10^6 km^2 of sea ice covers the Southern Ocean surrounding Antarctica (11). Microbial communities are found in the numerous brine pockets and drainage channels that occur throughout sea ice and at the ice-seawater interface (6, 21, 28, 38). From a biological perspective, this microhabitat presents a harsh physiochemical environment. Temperatures are no greater than −1.9°C, salinities range from that of seawater to 5× that of seawater (unpublished data), and ambient light is typically less than 13 microeinsteins m^{-2} s^{-1} (C. W. Sullivan, A. C. Palmisano, S. Kottmeier, S. McGrath-Grossi, and R. Moe, *in Fourth Symposium on Antarctic Biology: Nutrient Cycles and Food Chains*, in press). The ice microhabitat, however, appears to provide sufficient light and nutrients as well as a safe refugium to allow high concentrations of microorganisms to accumulate within its boundaries.

Primary production in sea ice has been described in both the Antarctic (6, 7, 22, 38–40; Sullivan et al., in press) and in the Arctic (3–5, 21, 27). Although bacterioplankton production has been demonstrated in the water column of polar seas (9, 15, 18), secondary production by bacteria in sea ice has received little attention. The earliest report, to our knowledge, is that of Iizuka et al. (23) who isolated species of *Brevibacterium* and *Achromobacter* from surface ice melt water near Syowa Station, Antarctica. Their samples, however, were stored for 5 months at −5°C and then incubated on agar plates at 25°C, a procedure that would eliminate any psychrophilic bacteria (30). Kaneko et al. (24) studied bacteria from the Beaufort Sea in the Arctic in April 1976. Samples were collected by chipping out pieces of surface ice and placing them in a sterile container. They found that although the total numbers (acridine orange direct counting) of bacteria were not significantly lower in sea ice than in underlying water, viable counts were much lower in the ice. In a preliminary report, Sullivan and Palmisano (38) noted the presence of ice bacteria in the bottom of annual sea ice in McMurdo Sound, Antarctica. As part of our effort to understand the ecology of sea ice microbial community (SIMCO) production in McMurdo Sound, annual sea ice cores were collected in 1980 to examine the nature of microalgal (33) and bacterial components of the ice. Here we describe the distribution, abundance, and morphological diversity of ice bacteria in McMurdo Sound during the austral spring of 1980.

MATERIALS AND METHODS

Field studies on sea ice bacteria were conducted from 10 November to 10 December 1980 at three sites in McMurdo Sound, Antarctica (Fig. 1). Samples were collected with a SIPRE ice auger to obtain one to three cores at each site from annual sea ice with thicknesses of 1.5 to 2.5 m. In addition to ice core samples, ice-seawater interface samples were collected by SCUBA divers using sterile glass jars or plastic syringes. Water column samples were collected below the ice through dive holes or seal breathing holes with an ethanol-cleaned Kemmerer PVC sampling device. All samples were maintained in darkness by wrapping them in opaque black plastic and placing them in Freezesafe styrofoam containers to avoid exposure to high light or temperatures less than −2°C. After transport by helicopter or track vehicle to the Eklund Biological Laboratory at McMurdo Station, Ross Island, the ice core samples were cut with an ethanol-cleaned stainless steel hand saw into one 20-cm section (bottom ice) and several 50-cm sections (upper ice) for estimation of bacterial biomass.

Thin (2 to 5 mm) horizontal sections of the bottom 5 cm of ice cores were prepared in a cold room (−10°C) by the method of Gow and Weeks (12) to examine the physical structure of the ice microhabitat and to determine the relationship of the microorganisms to the ice structure. The

* Corresponding author.

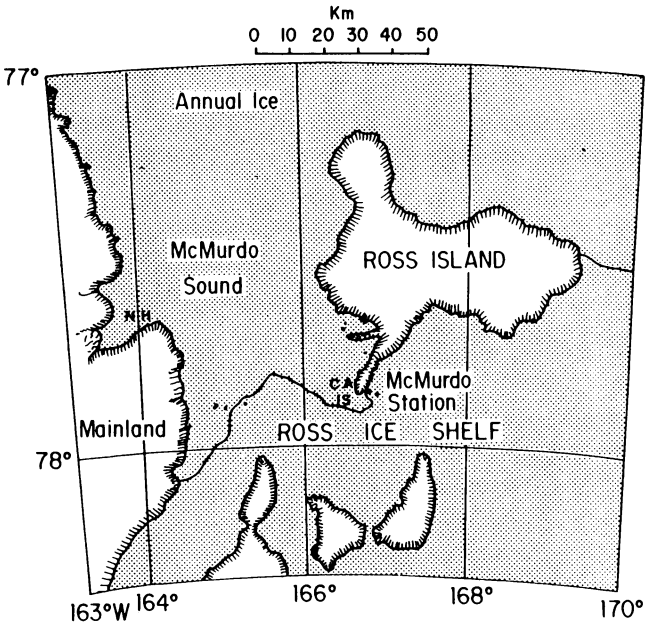

FIG. 1. Location of sea ice sampling stations in McMurdo Sound, Ross Sea, Antarctica. IS, Ross Ice Shelf; CA, Cape Armitage; NH, New Harbor.

birefringence pattern of thin sections was photographed with a 35-mm camera by using crossed polarizing filters to define crystal structure and brine drainage channel location. These sections were also examined in a −2°C room with a Zeiss phase-contrast microscope.

