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Bacteria in sea ice and underlying water of the eastern Weddell Sea in midwinter

Elisabeth Helmke, Horst Weyland

Alfred-Wegener-Institute for Polar and Marine Research, PO Box 120161, D-27515 Bremerhaven, Germany

ABSTRACT: Bacteria in the water beneath the sea ice of the eastern Weddell Sea were homogeneously distributed. Direct counts resembled values from spring and autumn, whereas viable cell counts, total ATP concentrations, as well as heterotrophic assimilation and extracellular enzymatic activities were very low, implying a metabolic inactive bacterioplankton. The consolidated sea ice had a very heterogeneous horizontal distribution of microbes on large as well as small scales but vertical profiles in low and densely populated ice cores exhibited similar patterns. A close relation between bacterial colonization of sea ice and genetic ice classes was revealed. Sea ice of the 'predominantly congelation ice' had the lowest bacterial biomass and displayed very low heterotrophic activities which were comparable to those of the water column. Samples of older sea ice belonging to the 'mainly frazil' and 'mixed ice' had maximal numbers of bacteria. They often included high proportions of culturable cells and dividing cells as well as large bacteria. The bacteria of these ice classes were active and contributed significantly to the productivity in the Weddell Sea during winter. 'Predominantly frazil ice' was less colonized; however, selective bacterial growth was also indicated in this typical winter ice by an increase in the proportions of culturable and psychrophilic bacteria with advancing age of the ice. Psychrophilic bacteria dominated in consolidated sea-ice whereas facultative psychrophiles prevailed in young sea-ice and water, corroborating a strict partitioning in a microbial sea-ice and a seawater regime. Generally, temperature does not appear to be the significant factor for the development of bacterial communities in the surface layer of the eastern Weddell Sea in winter since the metabolically active bacterial flora develops in the very cold sea-ice environment. The organic matter supply and its improved usability obviously controls bacterial activity as well as the selective enrichment of psychrophiles.

KEY WORDS: Bacteria · Sea ice · Winter · Antarctica · Weddell Sea

INTRODUCTION

The Southern Ocean is characterized by a very large seasonal fluctuation of ice cover ranging from about 4 to 20×10^6 km^2 (Zwally et al. 1983). Despite the transient existence of most of the Antarctic sea ice, it nevertheless provides a vast habitat for a variety of organisms, mainly diatoms, bacteria, flagellates, and protozoa, which reside within the internal brine pocket system (for reviews see Horner 1985, Garrison et al. 1986, Horner et al. 1992, Palmisano & Garrison 1993). Studies during the last decade have established that algae of sea-ice communities contribute significantly to the primary productivity of the polar seas (literature compiled by Palmisano & Garrison 1993), despite the multitude of environmental stress factors such as low temperature and irradiance coupled with high salinities. Bacterial production within the sea ice was also demonstrated (Grossi et al. 1984, Sullivan et al. 1985, Kottmeier & Sullivan 1987, 1990, Kottmeier et al. 1987, Rivkin et al. 1989, Grossmann & Dieckmann 1994) and there is evidence of a functioning microbial loop (Kottmeier et al 1987, Garrison & Buck 1989, Kottmeier & Sullivan 1990). Most of the extensive studies of the bacterial component of Antarctic sea-ice communities have taken place during spring, autumn, and summer. They focussed on bacterial abundance, biomass, and growth activities but some studies also dealt with the diversity, taxonomical and physiological characterization, as well as nutritional requirement of sea-ice bacteria (Delille 1992, Zdanowski & Donachie 1993). However, to evaluate the role of bacteria in the sea ice of the Antarctic Ocean and to understand their seasonal importance, more information is needed particularly on winter sea ice which comprises the major

part of pack ice. Due to the difficulties in accessing winter ice, studies of the bacterial sea-ice flora during this season are scarce. A limited data set is available from the fast ice including associated water of the McMurdo Sound (Kottmeier et al. 1985, Rivkin et al. 1989) as well as near Syowa station (Satoh et al. 1989) and from the marginal pack-ice zone of the Weddell Sea (Marra et al. 1982, Garrison & Close 1993). The most extensive bacteriological winter study was conducted in the diffusive pack ice west off the Antarctic Peninsula (Kottmeier & Sullivan 1987). However, the sea-ice situation there is quite different from that of the central, open-ocean pack-ice region of the eastern Weddell Sea.

