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Bacteria in the sea-ice zone between Elephant Island and the South Orkneys during the Polish sea-ice zone expedition, (December 1988 to January 1989)

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Abstract. During austral summer 1988/89, total bacterial Acridine Orange Direct Counts (AODC) in seawater, mean $6.0 \times 10^6\ l^{-1}$, were three to ten times lower than generally reported for the Bransfield Strait to north Weddell Sea area. In contrast, numbers of viable bacteria (Colony Forming Units, CFU), mean $10.6 \times 10^3\ l^{-1}$, were two to three times higher than reported. Bacterial abundance here shows large seasonal and spatial changes. On the basis of bacterial, diatom, detritus, and amino acid data from this study, two main regions were defined: 'Cold winter water' in the west with high salinity and low CFU, AODC, and other parameters. In the east, lower salinity and higher values for all parameters were found in warmer meltwater at the surface. CFU and AODC values in ice were respectively six and 85 times higher than in surrounding seawater. Taxonomic studies indicate considerable diversity in genera and nutritional requirements of isolated bacteria. Sea-ice and water column bacterial communities differed. Many isolated strains, however, were found in both habitats. Sea-ice seems to be important in regulating surrounding bacterioplankton.

The Antarctic climate is harsh, with low average annual air and sea temperatures. The onset of winter promotes the expansion of annual sea-ice cover from summer minima of around 3 million sq. Km in February, to ca. 20 million sq. Km in September (Doake, 1987). One may be of the opinion that sea-ice constitutes a hostile environment for micro-organisms, with temperatures around $-1.9°C$ (Kottmeier and Sullivan 1988), and salinity levels in the brine channels up to five times that of the surrounding seawater (Sullivan and Palmisano 1984). This, however, is not the case. Kottmeier and Sullivan (1990) report bacterial Carbon production in surface ponds and porewater ranging from 45–221% of primary production. In addition, high levels of algal production in ice, west of the Antarctic Peninsula, during the austral winter have been reported (Kottmeier and Sullivan 1987). These diverse algal and bacterial communities (Kottmeier et al. 1987; Horner et al. 1988; Ligowski et al. 1988; Zdanowski 1988a) may provide a source of food for wintering krill, *Euphausia superba*, (Stretch et al. 1988). Further, a detrital food web associated with polar ice was proposed by Sullivan and Palmisano (1984), who observed that many bacteria were attached to living algal cells or detritus. These communities break down as the ice melts during the spring and summer, and all material is released to the water column.

In order to investigate bacterial diversity in sea-ice and seawater, and the effects that the melting of annual ice has on adjacent water columns in biological terms, various bacterial parameters in sea-ice, and water close to and distant from the ice edge were compared along a 250 mile ice edge. Numbers of viable bacteria (CFU) were determined on different media in each of these habitats. Further, from a wide range of dissolved organic matter we selected free and combined amino acids for their high biological significance. These are generally in low concentrations in the water column as a result of their short turnover times (Williams et al. 1976), but as indicators of biological activity they may reflect local hydrological conditions. Taxonomic studies were also carried out on bacteria isolated from *E. superba* stomach.

Materials and methods

All sampling was carried out along the edge of the drifting pack ice between Elephant Island, South Shetlands, and Coronation Island, South Orkneys, from December 1988 to January 1989, (Fig. 1), from RV *Profesor Siedlecki*.

Stations were described on the basis of their location with respect to the edge of the drifting pack. They were divided into three categories: Stations 25, 33, 43, 46 and 50 were located at up to 60 miles seaward of the ice-edge (Open Water, OW). Stations 24, 31, 35, 39, 45, 48, 52, 57, 61, and 63 were close to the ice (Water near Ice, IW) at ca. 500 m from the ice edge. Those located at the ice edge, 27, 68, 70, and 75, were termed 'ice stations' (I), with additional samples at the latter three from water below the ice (WBI).

Seawater samples were collected at all but three stations with a six litre ethanol rinsed Van Dorn bottle. Samples for all analyses were drawn from an integrated sample prepared by aseptically combining 100 ml samples taken from 10, 20, 30, 40, and 50 m. At

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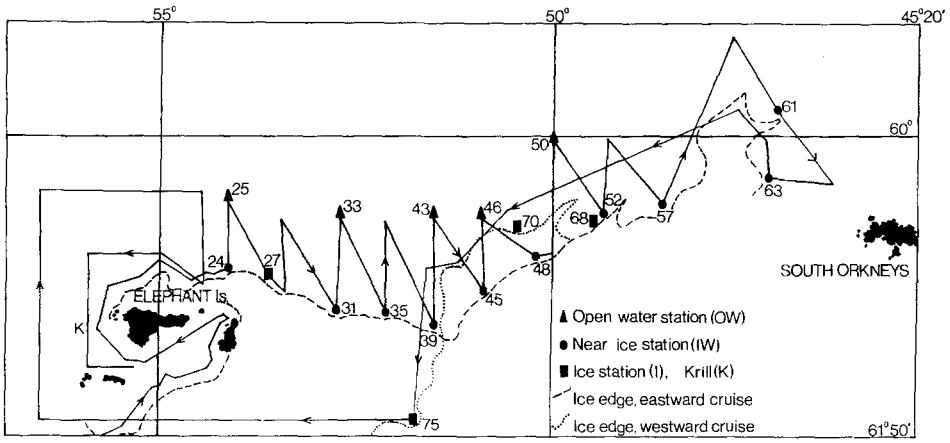

Fig. 1. Location of sampling stations

stations 68, 70, and 75, a diver collected water samples within 10 cm of the bottom of the ice using a sterile screw capped bottle which was opened and closed in situ, and from which samples for analysis were aseptically drawn.

