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## Bacteria in pack-ice north of Elephant Island (BIOMASS III, October 1986)

**ABSTRACT:** Total count (TC) of bacteria in drifting annual pack-ice in austral spring fluctuated between  $2.8 \cdot 10^6$  and  $2.09 \cdot 10^9 \text{ dm}^{-3}$ . TC of bacteria was lowest in the upper layer of a large pack-ice fragment, emersed above water surface and almost completely free of diatoms; it was comparable to TC of bacteria in surrounding sea water, which was very low at this time ( $1.92 \cdot 10^6$  —  $5.8 \cdot 10^6 \text{ dm}^{-3}$ ). TC of bacteria increased in the deeper layers of pack-ice, attaining a maximum in the middle layer characterized by a high count of diatoms. TC of bacteria was highest in small pack-ice pieces 10—20 kg in being and densely overgrown with diatoms. Bacterial population in pack-ice was dominated by rods (62%), and it contained filamentous bacteria (2.4%) and prosthecate forms (4.8%), rarely present in deep sea. Mean volume of bacterial cell ( $0.206 \mu\text{m}^3$ ) was small, only slightly exceeding that of cells of free-living bacteria in sea water in summer.

Key words: Antarctic, bacteria, pack-ice, BIOMASS III.

### 1. Introduction

In winter, as a result of phytoplankton mortality, the basic substrate for metabolic conversions of bacterioplankton disappears, i.e. the photosynthesized organic matter being supravivally released by phytoplankton to the environment (Hellebust 1965; Sorokin 1971; Larsson and Hagström 1982). Under conditions of drastic food deficiency, in the water column a new phycosphere appears (Bell, Lang and Mitchell 1974), i.e. pack-ice cumulating immense amounts of phytoplankton (Bunt and Wood 1963) and bacteria (Sullivan and Palmisano 1981; Marra, Burckle and Ducklow 1982). Owing

to the marked adaptation of some phytoplankton species to the life in darkness, under light conditions being even below the level of compensation intensity for phytoplankton (Sullivan et al. 1982), already in early Antarctic spring this phycosphere becomes the site of intense primary production (Sullivan et al. 1982) and of parallel secondary production (Kottmeier et al. 1985). This phycosphere represents a concentrated source of microbial carbon and thus it plays an important role in zooplankton nutrition (Rakusa-Suszczewski 1972; Sullivan 1987).

So far, the major part of studies of the pack-ice community have dealt with regions situated inside the polar circle. The present studies aimed at the preliminary examination of the pack-ice bacterial community in drifting pack-ice in Antarctic spring at lower latitudes.

## 2. Material and methods

For the determination of total count of bacteria pack-ice was collected in the vicinity of Elephant Island (Fig. 1) on 28 and 29 October 1986. One batch consisted of ice samples (B17, B19, B22, B23, B24, B25 and B30) taken from inside of small free-floating pack-ice pieces weighing 10–20 kg, taken from the board of r/v "Profesor Siedlecki". Another batch comprised ice samples taken from a big pack-ice fragment, several meters in diameter and about 1,5 m in thickness. Ice samples were taken as follows: B27 from the upper, about 30-cm thick layer covered with snow; B28 from the middle, about 40-cm thick layer; B29 from the lowest, about 70-cm thick layer. Water samples were taken as follows: B23<sub>1</sub> was taken near B23, and B26 was collected from under big pack-ice fragment. Moreover, water samples were taken from the water column at sampling stations 37–48 from a total of 48 sampling points (Fig. 1), every day between 30 Oct. and 3 Nov. 1986, as well as in the Admiralty Bay (King George Island — sampling station 77) on 9 Nov. 1986.