Within the framework of the 'Winter Weddell Sea Project 1986' (WWSP 86), an international, multidisciplinary program, the cruise ANT V/2 of the icebreaking RV 'Polarstern' provided an opportunity to study the microbial community across the entire width of the annual sea-ice cover of the Weddell Sea in midwinter. We focussed on the bacterial components of the sea-ice and underlying water biota and investigated their distribution and biomass as well as metabolic state and activity, in order to contribute to the understanding of the seasonal dynamics and role of microheterotrophs in annual sea-ice communities.

MATERIALS AND METHODS

Ice situation and sampling. During the cruise ANT V/2 of the RV 'Polarstern' the sea ice in the open Weddell Sea was traversed (Fig. 1) in the vicinity of the 0° meridian from the ice edge (~59° S) southward close to the continental shelf ice (~69° S), and to the ice edge at ~56° S on the return journey, in the period July 18 to September 10, 1986. Detailed information about ice situation, ice structure, and ice history is given by Casarini & Massom (1987), Wadhams et al. (1987), and Lange et al. (1989, 1990). Lange et al. (1989) defined 5 genetic ice classes which are indicative of the history of sea-ice formation and evolution. The classification of the ice cores into genetic ice classes related to the proportions of the 3 textural ice classes: granular ice, columnar ice, and mixed granular/columnar ice. Ice cores representing the predominantly and mainly frazil ice contained more than 80 or 60% granular ice, respectively; predominantly and mainly congelation ice cores contained more than 80 or 60% columnar ice, respectively; mixed frazil/congelation ice cores contained more than 40% of both granular and columnar ice. The ice classes of the ice cores studied are given in Fig. 1. Raabe (1987) described the weather and synoptic situation during ANT V/2. Temperatures within the sea ice ranged from –15°C at the top to about –2.5°C at the bottom of the ice cover (Bartsch 1989). Brine salinities (178‰ top to 35‰ bottom) are a function of temperature and can be calculated from brine temperature by the equations of Assur (1958). Bulk salinities are indicative of vertical brine movement and therefore of the age of the floe. During ANT V/2, mean values of 10.6‰ in the upper ice layers and of 5.4‰ in the bottom layers were determined (Bartsch 1989).

Ice cores from consolidated ice, chunks of brown coloured ice, samples of grease ice, young pancake ice, and underlying water were taken along the route (Fig. 1). Chunks of ice as well as grease ice and young pancake ice were collected by means of a wire net. Ice cores were drilled with a modified SIPRE ice auger (7.5 cm diameter). The ice cores used for bacteriological examination were taken close to those cores sampled for the assessment of ice structure, physical, chemical, and biological properties (Bartsch 1989, Lange et al. 1989, 1990, Spindler et al. 1990, Dieckmann et al. 1991a, b). Due to the small-scale patchiness