Individual core sections, either 20 or 50 cm in length, were allowed to thaw at 0 to 4°C in covered, opaque containers. The resulting ice core melt water was gently mixed and subsampled for biomass estimates or incubation with $H^{14}CO_3$ for microautoradiography. Chlorophyll *a*, phaeopigments (20), and particulate organic carbon (29) were determined for the ice samples as described by Palmisano and Sullivan (33). For microautoradiography, samples of ice core melt water from the bottom 20-cm sections were incubated with $H^{14}CO_3$ in a chamber designed to simulate the environment under the ice (32) at 0.3 and 13 microeinsteins m^{-2} s^{-1} at −2°C. After 4 to 6 h of exposure, samples were fixed in 1% glutaraldehyde, refrigerated at 0°C, and then returned on ice to the University of Southern California, Los Angeles, for processing. Samples (15 ml) were filtered onto 1.0-μm pore-sized Nucleopore filters, rinsed with 5 ml of distilled water, and placed on a Kodak ARIO fine-grain stripping film. Film was incubated in the dark for 3 weeks, developed in Kodak D19, and examined with a Zeiss research phase-contrast photomicroscope. Photomicrographs were taken with Kodak Plus X film developed with D19.

Live specimens from both core and ice-seawater interface samples were examined with a Zeiss research phase-contrast microscope maintained at −2°C in a walk-in refrigerator at the Eklund laboratory. Samples for epifluorescence or electron microscopy were immediately fixed with 2% glutaraldehyde in seawater which had been filtered through a 0.2-μm pore-sized membrane filter (Nuclepore). Samples from the ice-seawater interface were collected with a sterile syringe and fixed in situ by including sufficient glutaraldehyde in the syringe to give a final concentration of 2%.

The presence and enumeration of bacteria in ice core melt water, ice-seawater interface, and under ice-seawater samples were determined by acridine orange direct counting with an epifluorescence microscope by the method of Hobbie et al. (17). Bacterial cell volumes were estimated from measurements of individual cells observed by light and scanning electron microscopy (SEM). The factors for conversion of bacterial volumes to biomass reported by Krempin and Sullivan (25) were employed.

Preserved samples of ice core melt water were returned to the University of Southern California where they were prepared for SEM. Fixed samples on 0.2-μm pore-sized Nuclepore filters were desalted by successive passage through 100, 75, 50, and 25% seawater and deionized distilled water (31). Samples were then dehydrated by successive passage through 10, 25, 50, 75, and 100% ethanol. The dehydrated sample was subjected to critical-point drying, mounted on stubs with double-stick tape, sputter-coated with gold-paladium, and examined on a Cambridge SEM or, for high-resolution observations, the Hitachi model 5000 scanning-transmission electron microscope in the scanning mode at the Scripps Institution of Oceanography, La Jolla, Calif.

Samples for transmission electron microscopy, which were stored at 5°C for 11 months in 2% glutaraldehyde, were prepared by a modification of the technique of Tipet and Pickett-Heaps (41). Glutaraldehyde was decanted, and samples were postfixed in 1% osmium tetroxide for 15 min. Samples were then dehydrated by sequential passage through 5, 10, 20, 30, 40, 60, 80, and 100% acetone. Spurr low-viscosity embedding medium (1 ml) was added to 1 ml of cells in acetone. After 24 h, fresh resin was added to replace the old. Preparations in Spurr medium were flat-mounted between Teflon-coated glass microscope slides and dried overnight at 68°C. Individual diatoms of the genus *Amphiprora* were located in the mount with a phase-contrast microscope and cut out with a diamond scribe or scalpel. Each cell was then mounted on a resin stub with Epoxy cement and allowed to dry overnight. The resin block was trimmed, and the selected cell was sectioned at a known orientation. Sections were stained for 15 min in 2% uranyl acetate–1% dimethyl sulfoxide in 70% methanol, washed in 70% methanol followed by washing in water, and then stained for 3 min in Wray's lead tartarate and washed with water. Stained sections were floated onto Formvar-coated copper grids and examined in an Hitachi model H6000 transmission electron microscope at 50 kV at the Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder.

RESULTS

Horizontal sections of ice cores approximately 1- to 2-mm thick revealed the petrographic features of the sea ice microhabitat. Figure 2 shows a cross section 5 cm from the bottom of an ice core collected near the edge of the Ross Ice Shelf on 10 November 1980. The dark areas between crystals are brine channels which maintain a liquid environment for the SIMCO. Light microscopic observations of these ice core sections revealed brine channels and pockets containing a rich assemblage of ice algae and bacteria, some of which were attached to ice crystals; however, these organisms were not observed to occur within ice crystals as reported by Ackley (EOS **63**.1982, p. 47) for Weddell Sea ice.

The distribution and abundance of bacteria were determined for seven ice core sections collected at three stations in McMurdo Sound between 10 November and 28 November 1980 (Fig. 3). Vertical profiles of the ice column showed that