Ice samples described as 'coloured' (IB), or 'uncoloured' (IU), were taken from pieces weighing up to 100 kg collected from the ship or small boat. Cores measuring approximately 1 m were taken from floes of ca. 2.5 m thickness with a 20 cm diameter ice auger. All samples were transported to the laboratory in opaque sterile plastic bags within one hour of collection. The surface was cleaned with a sterile scraper and a sterile stainless steel ice auger of 2.5 cm diameter, driven by a hand-held electric drill, was used to take between twenty and thirty cores of 20 ml. Cores were aseptically transferred to a sterile glass beaker and partially melted in the dark at room temperature, with occasional gentle agitation. Samples were transferred to a refrigerator prior to full melting, and processed immediately when melting was complete. We had previously determined that the temperature of the melting sample did not exceed 2°C when transferred to the refrigerator as described. In addition, we found that to fully melt large samples in the refrigerator required more than 24 h.

Colony forming units

Colony forming units were enumerated in 1 to 20 ml of sample filtered through 47 µm diameter 'Millipore' HA filters, pore size 0.22 µm, settled on Natural Seawater Nutrient Agar, NASW (Zdanowski 1982), after 15 days incubation at 0°C and 10°C. CFU were also enumerated on Pseudomonas Selective Agar, PSA (Oxoid), and TCBS Vibrio Agar (Labm) to aid classification. Bearing in mind that some bacteria may be inhibited by complex media, a 'simple' medium of 0.22 µm filtered seawater gelled with Gelatin, GSW (Oxoid), was also used. The salinity levels of NASW, PSA and TCBS, were adjusted to 3.4% prior to autoclaving.

Viable bacteria were also isolated from aseptically excised fresh krill, *E. superba*, stomachs (K). Krill selected at random from a haul of ca. one tonne made by a standard industrial krill fishing net 60 miles west of Elephant Island, were manipulated aseptically from collection. Spread plates on NASW, PSA, TCBS, and GSW were prepared from a dilution series of homogenised krill stomachs in filter sterile (0.2 µm, 'Millex GV', Millipore), autoclaved seawater, and incubated at 0°C and 10°C for 15 days.

Purification of isolates

Ninety eight isolates were randomly selected from ice (39), water below ice (8), water near ice (23), open water (20), and krill (8), from all media at 0°C and 10°C, and subcultured to NASW (5–8 days, 10°C, 1 to 3 transfers). Microscopic investigations included Gram, cell morphology, and motility tests.

The API 20NE system (API Biomerieux) allowed twenty two biochemical and physiological tests to be conducted on each isolate. Those selected were suspended in 3.4% NaCl or Aux medium (API) for inoculation of the tests. To aid the physiological description of twenty five of these bacteria (12 from ice, 1 from water below ice, 7 from water close to ice, 5 from open water), nineteen enzyme reactions were investigated using API Zym (Humble et al. 1977). API Zym was inoculated with selected colonies suspended in 3.4% NaCl. The recommended incubation temperatures of 30°C (API 20NE) and 37°C (Zym) were considered too high for Antarctic marine bacteria; all strips were incubated at 10°C (Inoue 1977). API 20NE profiles are based on results after 20 days incubation. API reagents were added to appropriate cupules after 10 days, after they had been removed from the incubation tray to prevent spurious acidification and/or inhibition of bacterial growth in the other cupules (Tearle and Richard 1987). For API Zym, reagents (API) were added to cupules after 10 days incubation. Controls for both API systems inoculated only with saline were incubated alongside the test strips and processed in the same way with respect to API reagents.

Total counts

Total bacteria were determined by Acridine Orange Direct Count (AODC) by epifluorescence microscopy according to Zimmerman (1977), with a Carl Zeiss Jena FLUOVAL 2, fitted with an Apochromat HI 100/1.32; 160/0.17 objective. Samples of 2 to 20 ml were filtered through 0.2 µm 'Nuclepore' polycarbonate membranes, prestained with Sudan Black, (Hobbie et al. 1977). All bacteria in a minimum of twenty fields (Cassel 1965) including those associated with detritus and diatom frustules were counted. All fields, including those from seawater with no bacteria, to more than one hundred for sea-ice, were considered.

Total diatoms (Td) were determined parallely in the AODC samples, using 16/0.40; 160/0.17 objectives. Detritus content (DTR) was expressed as per cent of filter area covered during AODC determinations, taking into account the volume of sample filtered.

Amino acids

Amino acids were determined in 1 ml samples using o-phthaldehyde (OPA) in a fluorometric method after Dawson and Liebezeit (1980), with a 'SpectroGlo' filter fluorimeter, (Gilson Medical, USA), fitted with λ_{exc} 340 nm, and λ_{em} 455 nm filters.