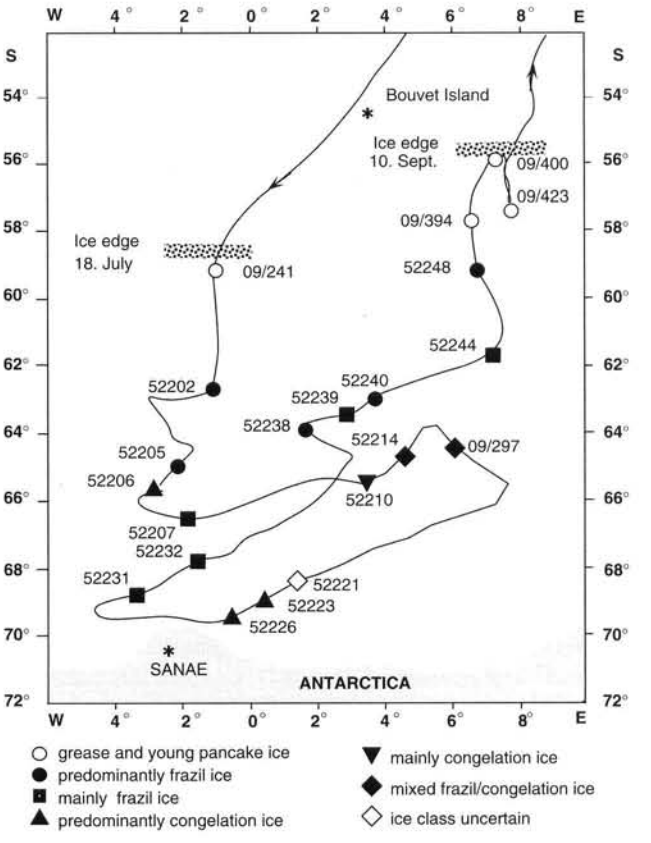

Fig. 1. Location of bacteriological sampling stations in the eastern Weddell Sea during the Winter Weddell Sea Project 1986 (WWSP 86) cruise ANT V/2 of RV 'Polarstern'. Sea-ice core stations are numbered with the first digits 52. Grease and small pancake ice as well as chunks of brown ice were sampled at the locations numbered with the first digits 09. The genetic ice classes of the cores are indicated by different symbols

of the sea ice, parallel cores may differ considerably. Nevertheless, to simplify the comparison between the different data sets, the bacteriological ice cores were numbered just as the parallel cores. Careful attention was paid to maintain sterile conditions during sampling and subsequent processing. The bacteriological cores were cut into 10 cm sections which were often subdivided into 2 cm sections. Unless stated otherwise the ice sections as well as the other ice samples were mechanically crushed and melted in measured volumes of filtered (0.2 µm pore size) autoclaved seawater (ratio seawater to sea ice 1:1, final salinities 20 to 23‰). The ice had melted within 1 h.

Seawater underlying the pack ice was pumped manually with a plastic bilge pump which had been rinsed with 95% ethanol and flushed several times with seawater from the sampling site. Ice-core drilling holes served as sampling sites. Surface seawater from the marginal ice zone was collected with sterile glass bottles (500 ml). Seawater from greater depth was taken with 10 l Niskin bottles on a rosette sampler.

Total bacterial counts and bacterial biomass. Sea-ice and water samples were fixed with formalin (3% final concentration) and stored at 2°C prior to enumeration. Bacteria were enumerated by epifluorescence microscopy according to the acridine orange direct count method (AODC) of Hobbie et al. (1977). The microscopic counting and sizing was carried out in the home laboratory 3 mo after sampling; due to vibration and movement of the ship, this work was not conducted aboard directly after sampling.

Bacterial cell concentrations were determined at least from each 10 cm section of an ice core. Biovolumes were estimated from 3 to 6 selected sections per core. Bacterial cell volumes were grouped into size classes which correspond to average nominal volumes of: 0.065 μm^3 (small cocci, diameter 0.5 µm); 0.39 μm^3 (short rods, diameter 0.7 µm, length 1.0 µm); 0.58 μm^3 (medium rods, diameter 0.6 µm, length 2.0 µm); 1.33 μm^3 (long rods, diameter 0.65 µm, length 4.0 µm). The volume of rods longer than 4 µm were individually calculated. To convert cell volumes to carbon, a factor of 220 fg C μm^{-3} (Bratbak 1985) was used. The bacterial organic carbon was multiplied with the conversion factor of 0.004 (Karl 1980) to obtain values of bacterial ATP.

ATP. For measurements of particulate ATP concentrations, volumes of 50 to 100 ml of the various sea-ice (immediately after melting) and water samples were filtered through Nuclepore filters (0.2 µm pore size). The particulate material of some densely populated ice-core sections were size fractionated by passing 100 ml of melted ice through 12.0 µm, 3 µm, and 0.2 µm pore size Nuclepore filters in succession. The filters were extracted in TRIS-buffer after Holm-Hansen (1973). Extracts were stored frozen at –27°C prior to analysis. ATP was assayed with the ATP-luciferase test combination HS (Boehringer) and the ATP-biophotometer SAI model 3000.

Viable counts. The number of culturable heterotrophic bacteria was estimated by the most probable number (MPN) technique as well as by the spread plate and membrane filtration method. For MPN calculations, 3 replicates of successive 10-fold dilutions were prepared using ZoBell medium 2216 E with 100% seawater content (SW).