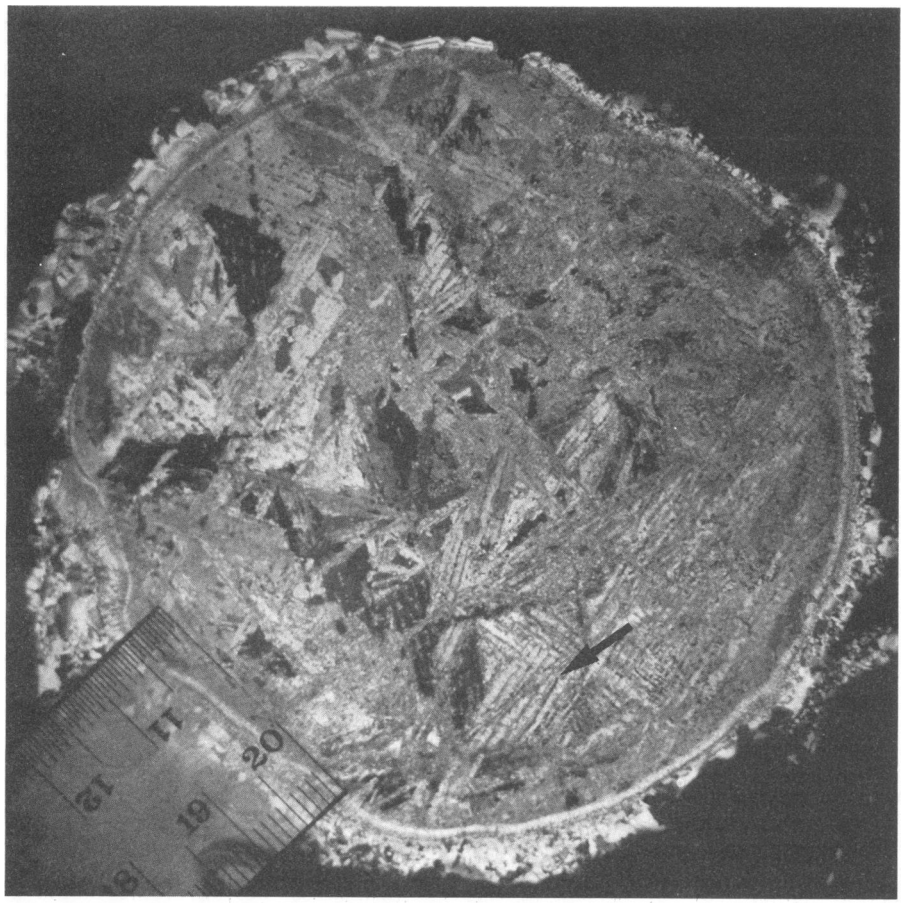

FIG. 2. Photograph of a horizontal section of an ice core. The section was located 5 cm from the bottom of core collected from annual sea ice in McMurdo Sound near the Ross Ice Shelf (IS, Fig. 1) on 10 November 1980. Light areas are cross sections of ice crystals. Between ice crystals are rows of liquid-filled brine channels (arrow) in which SIMCO were observed by phase-contrast microscopy. Centimeter marker is shown in lower left corner.

the highest concentration of bacterial numbers (Fig. 3a) and bacterial carbon (Fig. 3b) was located in the bottom 20 cm of the ice. A sharper gradient was observed for bacterial carbon than bacterial numbers because cells in the bottom 20 cm of ice were at least 10-fold larger in volume than those in the upper ice. Bacteria in the bottom ice reached concentrations as high as 1.02×10^{12} cells m^{-3}. For seven core samples, bacterial concentrations in the bottom section were positively correlated with both chlorophyll *a* ($P \leq 0.025$) and particulate organic carbon ($P < 0.05$). Bacteria in the bottom section were more morphologically diverse than those in the upper ice or water column.

In the upper sections of ice, bacterial concentrations ranged from 0.20×10^{11} to 3.60×10^{11} cells m^{-3}, and cell morphologies resembled those of the bacterioplankton in the underlying water column. Upper ice bacteria usually occurred as free-living cells of small size (≤0.5 by ≤0.2 μm); only short rods and cocci were observed.

The mean standing crop for the entire ice column based on core samples was 1.4×10^{11} bacteria m^{-2} of annual ice. By taking into account the different sizes of bacteria at various depths in the ice column, this standing crop contains 9.8 mg of bacterial carbon m^{-2} of annual ice. About 47% of the bacterial cell numbers and 93% of the biomass were located in the bottom 20 cm of the ice cores.

Samples collected by divers on 16 November to 10 December 1980 at the ice-seawater interface averaged 5.27 (± 2.41) $\times 10^{11}$ bacteria m^{-3}, with an estimated biomass of 36.6 mg of C m^{-3}. Since these samples were not included in the estimation of standing crop due to the uncertainty of quantitatively relating interface samples to ice cores, biomass values are conservative.

Both free-living and attached bacteria were present in the bottom 20 cm of annual ice (Table 1). Approximately 70% of the total bacteria were free-living, whereas 30% were attached to particles, including living algal cells and detritus. Epibacteria were primarily associated with diatoms of the genus *Amphiprora*. Although *Amphiprora* spp. represented only 13% of algal cell numbers, 19% of attached bacteria in core samples and 54% of attached bacteria in interface samples were associated with diatoms of this genus. Diatoms colonized by epibacteria were determined to be neither dead nor senescent by the following criteria: (i) chlorophyll *a* fluorescence of cells in unstained preparations under epifluorescence microscopy; (ii) the presence of subcellular structures including nuclei, nucleoli, and cytoplasmic membranes which appeared to be intact in acridine orange-stained preparations; and (iii) silver grain production in microautoradiographs of algal cells incubated with [^{14}C]bicarbonate under simulated in situ conditions (Fig. 4).

SEM revealed the cellular morphology of ice bacteria in the bottom 20 cm of ice. Free-living bacteria included short (≤0.5 μm) and long (≤1 μm) rods, some of which were dividing (Fig. 5a). The surface of some of these bacteria

TABLE 1. Free and attached bacteria in sea ice cores and at the ice-seawater interface from samples collected in McMurdo Sound, Antarctica, in 1980

Site	Date	Total bacteria		% Bacteria attached to following		
		% Free	% Attached	Living algae	Dead algae	*Amphiprora* spp.
Core Samples						
Ice Shelf	11–10–80	64	36	88	11	35
New Harbor no. 1	11–28–80	65	35	74	26	42
New Harbor no. 2	11–28–80	74	25	8	92	0
Cape Royds	12– 1–80	81	18	66	34	0
$\bar{x}$ of core samples		71 ± 8	28 ± 9	59 ± 35	41 ± 35	19 ± 22
Ice-seawater interface samples						
Cape Armitage	11–16–80	64	35	95	5	71
	11–16–80	74	26	21	79	17
	12–10–80	53	46	89	11	75
$\bar{x}$ ± SD of interface samples		64 ± 11	36 ± 10	68 ± 41	32 ± 41	54 ± 33

possessed a striking texture which suggested the presence of exocellular polymeric substances (EPS) (10) (Fig. 5b and c). Epibacteria colonizing *Amphiprora* spp. often occurred in chains of 10 to 30 cells, as well as in long filaments which sometimes branched. The diversity of epibacteria is shown in Fig. 6a through d; they included cocci, rod, straight and branching filamentous, fusiform, and prosthecate forms. The stalked bacterium (Fig. 6b and c) resembled *Prosthecobacter fusiformis* (37); it had a well-defined holdfast on the stalked end of both symmetrical daughter cells of dividing pairs, which is characteristic for this genus.