Ice was melted at 0 C. For counting of colony-forming bacteria, samples (0,2 cm<sup>3</sup>) were inoculated on nutrient agar incubated for 4 days at 9 C (Zdanowski 1981). For measurement of the total count (TC) of bacteria and evaluation of the approximate count of diatoms, samples (10 cm<sup>3</sup>) were filtered through 0,2  $\mu$ m-porosity polycarbonate filters (Nuclepore); after Acridine Orange (AO) staining (Zimmermann 1977) cells were counted by epifluorescence microscopy (EFM) using a Fluoval 2 unit. Carl Zeiss, Jena (Zdanowski 1986). Bacterial volume was assessed according to Zimmermann (1977). The biomass was evaluated on the assumptions that the specific weight of bacteria is 1,1 g cm<sup>-3</sup> and that C accounts for 15% of weight (Hagström et al. 1979). Parallel samples (10 cm<sup>3</sup>) preserved in formaline (1% final concentration) and sealed over a flame in vials were transported

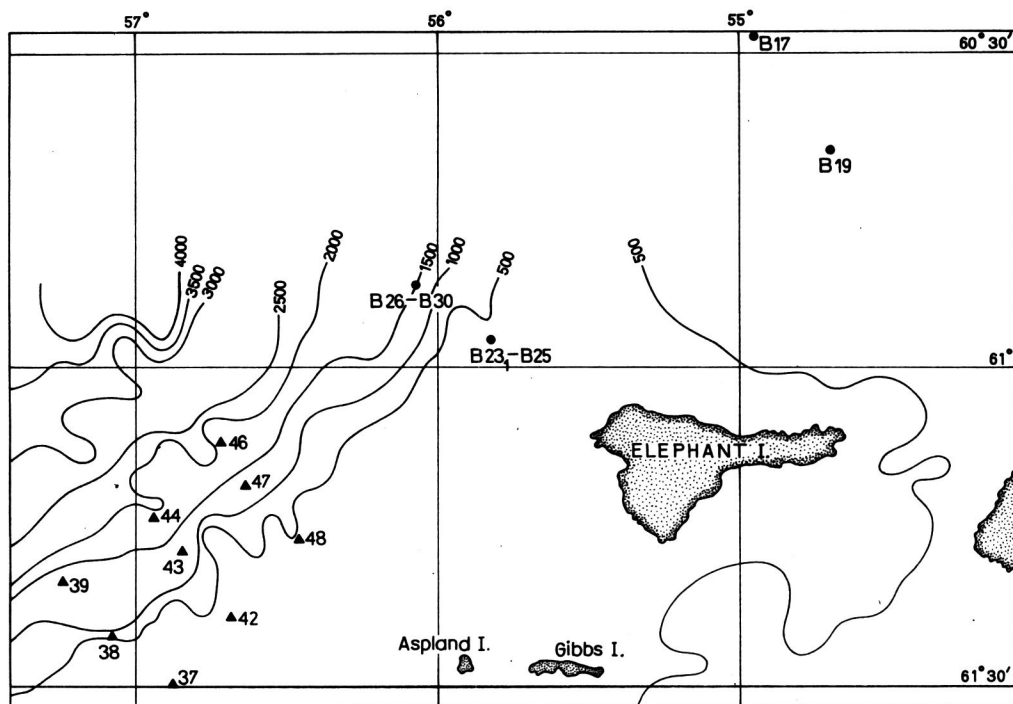


Fig. 1. Location of sampling stations (for pack-ice; B17—B30) and oceanographic stations (for sea water samples) in Scotia Front (st. 37—48)

to Poland, in order to repeat direct counts by EFM and to take EF microphotographs under normal laboratory conditions. A part of samples were assigned for taking scanning electron micrographs (SEM) of cells sedimented on polycarbonate filters (pore size  $0.2 \mu\text{m}$ ). Total counts (Tab. 1) represent the mean values of measurements performed immediately after sample collection and repeated after several months in Poland.