The number of colony forming units (CFU) was determined by spreading 0.1 ml of the original sample or the decimal dilutions onto the surface of Chitin agar (Weyland et al. 1970). Furthermore 1 ml, 10 ml, or 100 ml sample volumes, respectively, were filtered onto membrane filters (0.1 µm pore size, mixed esters of cellulose, Schleicher & Schuell) and these were also placed onto Chitin agar. Chitin medium yielded from our experience higher CFUs than the commonly used ZoBell agar. In order to obtain indications about the salinity tolerance of sea-ice and water bacteria, the medium was applied at 3 different salinity concentrations: Chitin agar 100%SW prepared with 100% natural seawater, Chitin agar 0%SW with 100% distilled water, and Chitin agar 300%SW with 100% natural seawater plus 46.0 g NaCl, 1.4 g KCl, and 10.6 g $MgCl_2 \cdot 6H_2O$, 3.0 g $CaCl_2 \cdot 2H_2O$ l^{-1}.

The susceptibility of the bacterial ice flora to the reduction of the osmotic pressure occurring during ice melting was tested by thawing replicate ice samples from 4 different stations as follows: (1) without seawater addition (final salinity 5 to 10‰); (2) with an equivalent volume of sterile, particle-free seawater (final salinity 20 to 23‰). Aliquots of both preparations were spread on Chitin agar 100%SW to assess viable bacterial counts.

Samples and cultures were not exposed to temperatures exceeding 4°C during the different operation steps. After 3 to 4 mo incubation at 1°C, colonies were counted and growth of the MPN replicates evaluated by turbidity. Vials with very low or no turbidity were examined microscopically. MPNs were calculated according to statistical tables (Rodina 1972).

Organisms and temperature tolerance. Pure cultures for taxonomical and ecophysiological studies were obtained from colonies which developed on solid media. The isolated strains were maintained on ZoBell agar 2216 E (75%SW) at 1°C.

Temperature tolerance of these strains was studied in liquid ZoBell medium 2216 E at 0, 5, 10, 15, 18, 24, and 30°C. For growth determination at –5°C and growth rate calculations the ZoBell medium 2216 E was supplemented with 1% glycerol. The ability of organisms to grow at the different temperatures was examined by means of turbidity measurements at 590 nm with a Mul-

tiskan plus MK II (Flow Laboratories). Direct counts were used for growth rate calculations (see Fig. 8). According to Morita (1975) psychrophiles are defined by growth at or below 0°C, optimum <15°C, maximum <20°C. Psychrotrophs (facultative psychrophiles) are characterized by growth at or below 0°C, optimum >15°C, maximum >20°C. Instead of the term psychrotrophs we use the term facultative psychrophiles.

Turnover times and bacterial production. Turnover times of dissolved organic material (DOM) were estimated by ^{3}H-leucine as well as ^{14}C-glucose uptake. Consolidated sea ice is a heterogeneous physical environment composed of several distinct microenvironments with partly separated substrate pools, therefore, the influence of sea-ice preparation on turnover times was tested. The following 4 sea-ice preparations were applied: (1) small ice cubes soaked with labelled substrate; (2) small ice cubes suspended in sterile, filtered seawater (ratio seawater to ice 1:1), plus tracer; (3) sea ice completely melted in seawater (ratio seawater to ice 1:1), plus tracer; and (4) melted ice without seawater addition, plus tracer.

The heterotrophic utilization of dissolved substrates was determined by the tracer approach (Williams & Askew 1968) with final concentrations of 0.4 to 0.5 nmol l^{-1} L-[4,5-^{3}H]leucine (sp. act. 1.74 TBq $mmol^{-1}$, Amersham) and 7.1 to 11.6 nmol l^{-1} D-[U-^{14}C]-glucose (sp. act. 10 GBq $mmol^{-1}$, Amersham). Uptake rates were measured as a function of time to establish linearity. Eight replicates were prepared using Method 2. At the beginning of the experiment, 2 of the replicates (blanks) were melted and poisoned with $HgCl_2$ (final concentration 7.7 µmol l^{-1}). The remaining samples were incubated at –2°C in the dark and reactions were stopped after 6, 12, and 18 h. Cells were collected on cellulose nitrate filters (pore size 0.2 µm), rinsed with particle-free seawater and subsequently assayed for radioactivity by liquid scintillation spectrometry in a Beckman LS-1000C with Filtercount (Canberra-Packard) as scintillation medium. Quenching was determined by the external standard ratio method.