Some of the epibacteria colonizing the *Amphiprora* cell wall appeared to have structural modifications of their own cell wall or EPS that may aid their attachment to diatoms. The chain-forming rod showed structural evidence of cell surface modification at one pole for attachment (Fig. 6d). Figure 7 shows a transmission electron micrograph of a thin section through the point of attachment of a bacterium and the siliceous portion of the cell wall of an *Amphiprora* cell. The bacterium was surrounded by an EPS layer which appeared to be embedded in the puncta of the diatom wall, presumably serving to anchor the bacterium to its host.

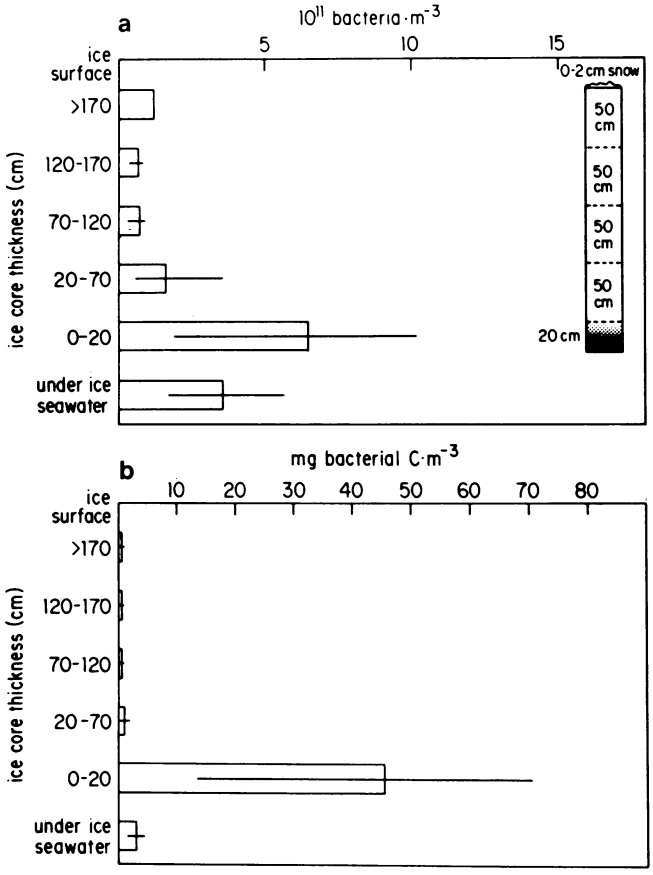

FIG. 3. Vertical profiles of bacterial numbers (a) and bacterial carbon (b) in annual sea ice cores from McMurdo Sound in 1980. $\bar{x}$ ± range of seven ice core samples is shown.

DISCUSSION

The bacterial community inhabiting bay-fast annual sea ice in McMurdo Sound in 1980 was found to be abundant and morphologically diverse. Although bacteria were distributed throughout the 1.5- to 2.5-m thick annual ice, those in the upper portion of ice were relatively small in size and low in concentration (4×10^{11} cells m^{-3}). In the bottom 20 cm of annual ice, in which 99% of the chlorophyll in the ice column was found by Palmisano and Sullivan (33), bacterial numbers were as high as 1.0×10^{12} cells m^{-3}, with a biomass more

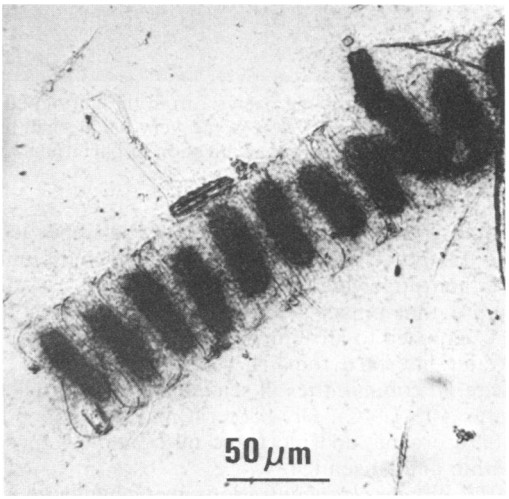

FIG. 4. Microautoradiograph of ice diatom *Amphiprora* sp. exposed to $H^{14}CO_3$ for 6 h at −1.8°C and 13 microeinsteins m^{-2} s^{-1}.