Dissolved free amino acid (DFAA) concentrations were determined directly in non-filtered samples. Concentrations of dissolved combined amino acids (DCAA) were determined in 0.2 μm 'Millex GV' filtered samples, with particulate combined amino acid concentrations (PCAA) being determined in non-filtered samples, both after complete acid hydrolysis (1 ml sample combined with 1 ml 8M HCl for 24 h at 100°C, and neutralised with an equal volume of 8M NaOH, to pH 6–8) of the combined amino acids into free amino acids, FAA (Bolter and Dawson 1982; Zdanowski 1985); these were determined with OPA and 2-Mercaptoethanol in boric buffer against glycine standards. DCAA was considered to be the difference between total amino acid and DFAA content in filtered samples before hydrolysis. All glassware was cleaned by 24 h combustion at 450°C.

Statistical analyses

On the basis of 22 results from API 20NE and 3 morphological tests covering motility, Gram reaction, and cell morphology for 98 isolates, a 'Genstat 5' cluster analysis computed similarity coefficients using the average linkage method. Results are presented as a dendrogram with groups determined at the 70% similarity level. Chi-square tests compared the frequency of positive responses for isolates from each habitat in order to detect physiological differences between populations. Within seawater and ice, all other determinations were compared using a Spearman rank correlation matrix. Included into the latter were organic Carbon data provided by R. Prego (pers. comm.).

Results

Distribution of microbial and amino acid parameters

In contrast to the low mean values of CFU, AODC, diatom numbers, detritus content and amino acid concentrations in the water column (Table 1a), corresponding values in sea-ice were from 2 times (amino acids) to eighty times (AODC) greater (Table 1b).

Within sea-ice 54% of parameters correlated positively, compared to 20% positive, and 1.8% negative correlations in seawater. Moreover, parameters in the sea-ice samples tended to show more highly significant correlations, with 40% at >0.025 significant level (Table 2a, 2b).

The average salinity (%) of each melted ice sample was 2.07 ± 0.88 (coloured ice, 2.2; uncoloured ice 1.6). Only DCAA showed significant correlation with salinity, at the 98% confidence level.

Values for all parameters were higher in coloured than uncoloured sea-ice, generally by two to six times. The exception was the number of CFU on GSW upon which 1.6 times fewer CFU were enumerated from coloured ice. An exceptional sample was very brown, very hard ice from station 75 (sample 75-1) within which values of all parameters except DCAA greatly exceeded those in other ice samples (Table 1b). Further, its AODC exceeded that in the average seawater sample by more than 7300 times. CFU on NASW was 86 times higher, on GSW 42.8 times, 3.5 on PSA, and 1.7 times higher on TCBS. In addition, the detritus level was over 100 times greater, and diatom numbers were exceeded by more than 150 times. DCAA and PCAA concentrations were respectively of the order of 60 and 400 times higher in this sample than in seawater. All observed microscopic fields were covered by detritus. There was a diverse diatom population within which well stained intracellular structures were visible, and attached bacteria were much in evidence. Dividing bacteria were also present. In all ice samples, bacteria were often associated with intact and damaged diatom frustules.

On the basis of the distribution of high and low values for these parameters, four distinct regions described as A, B, C, and D were defined between Elephant Island and the South Orkneys, (Fig. 2).

Region A; seawater was characterised by low numbers of viable bacteria, diatoms, detritus, and a low DFAA concentration. DCAA and PCAA were undetectable.

Region B; most parameters in sea-ice and water had higher values. Of particular note, however, were the highest mean numbers of total bacteria (AODC).

Table 1a, b. Average number of Total bacteria (AODC), Colony Forming Units (CFU) on different media, total number of diatoms (Td), detritus content (DTR%) and amino acid concentrations in samples characterising four sub-regions between Elephant Island and Coronation Island, (December 1988 to January 1989). (**a**) 10–50 m integrated seawater sample (**b**) sea-ice

a seawater

	Average in seawater	in sub-region A	B	C	D	Average in WBI	IW	OW
AODC, $\times 10^7 l^{-1}$	0.60	0.30	0.90	0.40	0.06	0.60	0.50	0.98
NASW, $\times 10^3 l^{-1}$	10.60	2.70	8.7	21.90	11.70	14.80	10.90	8.90
GSW, $\times 10^3 l^{-1}$	14.20	2.30[a]	6.40	23.70	15.30	42.20	15.70	10.10
PSA, $\times 10^3 l^{-1}$	8.90	nd	7.90	9.80	10.50	18.20	9.50	7.00
TCBS, $\times 10^3 l^{-1}$	24.90	nd	12.90	84.10	13.40	24.40	30.10	9.20
Td, $\times 10^7 l^{-1}$	0.18	0.06	0.15	0.44	0.06	0.39	0.21	0.16
DTR, %	0.62	0.06	0.3	2.17	0.21	1.20	0.90	0.21
DFAA, $\mu M l^{-1}$	0.18	0.15	0.13	0.24	0.35	1.25	0.16	0.16
DCAA, $\mu M l^{-1}$	0.80	0	1.03	0.99	0.98	2.47	0.55	1.37
PCAA, $\mu M l^{-1}$	0.09	0	0.06	0	0.44	0.00	0.03	0.11