To differentiate between the heterotrophic activity of bacteria and eukaryotes, replicate activity experiments were treated with the antibiotics D-threo-chloramphenicol (final conc. 300 µg ml^{-1}; Serva) and cycloheximide (final conc. 3 µg ml^{-1}; Sigma), respectively, according to Marten & Brandt (1984).

Rates of heterotrophic bacterial production were determined according to Fuhrman & Azam (1982) using [methyl-^{3}H]thymidine (sp. act. 2.81 TBq $mmol^{-1}$, Amersham) at a final concentration of 5 nmol l^{-1}. Bacterial production experiments were performed similar to the assimilation experiments described above but cells were collected on Nuclepore filters (pore size 0.2 µm) and extracted with ice-cold 5% trichloroacetic acid (TCA). Thymidine uptake was converted to bacterial cell production using a factor of 2.05×10^{18} cells mol^{-1} of thymidine incorporated (Riemann et al. 1982). Bacterial carbon production was calculated using the mean bacterial cell volume of the sample and the conversion factor 220 fg C $µm^{-3}$.

Extracellular enzyme activity. Activities of extracellular enzymes in ice and water samples were examined by means of dissolved fluorogenic model substrates after Hoppe (1983): leucine-methylcoumarinylamide (leu-MCA) for aminopeptidases; methylumbelliferylphosphate (MUF-phos.) for phosphatases; methylumbelliferyl-α–D-glucopyranoside (MUF-α–gluc.) for α-glucosidases; and methyl-β–D-glucopyranoside (MUF-β–gluc.) for β–glucosidases. Fluorescence was determined at 365 nm excitation and 455 nm emission by means of the spectrofluorophotometer SFB-23/B Kontron Instruments. The enzymatic tests were carried out with 10 to 15 ml of sea ice (melted without seawater) or seawater. To estimate the maximum hydrolysis velocity (V_{max}), 4 different substrate concentrations in the range of 1 to 26 µmol l^{-1} were used. The enzymatic activities were determined at 1°C, except the hydrolysis rates of the ice core no. 52 221 (see Table 6) which were done at 20°C due to extremely low activites.

RESULTS

Total bacterial counts and biomass

A large-scale patchy horizontal distribution of organisms in the pack ice was evident from visual inspections of overturned ice floes which showed a strong variation in the degree of discolouration by algae. Discrepancies in the biological features were, however, also apparent in the range of 1 to a few meters, as evidenced by measurements of adjacent cores.

Counts of bacteria within the ice ranged over 4 orders of magnitude (Fig. 2). Sea ice with bacterial cell concentrations not exceeding 1×10^{5} bacterial cells ml^{-1} (ice core nos. 52 202, 52 205, 52 238, 52 240, 52 248) predominated in the northern part of the study area and represented the 'predominantly frazil ice'. Elevated bacterial cell concentrations were determined in 'older sea ice' (term according to Dieckmann et al. 1991a) with discoloured zones belonging to the 'mainly frazil' and 'mixed frazil/congelation ice class' (Lange et al. 1989) (core nos. 52 207, 52 214, 52 231, 52 232, 52 239, 52 244). This densely populated sea ice was found mainly in the south of the study area (Fig. 1). 'Predominantly congelation ice' (core nos. 52 206, 52 226) representing refrozen leads (nilas ice) had the lowest bacterial concentrations.