### 3. Results and discussion

TC of bacteria was high in pack-ice and low in surrounding sea water (Tab. 1, Fig. 1) in the end of October 1986. Mean TC of bacteria in pack-ice ( $6.3 \cdot 10^8 \text{ dm}^{-3}$ ) was several times higher than the mean of TC of bacteria (per  $\text{dm}^3$ ) in the water column near Elephant Island in summer (Zdanowski 1985a). In general, TC of bacteria in pack-ice (of an order of  $10^8$ — $10^9$ ) was comparable to that found in summer in Antarctic sea waters, in the regions of Bransfield Strait and Drake Passage (Hanson et al. 1983; Zdanowski 1985 a, b; Bailiff et al. 1987; Mullins and Priddle 1987) and in the

Table 1

Microbial count in annual pack-ice and in surrounding sea water.

Morphological structure of bacterial populations in these habitats.

Abbreviations: spi — small pack-ice (brash ice), sw — sea water, bpi — big pack-ice fragment (ice cake), dc — disclosed, uc — uncoloured, ul — upper layer, ml — middle layer, bl — bottom layer; score of AO-stain: +<sup>5</sup> — very good, +<sup>4</sup> — good, +<sup>3</sup> — fair, +<sup>2</sup> — slight, +<sup>1</sup> — trace.

Source	Bacteria TC per dm <sup>3</sup> (·10 <sup>8</sup> )	Proportion (%)						Diatoms TC per dm <sup>3</sup>	Number of detritus particles overgrown by bacteria
		Cocci	Rods	Vibrios	Filaments	Pros- theate forms	AO-stain -ability		
B17 spi uc	3.8	43.5	46.0	6.8	3.6		10 <sup>6</sup>	10 <sup>6</sup>	
B19 spi dc	20.9	30.0	60.5	6.6	2.9		10 <sup>7</sup>	10 <sup>7</sup>	
B22 spi dc	11.9	37.5	55.9	6.4	0.5		10 <sup>7</sup>	1,3·10 <sup>7</sup>	
B23 spi dc	20.9	39.9	53.4	5.6	1.0		10 <sup>7</sup>	3,5·10 <sup>7</sup>	
B23 <sub>1</sub> sw	0.058	66.6	22.0	11.1			0	trace	
B24 spi dc	1.2	46.2	46.2	6.5	1.0		10 <sup>6</sup>	10 <sup>5</sup>	
B25 spi uc	0.9	48.5	40.6	10.9	0.5		trace	trace	
B26 sw	0.019	65.6	26.0				0	trace	
B27 bpi ul uc	0.028	60.0	30.0				10 <sup>4</sup>	10 <sup>5</sup>	
B28 bpi ml dc	1.9	24.4	62.0	6.2	2.4	4.9	3,8·10 <sup>7</sup>	10 <sup>7</sup>	
B29 bpi bl uc	0.202	11.0	61.0	22.0			3,2·10 <sup>5</sup>	10 <sup>4</sup>	
B30 spi dc	2.2	26.9	60.7	5.7	2.3	4.6	10 <sup>7</sup>	10 <sup>7</sup>	

region of McMurdo Sound (Hodson et al. 1981). TC of bacteria in sea water surrounding pack-ice was only from  $1.9 \cdot 10^6$  to  $5.8 \cdot 10^6 \text{ dm}^{-3}$  (Tab. 1). TC was highest in small drifting pack-ice pieces weighing from several to 10–20 kg, of a most intense brown colour, whose whole surface was exposed to sea water. They contained 100–1000 times more bacteria than the surrounding sea water. Lower number of bacteria occurred in a large fragment of pack-ice; a distinct differentiation of TC in vertical profile of pack-ice was found.