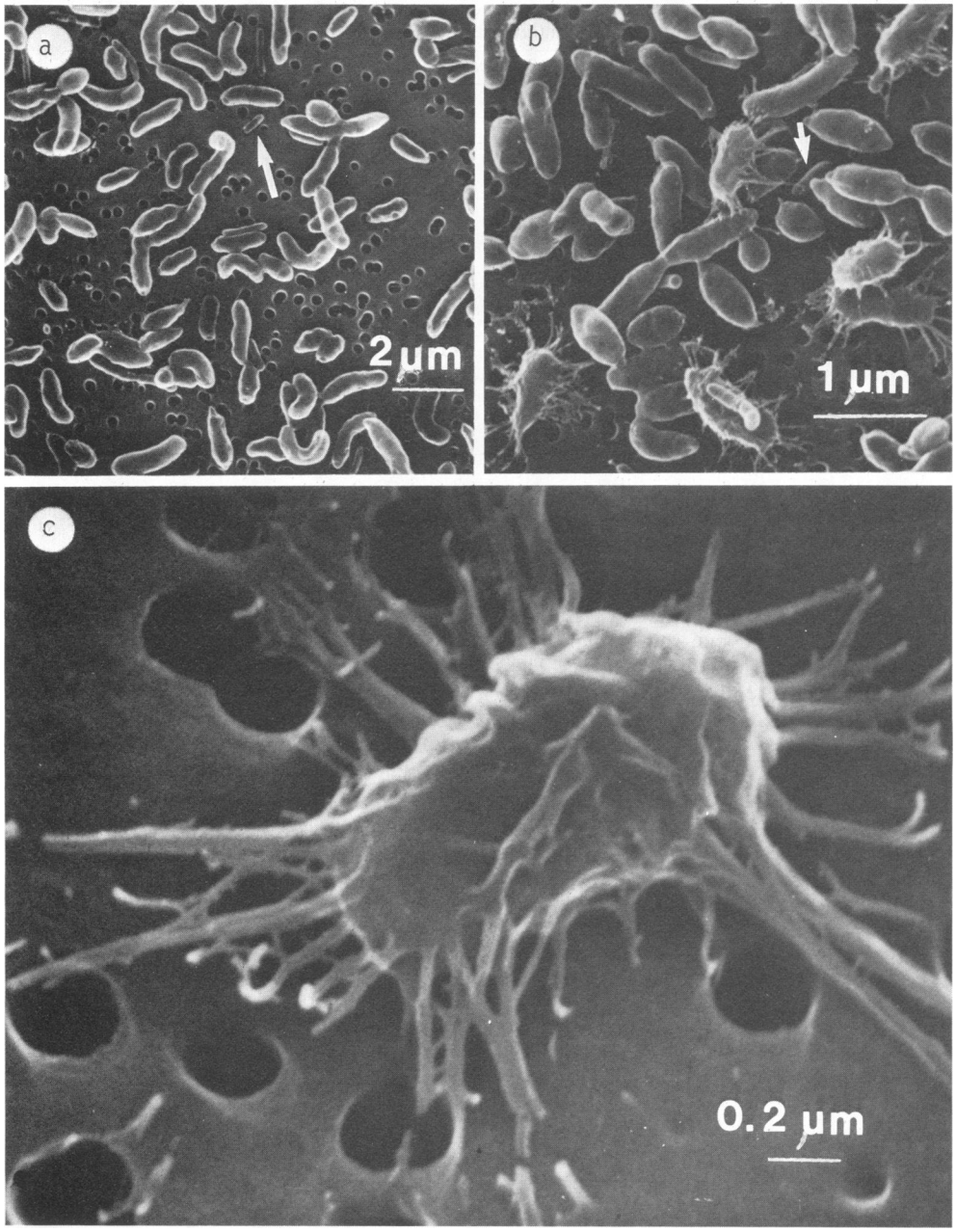

FIG. 5. SEMs of free-living bacteria from the bottom 20-cm section of an ice core collected from McMurdo Sound. (a) Free-living ice bacteria of large biovolume; note some very small bacteria present (arrow in a and b); (b) ice bacteria including those with a surface characteristic of EPS; (c) high magnification of bacterium with EPS.

than 10-fold greater than bacteria from the upper ice. The observed difference in bacterial cell number and biomass in the lower and upper ice may be a function of a gradient in temperature that exists across sea ice. Although the ice surface is exposed to air temperatures as low as −50°C, the ice-seawater interface remains relatively warm at −1.9°C (26). Bacterial communities in sea ice clearly flourish at low temperatures (−1.9°C). Or, bacteria may be responding to gradients in organic and inorganic nutrients (13) or salinity (1, 2) within annual sea ice.

Bacterial biomass concentrations were higher in sea ice than in the water column underneath. The mean concentration of bacteria in the bottom 20 cm of annual ice was 6.5×10^{11} cells m^{-3}, representing a biomass of about 46 mg of C m^{-3}. Bacterioplankton had a mean concentration of 3.6×10^{11}, with a biomass of only 3 mg of C m^{-3} due to small cell size. These ice bacterial crop sizes are greater than those reported by Kaneko et al. (24) for Arctic surface ice; however, they sampled the ice surface where bacterial numbers are at their lowest. Our estimates fall within the range reported by Hodson et al. (18) of 0.65×10^{11} to 6.5×10^{11} cells m^{-3} in surface waters of McMurdo Sound in 1977. In the lightless waters beneath the Ross Ice Shelf, Holm-Hansen et al. (19) found only 8.7×10^{9} to 12×10^{9} cells m^{-3} at depths of 20 to 200 m.