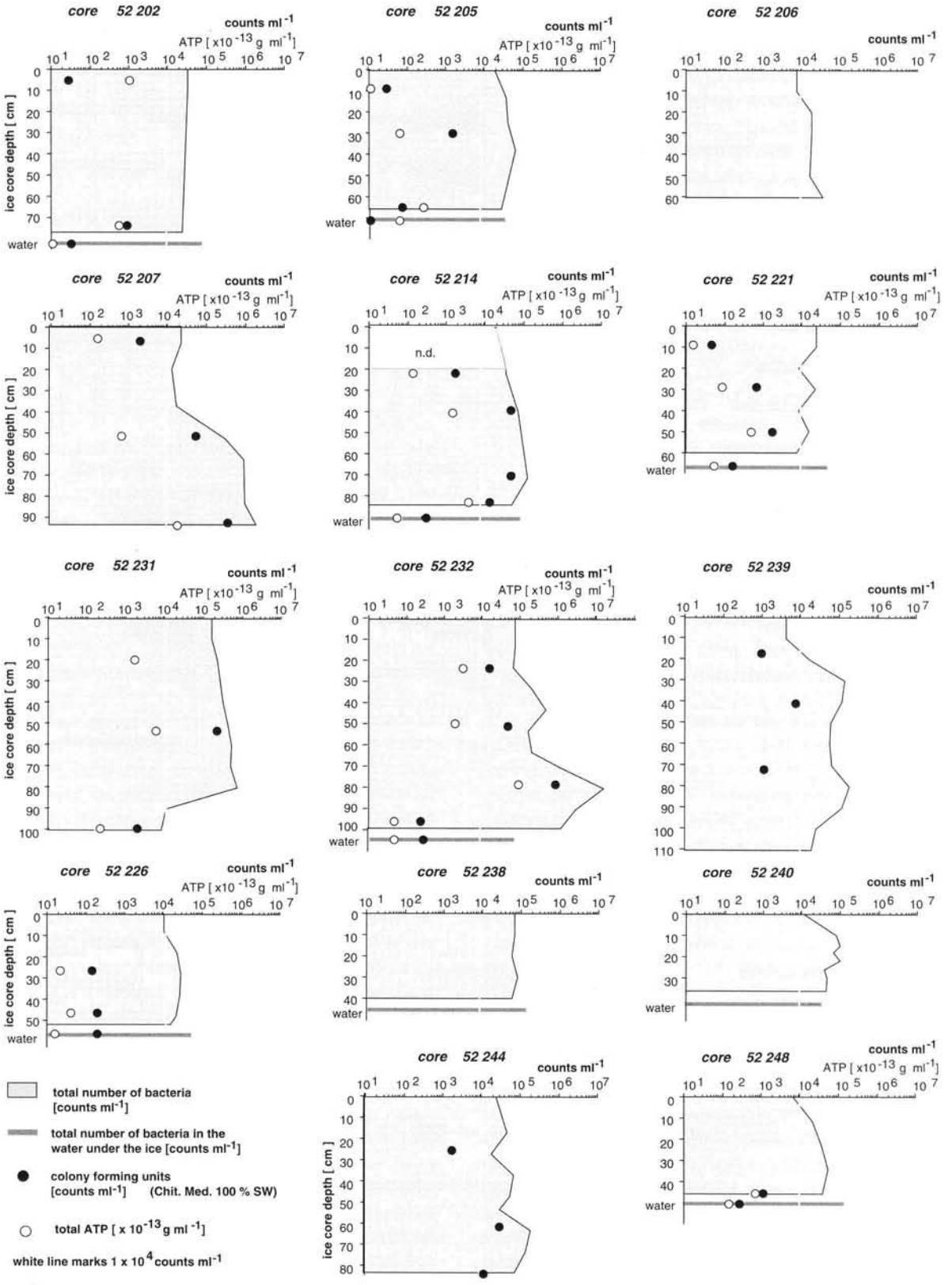

Fig. 2. Total counts of bacteria in sea ice and underlying water in comparison to colony forming units of bacteria and total ATP concentrations