There was an evident relationship between the count and general condition of the bacterial population in ice, on one hand, and the contents of diatoms and chlorophyll *a*, imparting colour to pack-ice fragments (Ligowski, Lipski and Zieliński 1988; Tab. 1). This confirms the hypothesis of Grossi, Kottmeier and Sullivan (1984), stating that microalgae stimulate bacterial growth in pack-ice supplying bacteria with organic substrate. This is well exemplified by the microbial community, as observed in the cross-section of a large pack-ice fragment (B27–B29; Tab. 1). Its middle, light brown layer (B28) contained most of diatoms. They were associated with numerous, morphologically differentiated (Tab. 2, Pl. 1), very well AO-stained bacterial population containing up to several percent of dividing cells, i.e. being in an excellent general condition. The uncoloured lower layer (B29) contained about 100 times fewer diatoms and about 10 times fewer bacteria, as compared with the middle layer. However, with respect to general condition (degree of AO-staining, distinctness of shape and frequency of divisions), both bacterial populations from layers B28 and B29 were similar. On the other hand, the upper layer B27 emerging above water level was completely different. It was almost fully devoid of diatoms (Tab. 1) and contained a very scarce bacterial population. With respect to the count and appearance, it resembled the bacterial community in the surroundings of ice. The low count, and particularly the poor general condition of the bacterial population in the upper layer of pack-ice and in surrounding sea water suggest that they exclusively contain starving transients. In contrast, bacterial populations neighbouring with diatoms contained — most likely — active forms.

As concerns the measurement procedure used, it is stressed that the composition and count of both — bacteria and diatoms, were determined by EFM in the same fields of vision, with the use of an Apochromat  $\times 100$  objective, i.e. under conditions intended for counting of fine objects (bacteria), but not of big diatoms. Possibly for this reason the present results concerning the count of diatoms are systematically underestimated by one order of magnitude, as compared with the measurements reported by Ligowski, Lipski and Zieliński (1988).

There is extensive evidence indicating that the pack-ice community comprising psychrophilic microalgae, bacteria and protozoans plays an important part in the primary and secondary production. In studies of the

pack-ice community in McMurdo Sound in October, i.e. under conditions of continuous daylight in the region situated near 78°S, Kottmeier et al. (1985) have observed a bloom of microalgae in pack-ice, resulting, within a week, in an increase — by one order of magnitude — in the extent of carbon fixation per square meter; this phenomenon was paralleled by bacterial production which, under 1 m<sup>2</sup> of ice, was equivalent to the production in a 1000—10000 m integrated water column in McMurdo Sound. It is assumed, moreover, that bacterial and diatom cells being in the phase of intense growth may — in parallel with ice breaking and melting — act as inoculum for pelagic populations (Garrison, Ackley and Buck 1983). They are also a source of food for the sub-ice community (Rakusa-Suszczewski 1972) and for pelagic consumers (Ackley, Buck and Taguchi 1979; Garrison, Buck and Silver 1982; Fuhrman and McManus 1984), including krill larvae (Sullivan 1987).

Table 2  
Mean volume of bacteria in relation to different cell length classes in the middle layer of a big pack-ice fragment (B28)

Bacteria	Mean cell length or cell diameter (μm)	Mean cell width (μm)	Mean cell volume (μm <sup>3</sup> )	Frequency in the class of cell length (%)
Cocci	0.2		0.004	8.5
	0.6		0.11	7.3
	0.74		0.21	8.5
Rods	0.8	0.37	0.09	34.1
	1.4	0.37	0.15	17.1
	3.0	0.29	0.20	2.4
	3.0	0.48	0.54	6.1
	2.2	0.70	0.85	2.4
Vibrios	1.5	0.37	0.16	6.1
Filaments	13.7	0.37	1.47	2.4
Prosthecate forms	3.3	0.37	0.38	4.9

The bacterial population in pack-ice (Tab. 2, Pl. 1) was evidently dominated by rods (62%), and contained filamentous forms (2,4%) — (rarely occurring in deep sea), sometimes attaining a length of 50 μm, as well as prosthecate forms (4,9%); it differed from the communities of bacterioplankton living in the water column, which was dominated by cocci (Tab. 1) (Rakusa-Suszczewski and Zdanowski 1988). Differences in the structure between bacterial populations living in pack-ice and in the water column may result from both — the mechanism of incorporation of bacteria during