[a] $n = 1$
nd = no data
See text for abbreviations

Table 1 (continued)
b Sea-ice

	Average in ice*	sub-region A	B	C	D	Sample IB[a]	IU	75-1
AODC, $\times 10^7 l^{-1}$	47.90	29.20	54.80	63.00	nd	92.10	24.10	4400.0
NASW, $\times 10^3 l^{-1}$	54.60	50.60	59.30	68.30	nd	83.6	50.00	825.8
GSW, $\times 10^3 l^{-1}$	56.30	2.00	80.80	54.20	nd	42.80	56.30	2190.0
PSA, $\times 10^3 l^{-1}$	77.60	nd	115.50	31.20	nd	123.00	77.60	427.3
TCBS, $\times 10^3 l^{-1}$	68.10	nd	93.70	36.10	nd	114.20	31.20	194.5
Td, $\times 10^7 l^{-1}$	3.50	1.20	1.20	4.20	nd	2.97	0.53	20.52
DTR, %	17.90	12.60	11.20	34.20	nd	31.00	4.90	100.00
DFAA, $\mu M l^{-1}$	2.15	nd	2.10	2.27	nd	4.30	1.06	11.35
DCAA, $\mu M l^{-1}$	1.52	nd	0.70	2.73	nd	2.85	1.15	0.21
PCAA, $\mu M l^{-1}$	3.80	nd	2.70	5.60	nd	8.78	1.51	39.20

nd = no data
[a] sample 75-1 not included

Table 2a, b. Spearman rank correlation matrix of significance levels comparing colony forming bacteria (CFU) on NASW, GSW, PSA, TCBS, Total bacterial count (AODC), total diatom number (Td), detritus content (DTR), dissolved free amino acids (DFAA), dissolved combined amino acids (DCAA), particulate combined amino acids (PCAA), dissolved organic Carbon (DOC), and particulate organic Carbon (POC)[+] in samples collected during sea-ice zone cruise. (**a**) 10–50 m integrated seawater sample (**b**) sea-ice
a seawater

	AODC	GSW	PSA	TCBS	Td	DTR	DFAA	DCAA	PCAA	DOC
NASW	0.1[a]	*0.048*	>0.1	>0.1	*0.025*	*0.025*	*0.005*	>0.1	*0.025*	>0.1[a]
AODC		*0.040*	0.06[a]	>0.1	>0.1	>0.1[a]	*0.005*[a]	>0.1	>0.1	>0.1
GSW			*0.024*	>0.1	>0.1	>0.1	*0.005*	>0.1	>0.1	>0.1
PSA				>0.1	>0.1	>0.1	*0.04*	>0.05	>0.1	>0.1
TCBS					>0.1	>0.1	>0.1	>0.1	>0.1	>0.1
Td						*0.025*	0.1	>0.1	>0.1[a]	>0.1[a]
DTR							*0.05*	>0.1	>0.1[a]	>0.1[a]
DFAA								>0.1	>0.1	>0.1
DCAA									>0.1	>0.1[a]
PCAA										>0.1

[a] negative correlation

Table 2 (continued)
b Sea-ice

	AODC	GSW	PSA	TCBS	Td	DTR	DFAA	DCAA	PCAA	POC
CFU	*0.001*	*0.004*	>0.1	*0.004*	*0.001*	*0.005*	*0.025*	*0.05*	*0.025*	*0.011*
AODC		*0.033*	*0.033*	*0.029*	*0.001*	*0.001*	*0.001*	>0.1	*0.001*	*0.004*
GSW			*0.023*	>0.1	*0.035*	0.067	>0.1	>0.1	>0.1	>0.1
PSA				*0.05*	>0.1	0.072	>0.1	>0.1	>0.1	>0.1
TCBS					0.097	0.077	0.053	>0.1	>0.1	0.027
Td						*0.001*	*0.005*	>0.05	*0.005*	*0.006*
DTR							*0.005*	>0.1	*0.001*	*0.0017*
DFAA								>0.1	*0.001*	*0.003*
DCAA									>0.1	>0.1
PCAA										*0.006*

Region C; a small area encompassing three stations in the vicinity of the ice edge was considered as a third region. Here most parameters showed their highest values.

Region D; a lens of warm meltwater with lower salinity in the east of the sample area (Tokarczyk et al. 1991). Lowest AODC values compared to other regions occurred in tandem with high numbers of viable bacteria. Moreover, highest PCAA and DFAA concentrations also differentiated this region, the latter showing a strong increasing gradient from the west.

Water below ice tested at three stations along the edge of the pack, showed higher values for most parameters than distant seawater.

Numbers of viable bacteria isolated from the same sample showed considerable variation on different media. Counts on TCBS and PSA, and to a lesser extent GSW,

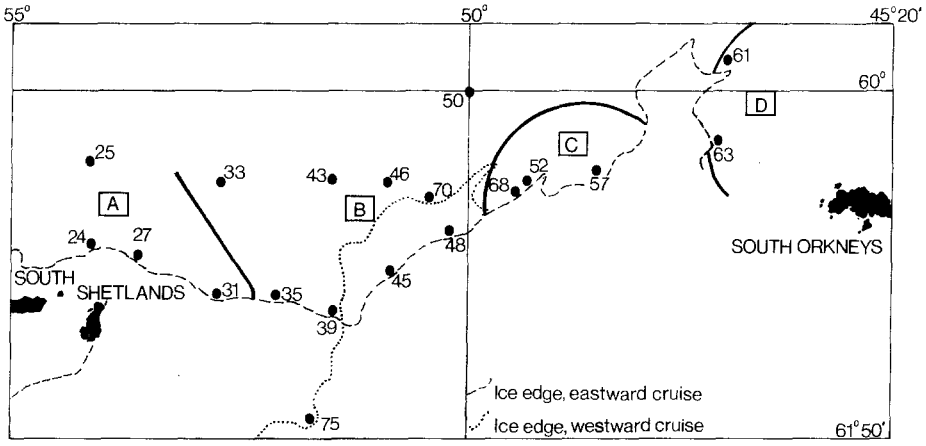

Fig. 2. Division of the sampling area into four subregions on the basis of microbial and amino acid data

occasionally exceeded by 2 to three times those on NASW. No particular medium or incubation temperature (0° or 10°C) consistently yielded more CFU. Colonies on GSW were frequently small and poorly developed.