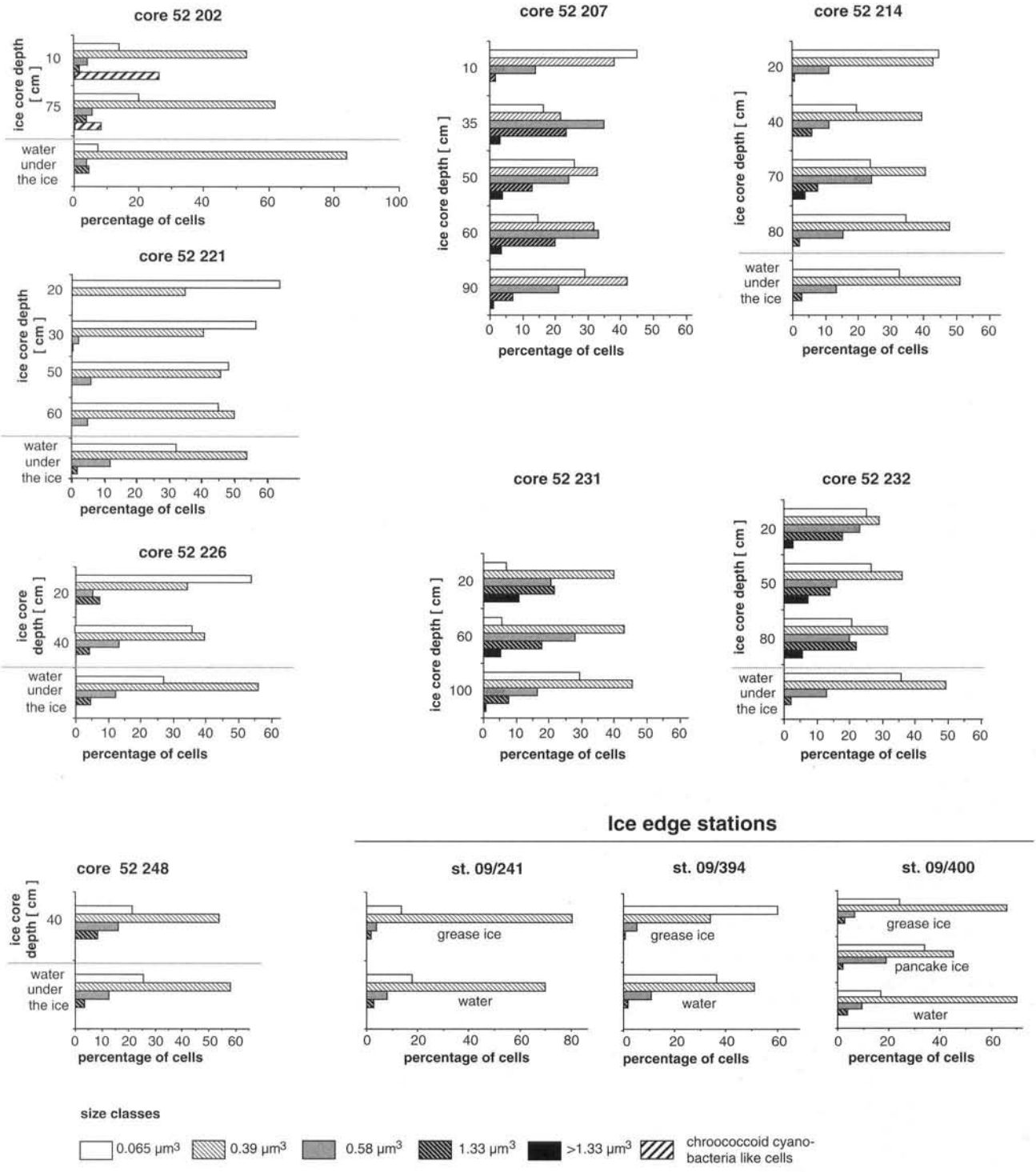

Fig. 3. Size pattern of bacterial sea-ice and water assemblages

The vertical distribution of bacteria throughout the ice displayed a rather uniform pattern with interior ice assemblages (for a description of different ice assemblages see Horner et al. 1992). Bacterial cell concentrations attained their maximum between about 10 to 30 cm above the bottom of the sea ice. The maximum cell concentration of bacteria was 1.8×10^7 cells ml^{-1}. Nearer to the bottom, bacterial cell concentrations were lower but lowest densities (minimum 4×10^3 cells ml^{-1}) were determined in the upper zone at the