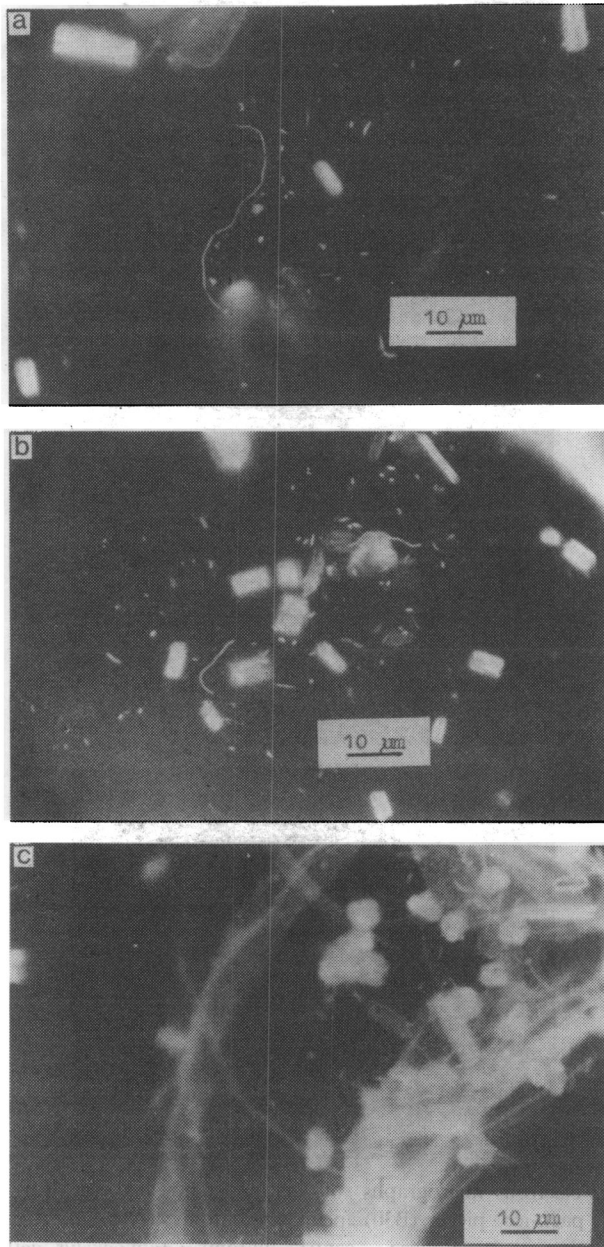


Plate 1. Epifluorescent photomicrographs of acridine orange-stained bacteria from the middle layer of a big pack-ice fragment (B28)

Note the diversity of size and shape of bacteria (a, b), the prevalence of pennate diatoms (a, b, c), sporadic *Amphiprora* sp. (a) and the lack of cylindric diatoms