Sea ice bacteria are likely to contribute to the overall

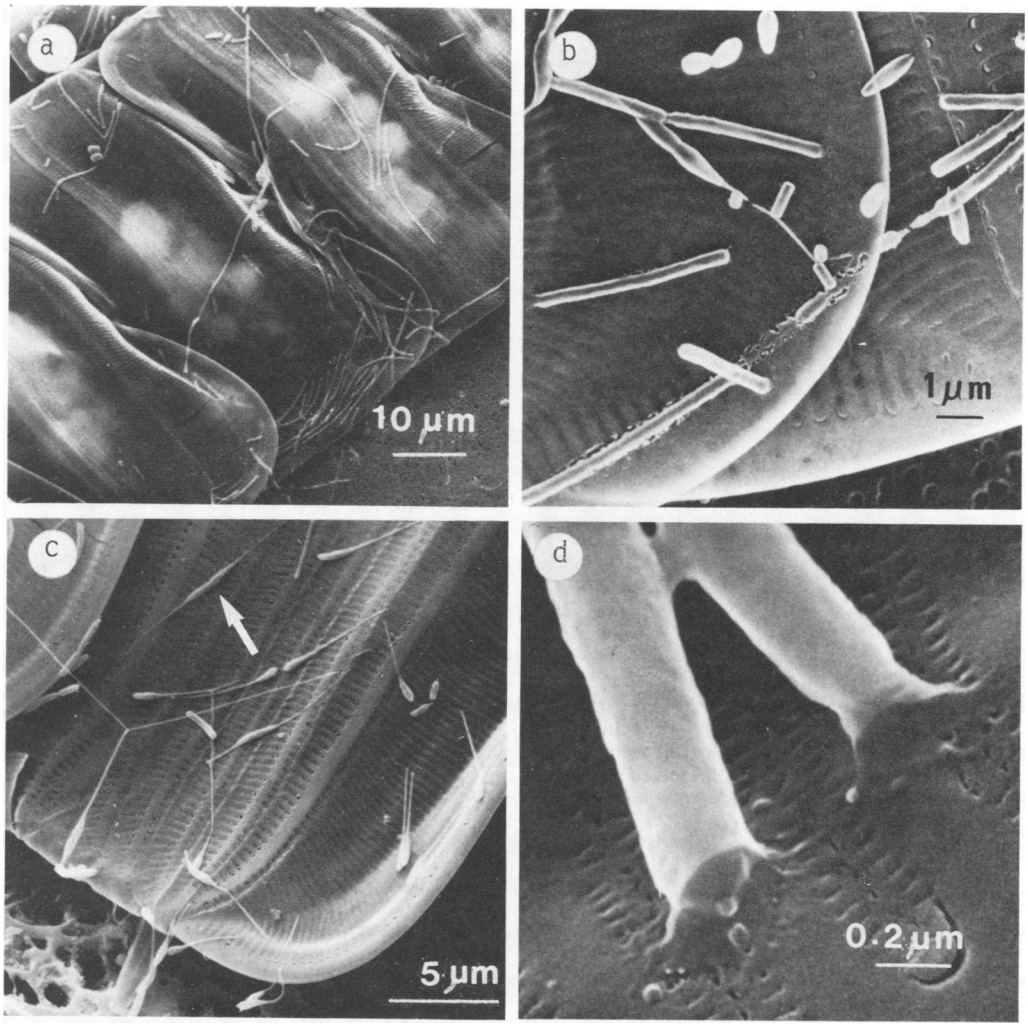

FIG. 6. SEMs of epibacteria colonizing the cell wall surface of diatom *Amphiprora* sp. taken from the 20-cm bottom section of an ice core collected from McMurdo Sound, Antarctica. (a) Filamentous and chain-forming bacteria; (b) fusiform, rod, and prosthecate bacteria; (c) prosthecate bacterium resembling *P. fusiformis*; note dividing cells (arrow) and symmetry of daughter cells; (d) Attachment site of chain-forming rod to cell wall surface of *Amphiprora*.

secondary production of McMurdo Sound (Sullivan et al., in press). By the end of November, the standing crop of bacteria was 9.8 mg of C m^{-2} of sea ice. This value, however, represents only 0.1% of the ice algal standing crop in McMurdo Sound during the same period (33). We believe these bacterial standing crop estimates are conservative for two reasons. First, bacterial production within sea ice becomes physically trapped as the ice grows, with some losses from grazing occurring primarily at the ice-seawater interface (14, 34, 35, 40). Microbial grazers such as ciliates (8) may invade brine channels in the ice from the water column later in the season when ice becomes more porous; however, during November and early December 1980, few ciliates were observed in our samples. Second, the SIMCO in McMurdo Sound may continue to grow until late December or early January (39).

Beyond their direct contribution to production, bacteria may be important in conditioning and maintaining the ice microenvironment for algal growth. Bacterial metabolic activities could provide vitamins and growth factors, or they could recycle organic nitrogen compounds to inorganic nutrients such as NO_3^-, NO_2^-, and NH^{4+}. Furthermore, through their respiratory activity, ice bacteria may lower ambient oxygen concentrations in brine cells, preventing oxygenic inhibition of algal photosynthesis in this restricted environment.

The ice bacterial community had two components: the free-living bacteria which comprised 70% and the attached or epibacteria which comprised 30% of the total bacteria. The free-living bacteria were mostly cocci or short rods; they were not morphologically diverse but did vary in cell volume. Most were relatively large with biovolumes in the range of 3 to 10 μm^3 $cell^{-1}$, whereas others were quite small (<0.5 μm^3 $cell^{-1}$) (Fig. 5a and b, arrow). Some of the larger free-living bacteria had a striking surface texture that suggested the presence of EPS (10).

Epibacteria primarily colonized the surfaces of living algal cells, in addition to dead cells and other detritus. Symbiotic relationships between ice algae and bacteria were suggested by the close physical association of epibacteria with *Amphiprora* spp. and by a positive correlation of bacterial concentration and chlorophyll *a* in the bottom of annual ice. Epibacteria appeared to be selective in the colonization of diatoms, as they were found predominantly on *Amphiprora*