Ecophysiological grouping

Chi-square results indicate significant differences between populations for four API 20NE responses. The responses of all isolates are shown in Fig. 3a. Gelatin hydrolysis, varying significantly at the 0.01% level, was prominent in isolates from water below ice (Fig. 3b) and from krill (Fig. 3c), although the small number of isolates from each of these should be stressed. The distribution of gluconate assimilating bacteria also varied at this level, with no such bacteria isolated from open water (Fig. 3d). Mannitol and adipate assimilation varied at 0.05% and 0.1% levels respectively. Malate assimilation, significant at the 0.5% level, was demonstrated by over half the isolates from ice (Fig. 3e), compared to 25% below ice, 23% close to ice (Fig. 3f), and 15% from open water.

Several isolates reduced NO_3 to NO_2, but further reduction to nitrogen was not shown; indole production was also absent. Further, no isolates assimilated caprate, an observation also reported by Richard (pers. comm.) during work with 16 marine isolates. Richard actually detected a low incidence of arginine hydrolysis but all isolates in this work proved negative.

In API 20NE, β-Glucosidase production was absent in isolates from water below ice, but was shown by ca. 45% of all others. The lower incidence of this enzyme demonstrated in API Zym may be a result of the fewer tests that were run. Alternatively, different cell concentrations used in each test or substrate specificity may be important.

In API Zym, differences between isolates from ice and water in terms of proteolytic enzyme specificity were evident (Figure 4). Trypsin and chymotrypsin were prominent in isolates from water. Leucine and Valine aminopeptidases, and Lipase activity were more frequent in ice isolates. α-Galactosidase and α-mannosidase activity, however, were restricted to isolates from ice.

Control strips of API 20NE and API Zym inoculated and treated as described above, showed no 'false positive' responses.

Motile Gram negative rods dominated each population. One Gram positive isolate was noted.

The 'Genstat 5' statistical analysis produced nine clusters at the 70% similarity level ranging in size from two to 47 isolates (Fig. 5). A poor range of positive responses in the API 20NE system, particularly an inability to assimilate sugar derivatives as sole carbon sources, characterised isolates in the largest cluster, II. This cluster, containing 60% of the isolates from water far from ice (Table 3), is considered to be composed of auxotrophic bacteria. Isolates in cluster III were also auxotrophic with respect to their responses in API 20NE, growing on complex nutrient media but not utilising monosaccharides as sole Carbon sources.

Prototrophic isolates which utilised sugars such as glucose, maltose, and malate as sole Carbon sources occurred in cluster IV. Of six prototrophic isolates additionally capable of glucose fermentation, in cluster V, five were from water below ice and the sixth from krill. The majority of krill isolates occurred in clusters II and IV, and those from water below ice only in clusters II and V.

Isolates in clusters VI, VII, VIII and IX showed the widest range of positive responses in API 20NE. These were predominantly from ice and water close to ice. Of seven unclustered isolates six were from ice and one from krill.

Five distinct clusters characterised the isolates from ice: four were provisionally identified as containing *Acinetobacter* (Buchanan and Gibbons 1974; Bio Merieux 1990), and the fifth as *Sphingobacterium*. Three genus' isolated in parallel from ice and water close to ice were assigned to *Aeromonas*, *Flavimonas*, and *Agrobacterium*; a fourth was unidentified. Those in cluster V from water below ice were considered to be *Vibrio* spp.

Discussion

The major objective of this study was to relate various bacteriological parameters, nutritional requirements,