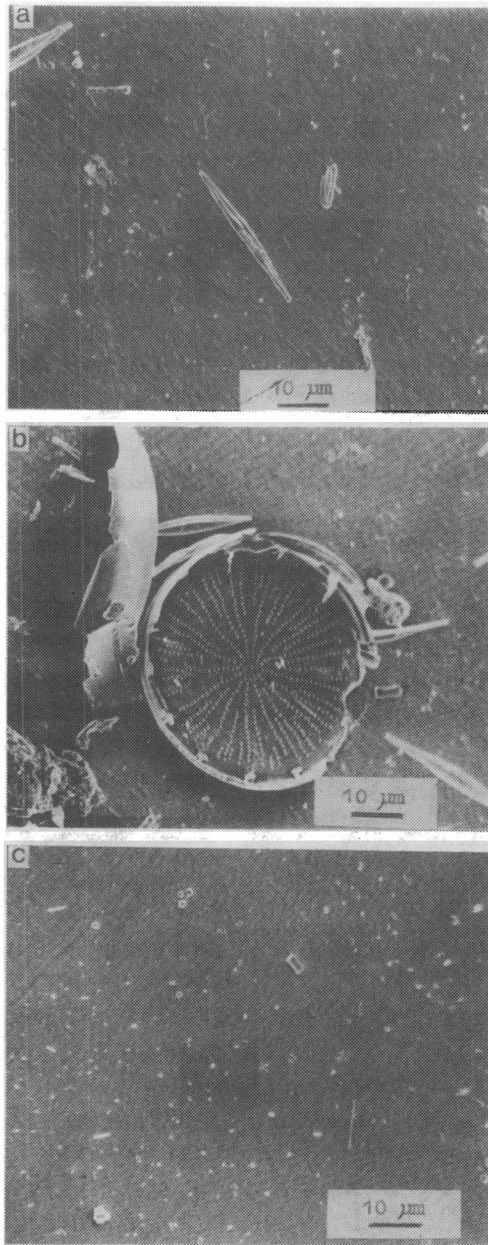


Plate 2. Scanning electron micrographs of diatoms and bacteria: (a) — in a small, separately floating pack-ice piece (B30) pennate diatoms (*Nitzschia lecontei*) — in prevalence; (b) — the same pack-ice piece — centric diatoms (*Actinocyclus actinochilus*) — rare; (c) — bacteria in a 10-cm<sup>3</sup> sample of sea water from sampling station 37, depth 200 m (approximately  $3.29 \cdot 10^8$  cells per dm<sup>3</sup>). The sample was filtered through a polycarbonate filter (por size 0.2 µm), air-dried and sputtered with gold. Note that in a parallel sample prepared for EFM (AO-stained) no bacteria were found



ice formation and subsequent modification of the population structure in the course of growth in ice. Among two mechanisms proposed for incorporation of algae in newly formed pack-ice: by preferential ice nucleation or by scavenging (Garrison, Ackley and Buck 1983), the former allows for formation of ice microbial community differing from that present in the water column. According to Parker et al. (1985), only few bacterial strains (only one of 11 strains isolated from pack-ice in the region of McMurdo Sound) have been found to exhibit an ice nucleation activity at temperatures higher than  $-10^{\circ}\text{C}$ . At the subsequent stage of ice "maturation", microalgae and bacteria must become mutually adapted; for example, bacteria sensitive to antibacterial substances produced by some algae are eliminated. In turn, bacterial metabolism may exert on algae either a favourable effect (e.g. via production of growth factors or lowering of the level of oxygen inhibiting the photosynthesis in microalgae, as reported by Sullivan and Palmisano, 1984) or an adverse one. For example, Garrison, Buck and Silver (1982) have shown that *Phaeocystis* being greatly antagonistic towards many bacteria (Sieburth 1965) and representing the dominant microalga in newly formed ice, is replaced by diatoms in older ice.

So far, the major part of the questions concerning the genesis and development of bacterial populations in pack-ice remain within the range of hypotheses. It is unclear whether these are — for sure — the same bacteria which populate ocean waters in summer and are bound in newly formed ice, or whether it is a specific population actively living within pack-ice in brine channels or being attached to the ice crystal surface, and — after ice melting — surviving in water as starving transients. The failure of an attempt at culturing these bacteria after inoculation of  $10^4$ — $10^5$  cells on NA (Zdanowski 1981) may e.g. indicate that their major part consists of planktonic bacteria with little affinity to nutrient-rich surfaces. However, a part of them doubtless belong to osmotrophic epibacteria; namely, our microscopic inspection pointed to the cases of colonization of living and dead diatoms by bacteria as well as to examples of the occurrence of small amounts of bacteria-overgrown amorphous detritus.