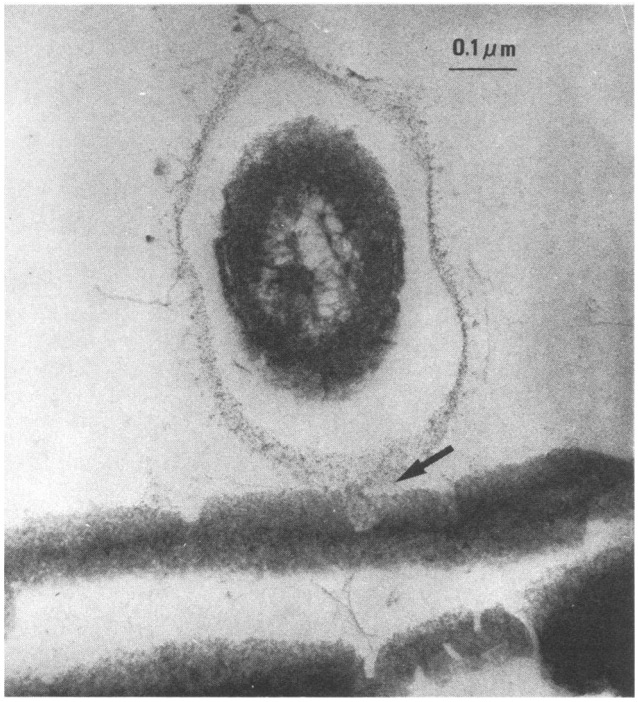

FIG. 7. Transmission electron micrograph of a thin section of an epibacterium attached to the external surface of the cell wall of *Amphiprora* sp. Dark electron opaque material in lower portion of micrograph is cross section of siliceous valve of diatom cell wall; note that EPS material fills punctae of valve (arrow).

spp. We first questioned whether this association was the result of bacterial attack on a senescent diatom population. However, the *Amphiprora* cells appeared healthy: the cytoplasm fluoresced in cells stained with acridine orange, subcellular organelles and membranes were intact, and cells were not vacuolated. Moreover, microautoradiography of the natural diatom community with $H^{14}CO_3$ under simulated in situ conditions showed that most algae including *Amphiprora* spp. were photosynthetically active in sea ice.

Two hypotheses concerning the *Amphiprora*-bacteria association may be advanced. *Amphiprora* spp. may leak or excrete a large portion of their photosynthate to the extracellular environment where it is taken up and utilized by epibacteria. Or, *Amphiprora* spp. may fail to produce certain antibacterial substances by which other ice diatoms inhibit growth or attachment of bacteria or both (35).

Among the morphologically diverse epibacteria colonizing the surface of *Amphiprora* spp., prosthecate bacteria were most unusual. A morphologically similar bacterium was first described from a natural freshwater population but was not isolated (16). More recently, four strains of a bacterium with this morphology were isolated from freshwater systems (lakes, ponds, and sewage) and identified by Staley et al. (37) as *P. fusiformis*. Often confused with *Caulobacter* spp., *Prosthecobacter* spp. lack a dimorphic stage of motile nonstalked daughter cells and immotile stalked mother cells. Instead, *Prosthecobacter* cells divide symmetrically with daughter cells as mirror image replicas of the stalked mother cell.

In conclusion, annual sea ice is a microhabitat for a previously undescribed, complex community of morphologically diverse marine bacteria. SIMCOs may now be considered not only as a source of primary production but also of secondary production in polar ecosystems (33). We propose that a detrital food web is associated with sea ice. This proposition is based on the presence in sea ice of utilizable organic substrates such as amino acids (1; D. Manahan et al., manuscript in preparation), ice bacteria, and bactivorous microzooplankters (8, 36; C. W. Sullivan and G. T. Taylor, unpublished data).

ACKNOWLEDGMENTS

We acknowledge S. McGrath Grossi and E. Flentye for expert technical assistance, J. Pickett-Heaps for use of laboratory facilities at the University of Colorado, Boulder, A. J. Gow for instruction in petrographic methods, and Ruth Toyama for manuscript preparation. U.S. Navy squadron VXE-6 provided helicopter support for field studies, and G. Simmons, D. Anderson, and J. Wood provided diving support.

This work was supported by National Science Foundation grant DPP79-20802 to C.W.S.

LITERATURE CITED

1. **Ackley, S. F., K. R. Buck, and S. Taguchi.** 1979. Standing crop of algae in the sea ice of the Weddell Sea region. Deep Sea Res. **26A**:269–381.
2. **Ackley, S. F., S. Taguchi, and K. R. Buck.** 1978. Primary productivity in ice of the Weddell region. Cold Regions Research and Engineering Laboratory Report 78-19, Hanover, N.H.
3. **Alexander, V.** 1974. Primary productivity regimes of the nearshore Beaufort Sea with reference to the potential role of ice biota, p. 609–632. Symposium on the Beaufort Sea, coastal and shelf research. Arctic Institute of North America, Arlington, Va.
4. **Apollonio, S.** 1961. The chlorophyll content of arctic sea ice. Arctic **14**:197–200.
5. **Apollonio, S.** 1965. Chlorophyll in arctic sea ice. Arctic **18**:118–122.
6. **Bunt, J. S., and C. C. Lee.** 1970. Seasonal primary production in Antarctic sea ice at McMurdo Sound in 1967. J. Mar. Res. **28**:304–320.
7. **Bunt, J. S., and E. J. F. Wood.** 1963. Microalgae and Antarctic sea ice. Nature (London) **199**:1254–1255.
8. **Fenchel, T., and C. C. Lee.** 1972. Studies on ciliates associated with sea ice from Antarctica. I. The nature of the fauna. Arch. Protistenkd. **114**:5231–5236.
9. **Fuhrman, J. A., and F. Azam.** 1980. Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. Appl. Environ. Microbiol. **39**:1085–1095.
10. **Geesey, G. G.** 1982. Microbial exopolymers: ecological and economic considerations. ASM News **48**:9–14.
11. **Gordon, A. L.** 1981. Seasonality of southern ocean sea ice. J. Geophys. Res. **86**:4193–4197.
12. **Gow, A. J., and W. F. Weeks.** 1977. The internal structure of fast ice near Narwhal Island, Beaufort Sea, Alaska. Cold Regions Research Laboratory Report 77-29, Dartmouth, N.H.
13. **Grainger, E. H.** 1977. The annual nutrient cycle in sea ice, p. 285–299. *In* M. J. Dunbar (ed.), Polar oceans. Arctic Institute of North America, Arlington, Va.
14. **Gruzov, E. N.** 1977. Seasonal alterations in coastal communities in the Davis Sea, p. 263–278. *In* G. A. Llano (ed.), Adaptations within Antarctic ecosystems. Smithsonian Institution Press, Washington, D.C.
15. **Hansen, R. B., and D. H. Pope.** 1981. Bacterioplankton adaptation and growth in different zones of the Southern Ocean. Antarct. J. U.S. **16**:130–132.
16. **Henrici, A. T., and D. E. Johnson.** 1935. Studies of freshwater bacteria. II. Stalked bacteria, a new order of Schizomycetes. J. Bacteriol. **30**:61–93.
17. **Hobbie, J. E., R. J. Daley, and S. Jasper.** 1977. Use of nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. **33**:1225–1228.
18. **Hodson, R. E., F. Azam, A. F. Carlucci, J. A. Fuhrman, D. M. Karl, and O. Holm-Hansen.** 1981. Microbial uptake of dissolved