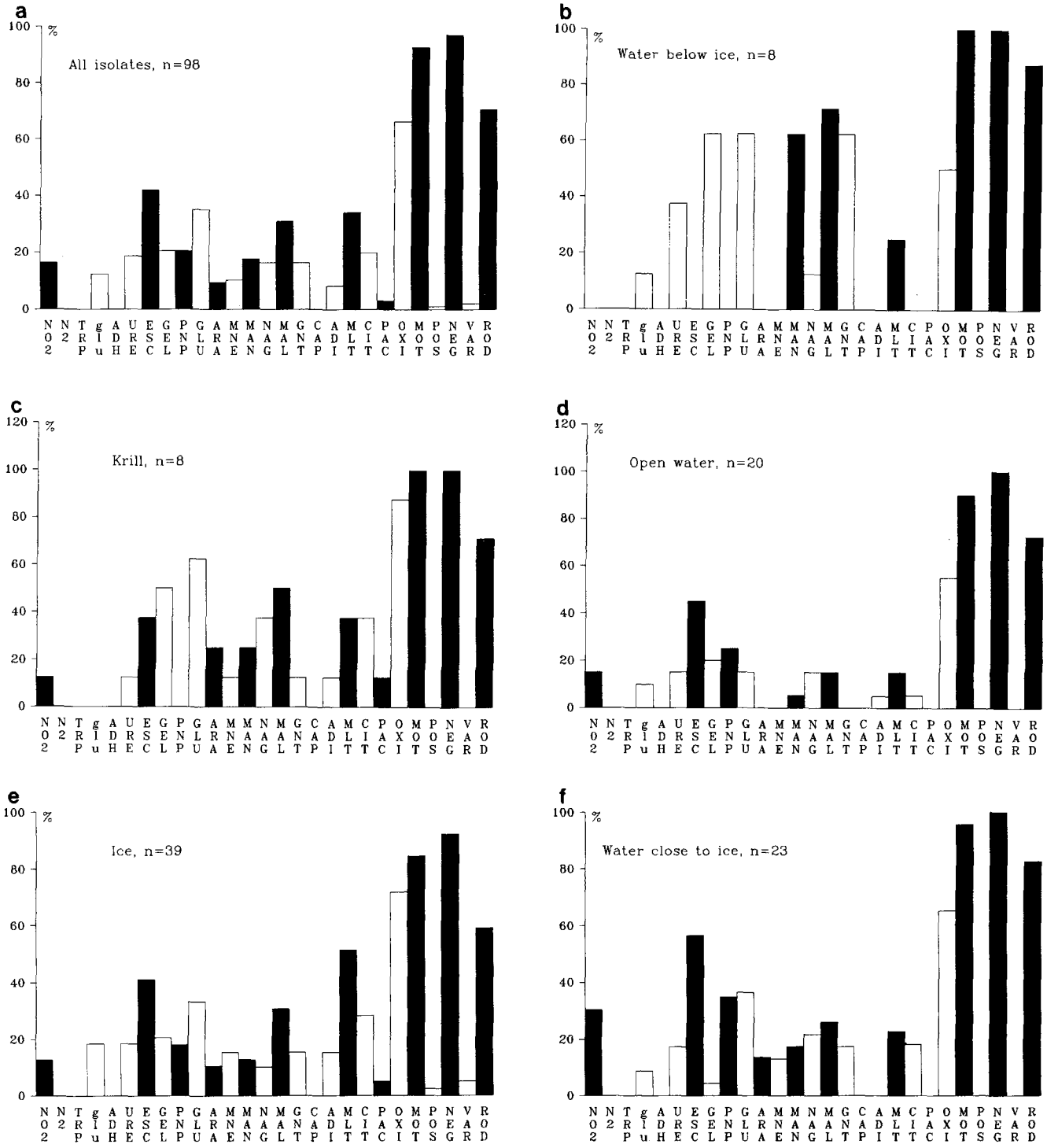

Fig. 3. Percentage of positive responses in API 20NE, and classical test results, for five bacterial populations. The following reactions or enzymes were investigated; reduction of nitrates to nitrites (NO_2); reduction of nitrites to Nitrogen (N_2); indole production (TRP); fermentation of glucose (glu); presence of arginine dihydrolase (ADH); urease (URE); β-glucosidase (ESC); gelatin hydrolysis (GEL); β-galactosidase (PNP); assimilation of glucose (GLU); arabinose (ARA); mannose (MNE); mannitol (MAN); N-acetyl glucosamine (NAG); malate (MLT); gluconate (GNT); caprate (CAP); adipate (ADI); maltose (MAL); citrate (CIT); phenyl-acetate (PAC); cytochrome oxidase (OXI); motility (MOT); Gram positive (POS); Gram negative (NEG); Gram variable (VAR); Morphology (ROD)

numbers, and amino acid data in sea-ice and seawater, with hydrological conditions in the vicinity of, and at varying distances from an ice edge.

A definition of water masses requires consideration of their biological history and oceanograhic parameters (Bolter 1987). In this respect, surface water in the investigated area was divided into two distinct parts: The western and to some extent the central region, with rather low indicator levels of biological activity, particularly chlorophyll *a* (Figueiras and Perez 1990; Tokarczyk et al. 1991), CFU

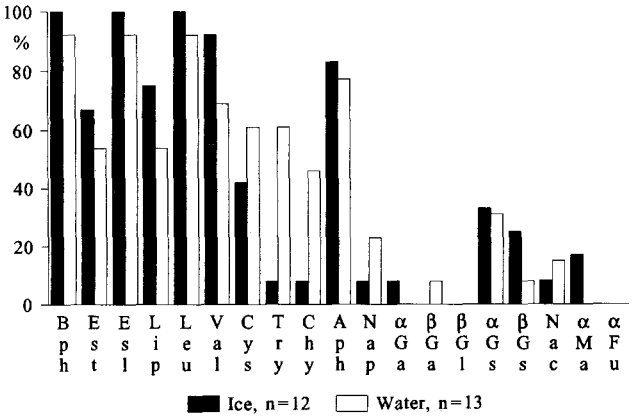

Fig. 4. Percentage positive API Zym responses of isolates from sea-ice, and from water. The following enzymes were assayed for; Alkaline phosphatase (Bph); Esterase C4 (Est); Esterase Lipase C8 (Es1); Lipase C14 (Lip); Leucine arylamidase (Leu); Valine arylamidase (Val); Cysteine arylamidase (Cys); Trypsin (Try); Chymotrypsin (Chy); Acid phosphatase (Aph); Napthol-AS-BI-phosphohydrolase (Nap); α-Galactosidase (αGa); β-Galactosidase (βGa); β-Glucouronidase (βG1); α-Glucosidase (αGs); β-Glucosidase (βGs); N-acetyl-β-glucosaminidase (Nac); α-Mannosidase (αMa); α-Fucosidase (αFu)