There were substantial differences, apart from some evident similarities (high count, occurrence of filamentous and prosthecate bacteria), in the pack-ice community between the region of the present studies (Tabs. 1 and 2) and McMurdo Sound (Grossi, Kottmeier and Sullivan 1984; Sullivan and Palmisano 1984). In contrast to the pack-ice population from McMurdo Sound, mainly comprising big bacteria ( $3$ — $10\ \mu\text{m}^3$  per cell), the bacteria observed in the present studies were mostly fine, approaching in length the free-living ones in the water column (Tab. 2). The biomass of bacteria in McMurdo Sound (in the most populated pack-ice layer, i.e. in the bottom 20 cm of ice) and in our region (in the middle layer of pack-ice) amounted to 46 and only  $6.5\ \text{mg C m}^{-3}$ , respectively; at the same time, TC of bacteria

was only 3,4 times higher in McMurdo Sound than in our region. There were differences between both regions in the decrease in TC of bacteria in the surface layer of pack-ice, as compared with that in the maximally populated layer; this decrease was about three-fold and about 70-fold in McMurdo Sound and our region, respectively (Tab. 1). The microalgal population in McMurdo Sound was dominated by *Amphiprora* sp. algae (Sullivan and Palmisano 1984) whose surface was supravitally colonized by the major part of epiphytic bacteria occurring in ice (Grossi, Kottmeier and Sullivan 1984). In our region genus *Amphiprora* has been found to be scarce in ice (Ligowski, Lipski and Zieliński 1988). Pennate diatom *Nitzschia cylindrus* and *Nitzschia lecontei* were most frequent in the preparations, whereas centric forms *Actinocyclus actinochilus* were more scarce (Pl. 2 a, b). It can thus be assumed that — irrespective of the latitude in the Antarctic — annual pack-ice is always a preferred habitat for bacteria and microalgae. However, the composition of the population and its distribution in ice are characteristic of the particular region. Studies in McMurdo Sound have concerned long-lasting stationary ice, whereas we investigated drifting pack-ice which could be derived from the vicinity of Elephant Island, although its origin from the Bellingshausen Sea or — less likely — from Bransfield Strait (Stein 1987) also cannot be ruled out.

The difference in TC between bacteria in pack-ice and in its immediate vicinity was much bigger in the region of the present studies (Tabs. 1 and 3), as compared with the earlier results concerning the pack-ice bacterial com-

Table 3

Total count of bacteria ( $10^6 \text{ dm}^{-3}$ ) in sea water at sampling stations in Scotia Front (37–48) and in Admiralty Bay (77)

Sampling points — depth (m)	Sampling stations									
	37	38	39	42	43	44	46	47	48	77
0	1.28	5.60	1.12	1.28	0.62	3.50	nf	nf	nf	3.68
20	0.64	nf	0.64	1.60	0.96	1.76	0.32	nf	nf	3.00
50	1.28				1.28	2.70	nf	0.48		
100	0.32				0.32	0.64	nf	nf		
200	nf				0.32	3.30	nf	nf		
400				<u>nf</u>						<u>1.76</u>
460	<u>nf</u>									
490									<u>nf</u>	
500					nf	0.64	nf	nf		
670		<u>nf</u>								
1000			<u>1.28</u>			0.43	nf	<u>nf</u>		
1200					<u>0.80</u>					
1450						<u>0.32</u>				
1500							nf			
1850							<u>1.28</u>			

Explanations: nf — not found; — over the bottom

Table 4

Seasonal variation of bacterial count (for AO-stained bacteria)  
in sea water  
(n = number of sampling points)

Expedition	Range of bacterial count ( $10^8 \text{ dm}^{-3}$ )		
<b>FIBEX Feb. March 1981</b>			
Drake Passage Bransfield Strait n — 94	0.16	—	3.63
<b>SIBEX Dec. 1983/Jan. 1984</b>			
Drake Passage Bransfield Strait n — 73	0.016	—	0.73
<b>BIOMASS III Oct./Nov. 1986</b>			
Scotia Front n — 49	0	—	0.056
Admiralty Bay n — 3	0.017	—	0.037

munity in McMurdo Sound (Sullivan and Palmisano 1981) and in Weddell Sea in late winter (Marra, Burckle and Ducklow 1982). The contrast between pack-ice and sea water was even more pronounced, when TC of bacteria was referred to that in water of the ice-free region of Scotia Front at a distance of more than 10 miles SW (Fig. 1). In the latter region (Tab. 3), at 26 sampling points TC remained between  $3 \cdot 10^5$  and  $5 \cdot 10^6 \text{ dm}^{-3}$ , and at the remaining 22 sampling points no bacteria were detected by EFM in 20-cm<sup>3</sup> water samples; also detritus overgrown by bacteria was rarely observed.