organic matter in McMurdo Sound, Antarctica. Mar. Biol. **61**:89–94.
19. **Holm-Hansen, O., F. Azam, L. Campbell, A. F. Carlucci, and D. M. Karl.** 1978. Microbial life beneath the Ross Ice Shelf. Antarct. J. U.S. **13**:129–130.
20. **Holm-Hansen, O., C. J. Lorenzen, R. W. Holmes, and J. D. H. Strickland.** 1965. Fluorometric determination of chlorophyll. Journal Cons. Cons. Int. Explor. Mer **30**:3–15.
21. **Horner, R. A.** 1976. Sea ice organisms. Oceanogr. Mar. Biol. Annu. Rev. **14**:167–182.
22. **Hoshiai, T.** 1977. Seasonal change of ice communities in sea ice near Syowa Station, Antarctica, p. 307–317. *In* M. J. Dunbar (ed.), Polar oceans. Arctic Institute of North America, Montreal.
23. **Iizuka, H., I. Tanabe, and H. Meguro.** 1966. Microorganisms of the Antarctic Ocean. J. Gen. Appl. Microbiol. **12**:101–102.
24. **Kaneko, T., G. Roubal, and R. M. Atlas.** 1978. Bacterial populations in the Beaufort Sea. Arctic **31**:97–107.
25. **Krempin, D. A., and C. W. Sullivan.** 1981. The seasonal abundance, vertical distribution, and relative microbial biomass of chroococcoid cyanobacteria at a station in southern California coastal waters. Can. J. Microbiol. **27**:1341–1344.
26. **Littlepage, J. L.** 1965. Oceanographic investigations in McMurdo Sound, Antarctica. Antarct. Res. Ser. **5**:1–37.
27. **Meguro, H., K. Ito, and H. Fukushima.** 1966. Diatoms and the ecological conditions of their growth in sea ice in the Arctic Ocean. Science **152**:1089–1090.
28. **Meguro, H., I. Kuniyuki, and H. Fukushima.** 1967. Ice flora (bottom type): a mechanism of primary production in the polar seas and growth of diatoms in sea ice. Arctic **20**:114–133.
29. **Menzel, D. W., and R. F. Vaccaro.** 1964. The measurement of dissolved organic and particulate carbon in seawater. Limnol. Oceanogr. **9**:138–142.
30. **Morita, R. Y.** 1975. Psychrophilic bacteria. Bacteriol. Rev. **39**:144–167.
31. **Paerl, H. W., and S. L. Shimp.** 1973. Preparation of filtered plankton and detritus for study with scanning electron microscopy. Limnol. Oceanogr. **18**:802–805.
32. **Palmisano, A. C., and C. W. Sullivan.** 1982. Physiology of sea ice diatoms. I. Response of three polar diatoms to a simulated summer-winter transition. J. Phycol. **18**:489–498.
33. **Palmisano, A. C., and C. W. Sullivan.** 1983. Antarctic sea ice microbial communities. I. Distribution, diversity and production of ice algae in McMurdo Sound. Polar Biol. **2**:171–177.
34. **Sagar, P. M.** 1980. Life cycle and growth of the Antarctic gammarid amphipod *Paramoera walteri* (Stebbing 1906). J. R. Soc. N.Z. **10**:259–470.
35. **Sieburth, J. McN.** 1967. Microbiology of Antarctica, p. 267–295. *In* J. Van Mieghem, P. Van Oye, and J. Schell (ed.), Biogeography and ecology in Antarctica. Dr. W. Junk Publishers, The Hague.
36. **Silver, M. W., J. M. Mitchell, and D. L. Ringo.** 1980. Siliceous nanoplankton. II. Newly discovered cyst and abundant choanoflagellates from the Weddell Sea, Antarctica. Mar. Biol. **58**:211–217.
37. **Staley, J. T., J. A. M. de Bont, and K. de Jonge.** 1976. *Prosthecobacter fusiformis* nov. gen. et sp., the fusiform caulobacter. Antonie van Leeuwenhoek J. Microbiol. Serol. **42**:333–342.
38. **Sullivan, C. W., and A. C. Palmisano.** 1981. Sea ice microbial communities in McMurdo Sound, Antarctica. Antarct. J. U.S. **16**:126–127.
39. **Sullivan, C. W., A. C. Palmisano, S. Kottmeier, S. Grossi, R. Moe, and G. T. Taylor.** 1983. The influence of light on development and growth of sea ice microbial communities in McMurdo Sound. Antarct. J. U.S. **18**:100–102.
40. **Sullivan, C. W., A. C. Palmisano, S. Kottmeier, and R. Moe.** 1982. Development of the sea ice microbial community (SIMCO) in McMurdo Sound, Antarctica. Antarct. J. U.S. **17**:155–157.
41. **Tipet, D. H., and J. D. Pickett-Heaps.** 1977. Cell division in the pennate diatom *Surirella ovalis*. J. Cell Biol. **73**:705–727.