and amino acids. Meltwater associated with an ice edge close to the South Orkneys with lower salinity, higher temperature and levels of viable bacteria, diatoms, detritus, chlorophyll *a*, and DFAA than water to the west. Bacterial growth in meltwater may have been encouraged by elevated temperatures, but local factors such as reduced vertical mixing afforded by the water columns stability are also important (Smith and Nelson 1985). In addition, viable bacteria and nutrients are released from melting ice, and it is likely that higher amounts of detritus and diatoms in the water column to the east can be partially accounted for in this way; high numbers of algal aggregates in the vicinity of melting ice were reported by Riebesell et al. (1989). Bacteria released in tandem respond by increasing their metabolic rates.

Sea-ice constitutes a quite unique habitat, with bacterial communities efficiently utilising dissolved organic carbon in spatially well defined compartments. This was demonstrated in brown ice at station 75 where bacterial numbers were extremely high, with POC (13.5 mg $C l^{-1}$) exceeding DOC by 6.6 times (Prego, unpub.), and total bacterial organic Carbon being equivalent to ca. 15% and 96% of POC and DOC respectively. This highlights the

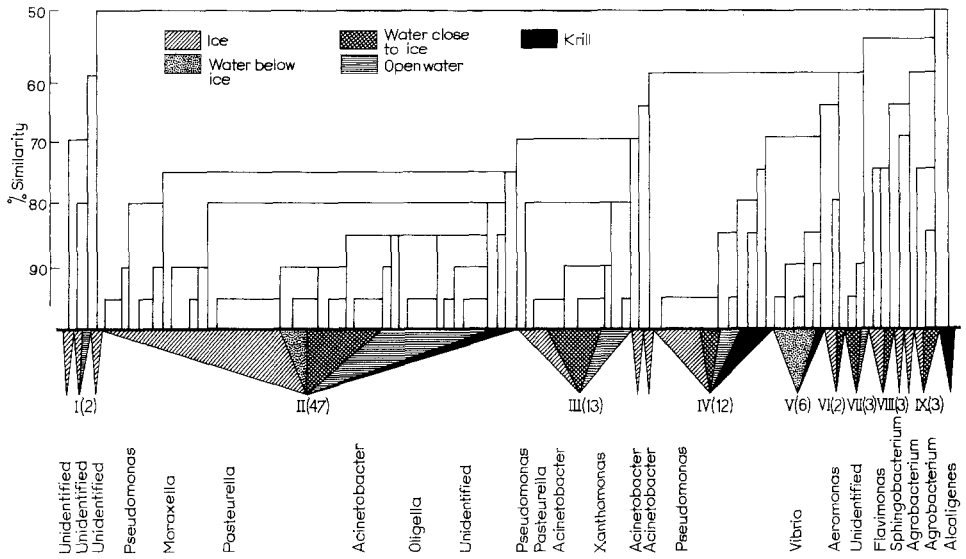

Fig. 5. Numerical taxonomic classification of 98 isolates from ice, water below ice, water close to ice, open water, and krill stomach, using a standard agglomerative approach. Numbers I, II, III, IV, V, VI, VII, VIII, IX correspond to clusters determined at the 70% level. (Number of isolates per cluster is noted in brackets. Shaded areas denote percentage of each cluster represented by each habitat)

Table 3. Habitat representation in each cluster

Cluster	% of isolates from Ice $n=39$[a]	% of isolates from WBI $n=8$	% of isolates from IW $n=23$	% of isolates from OW $n=20$	% of isolates from krill $n=8$[b]
I	2.56	0	0	5.00	0
II	48.71	37.50	43.47	60.00	37.50
III	10.25	0	21.74	20.00	0
IV	12.82	0	8.70	10.00	37.50
V	0	62.50	0	0	12.50
VI	2.56	0	4.34	0	0
VII	0	0	8.70	5.00	0
VIII	5.13	0	4.34	0	0
IX	2.56	0	8.70	0	0

[a] six isolates did not cluster
[b] one isolate did not cluster

accumulation of POC in sea-ice as opposed to DOC, which contrasts sharply with estimates by Zdanowski (1985) for free living bacteria in the water column: In seawater DOC exceeded POC values which were average for Antarctica, by thirteen times, with total bacterial Carbon being equivalent to only 0.9% of POC. Both these authors determined organic Carbon from the oxidation of organic matter to CO_2 (Prego and Fraga 1988).

Environmental conditions which are often considered to be unfavourable for many ecto- (Chróst 1990) and extracellular enzymes (Priest 1984), those involved in processes which decompose high molecular weight organic matter in aquatic habitats, do not necessarily prevail in sea-ice. The structure of sea-ice itself reduces the risk that ectoenzymes will be parted from the maternal cell for example, by providing conditions which are more stable than much of the water column. Within sea-ice refugia (Sullivan et al. 1985) bacterial cells may be exposed to high nutrient levels, promoting ectoenzyme activities and the uptake of small organic molecules (Payne 1980), and in turn the development of bacteria utilising these enzymes. It is unclear how the ambient temperature affects enzyme activity, but Antarctic bacteria respond quickly to small temperature changes in terms of respiratory activity (Vosjan and Olanczuk-Neyman 1991). We can presume that they respond in a similar way with respect to other enzyme activities, and this would be particularly important in organic matter decomposition (Zdanowski 1988b).