We attempted to explain the drastic drop in TC of free-living bacteria in the water column in this period by poor AO-stainability of "starved" bacterial cells, which prevents counting of all bacteria. The picture of bacteria examined in the present studies (during transition from an exceptionally severe winter to spring) completely differed from that of bacteria collected in summer. In the present studies, there were neither well stained nor at least fairly well stained bacteria (Tab. 1). Therefore, only slightly stained bacteria (within the limit of discernment) were counted. We assumed that perhaps the major part of bacteria, being starving transients, failed to be recorded. To elucidate this problem, preparations parallel to the AO-stained ones (showing no presence of bacteria upon EFM-inspection) were examined by the number of particles of the size and appearance of bacteria in all SEM-examined preparation remained within the range of  $1.45 \cdot 10^8$ — $5.08 \cdot 10^8 \text{ dm}^{-3}$ . Comparison of the results obtained for TC of bacteria during three

expeditions: FIBEX 1981 (Zdanowski, unpubl.), SIBEX (1983—84) (Zdanowski 1985a) and BIOMASS III (Tab. 4), testified to an evident relationship between TC of AO-stained free-living bacteria and season. TC of bacteria in the water column attained a maximum in summer and a minimum during winter-to-summer transition. The drastic drop in the count of the population of AO-stained bacteria — after retreat of a severe winter — in the water column remains in strong contrast with the very numerous and morphologically differentiated pack-ice bacterial community being very intensely AO-stained.

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## 5. Streszczenie

Zbadano zawartość bakterii w próbach rocznego lodu morskiego dryfującego w pobliżu wyspy Elephant i w otaczającej lód wodzie morskiej (rys. 1). Stwierdzono pozytywną zależność między zagęszczeniem i kondycją populacji bakterii w lodzie, a zawartością okrzemek barwiących lód (tab. 1). TC bakterii (EFM) w lodzie wahała się od  $2,8 \cdot 10^6$  dcm<sup>-3</sup> w wolnej od okrzemek, niezabarwionej wierzchniej warstwie kry lodowej, do  $1,9 \cdot 10^8$  dcm<sup>-3</sup> w lekko-brązowej warstwie środkowej. W małych 10–20 kg bryłach lodu pływającego w wodzie, intensywnie zabarwionych i przerośniętych okrzemkami, TC bakterii osiągało  $2,09 \cdot 10^9$  dcm<sup>-3</sup>.

Zawartość bakterii (EFM) w lodzie morskim zasiedlonym przez okrzemki była od 100 do 1000 razy wyższa niż w otaczającej wodzie morskiej. Woda morska zawierała zaskakująco niską liczbę bakterii (barwiących się OA), średnio około  $100 \times$  niższą niż w okresie letnim. Większość bakterii występujących w lodzie stanowiły pałeczki (62%), a następnie ziarniaki (24,4%) i przecinkowce (4,9%). Resztę stanowiły formy nitkowate o średniej długości 13,7  $\mu$ m (2,4%) (dochodzące niekiedy do 50  $\mu$ m długości) i formy z wyrostkami (4,9%) (tab. 2, pl. 1). Średnia objętość bakterii (0,206  $\mu$ m) była niska, porównywalna z objętością bakterii wolnożyjących w wodzie morskiej w okresie letnim. Zespoły bakterii lodu morskiego w badanym rejonie na północ od koła podbiegunowego, mimo szeregu podobieństw, różniły się od opisywanych wcześniej zespołów bakterii z lodu w rejonach usytuowanych za kołem polarnym.