In this work API Zym allows us to comment only on the capacity of an isolate to produce certain enzymes, not on in situ nutrient conditions in ice. Specific enzymes such as leucine (*Leu*) and valine (*Val*) aminopeptidases although widespread, were prevalent in isolates from ice. It is unclear why less specific proteases such as trypsin and chymotrypsin were not so common in sea-ice isolates as those from water. High nutrient levels in sea-ice comprise a wide range of organic material which requires specific enzymes to effect its full utilisation. In this respect enzymes such as *Leu* (Hałemejko and Chróst 1986), β-glucosidase (βGS), (Chróst 1991) a carbohydrase prevalent in sea-ice isolates, and lipases are actively produced. The demonstration of α-Galactosidase and α-mannosidase activity solely by ice isolates is evidence of proliferation of certain strains, galactose and mannose being prominent manosaccharides in marine particulate matter (Handa and Tominaga 1969).

Physiological differences between populations shown by the API 20NE system reflect differing spectra of available Carbon sources in each habitat. Microbial populations utilising organic acids such as malate, alcohols, amino acids, and sugars released by phytoplankton (Griffiths et al. 1982), change in tandem with these exudates (Newell et al. 1981). An indication of such phenomena in this work are the low numbers of gluconate and malate assimilating bacteria from open water compared to the ice and ice edge area.

Each habitat was strongly represented in the largest cluster by bacteria which showed a poor range of positive responses in the API 20NE test. Interestingly *Vibrio* species isolated from water below ice were only found in one cluster, together with one isolate from krill. These demonstrated broader spectra of positive responses. In general isolates which were best equipped to utilize the investigated range of sole Carbon sources, were those identified provisionally as *Aeromonas*, *Flavimonas*, *Sphingobacterium*, and *Agrobacterium*, all of which were found in ice or in water close to ice.

During EPOS 1 bacterial communities and numbers below ice differed to those in the water column at some stations (Larsson et al. 1989). Although we did not enter the pack, bacterial numbers and populations showed clear changes as we moved seaward from the ice edge, particularly as most open water isolates were incapable of assimilating a number of sugars as sole carbon sources.

Gram negative bacteria which are recognised as constituting the majority of isolates from the marine environment (Sieburth, 1979) were dominant in this study. Their additional prevalence in sea-ice confirms findings of Zdanowski (1988a).

The patchy distribution of ice communities in this work was also noted during EPOS I (Delille et al. 1989). We expected such patchiness in microbial numbers and amino acid concentrations to reflect the degree of colouration in ice samples. Coloured samples generally showed highest values, although higher than expected values were occasionally found in uncoloured ice. The colour does reflect the concentration of plant pigments (Hoshiai 1985), but the state of the specific variables discussed in this paper cannot be estimated in the same way.

Salinities and AODC values in melted sea-ice in this work were similar to those reported by Kottmeier and Sullivan (1990) during AMERIEZ I (Spring), and AMERIEZ II (Autumn) for the Weddell-Scotia Sea. Our ice sample in which AODC was of the order of 4.4×10^{10} per litre appears quite exceptional. Average AODC in seawater (Table 1) in this work, however, was over ten times lower than previously reported for the Bransfield Strait to north Weddell Sea area, (Zdanowski 1982; Hanson et al. 1983a, 1983b; Zdanowski 1985; Mullins and Priddle 1987; Kottmeier and Sullivan 1990; Kim 1991). A comparison with AODC results from three Polish expeditions, FIBEX (summer/autumn, 1981) with AODC of $10.5 \times 10^7 \pm 7.4$ per litre ($n=97$); SIBEX (summer, 1984), AODC $2.1 \times 10^7 \pm 1.5$ per litre ($n=73$); BIOMASS III (spring, 1986), $0.68 \times 10^7 \pm 1.1$ per litre ($n=43$) (Zdanowski, in prep.), each slightly west of the present area east of the Scotia Front, suggests a seasonal influence. Conversely, 3.5 times lower AODC enumerated in this study compared to SIBEX, conducted at the same time of the year but not in the vicinity of ice, suggests that larger spatial and temporal scales may exist.

In contrast to the accepted methods for AODC determination, viable bacteria are enumerated in various ways. With differences in methodology in mind, viable bacteria in seawater were up to two times more numerous in this study than previously reported for this area (Zdanowski 1982, 1985; Delille et al. 1989; Kim 1991). The fact that CFU values for the same sample varied on different media highlights the need for standard procedures in this respect.

CFU constituted 0.18% of AODC in seawater in this work, a percentage which has increased in four consecutive expeditions, from FIBEX and SIBEX (Zdanowski in

prep.), BIOMASS III (Zdanowski 1988a), to this work. CFU and AODC data from this and EPOS leg 1 cruises divided the South Shetland and South Orkney shelves into at least two areas, (Delille and Zdanowski unpub.). Although differences in the ice situations during each cruise were considerable, there was little change in the number of viable bacteria. A decrease in the total biomass in this study, however, may indicate an increase in grazing pressure.

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