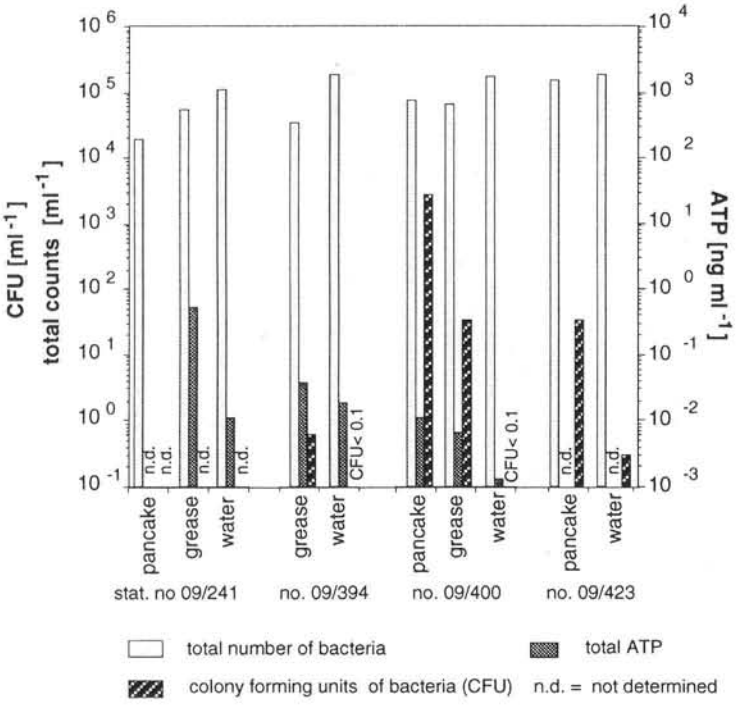

Fig. 4. Total counts and colony forming units of bacteria as well as total ATP concentrations in young sea-ice and water samples of the ice edge area

snow/ice interface. Although an accumulation of chroococcoid cyanobacteria-like cells were found in the upper part of the sea-ice core 52 202 (Fig. 3), a development of pond and infiltration communities was otherwise not evident during this season.

The patchy horizontal distribution in the sea ice was not reflected in the underlying water column. Bacterial densities in the water beneath the ice cover ranged from 0.4 to 2×10^5 cells ml^{-1} (Figs. 2 & 4). The higher cell concentrations were found in the marginal ice zone where the bacterial density in the water always surpassed that in the young grease or pancake ice (Fig. 4). In the water column the vertical gradient of the bacterial cell concentration was small. The total number of bacteria averaged 5×10^4 cells ml^{-1} to a depth of 25 m and 2×10^4 cells ml^{-1} to a depth of 1000 m.

The size patterns of different bacterial assemblages from sea ice and water are demonstrated in Fig. 3. The seawater samples and all sea-ice habitats with low bacterial concentrations had smaller cells with mean cell volumes of 0.17 to 0.35 µm^3. Brown coloured, densely populated ice cores were inhabited by high proportions of exceptionally large bacterial cells. Organisms longer than 10 µm and with a diameter exceeding 1 µm (Fig. 5A, B, C) were frequently encountered. In some of these middle ice-core sections, average cell volumes increased up to 0.9 µm^3. High proportions of dividing cells up to 6% (Fig. 5C, E) as well as flagellated cells (Fig. 5D, E) indicate *in situ* activity of these bacterial communities. The electron micrographs exhibit a variety of distinct morphological bacterial types including different epiphytic bacteria with appendages (Fig. 5F, G, H) as well as organisms with slime sheath (Fig. 5D). Slime formation appeared as a very common property (about 20%) among the sea-ice isolates.

Total bacterial biomass calculated on the basis of bacterial cell concentration and cell volume is compared to ATP (index of total living biomass) in Table 1. The bacterial biomass in the water beneath the ice had a low scatter similar to that of the bacterial cell concentration. In spite of the small cell volumes of the bacterioplankton, their biomass integrated over 100 m water depth exceeded, in any case, the integrated biomass in the sea ice above, even if densely populated sea-ice floes with larger bacterial cells were considered.

ATP concentrations

The ATP concentration of particles greater than 0.2 µm was determined as an index for living biomass of all the biota. Total particulate ATP in the sea-ice samples ranged from 2 ng l^{-1} in nilas ice and upper ice-core sections to a maximum of 9520 ng l^{-1} in a brown, near-bottom ice layer (Fig. 6). Assuming a carbon to ATP ratio of 250:1 (Karl 1980), the total living biomass ranged from 0.5 to 2380 µg C l^{-1}.

Total particulate ATP concentrations in the water column under the sea ice changed little and ranged from <1 to 6 ng l^{-1} (Fig. 6), i.e. <0.25 to 1.5 µg C l^{-1}. A slight increase of total ATP up to 20 ng l^{-1} was observed in the water at the ice edge and south up to 59° latitude, with the exception of Stn 09/400 (Fig. 6).

In contrast to the bacterial biomass, ATP in sea ice integrated over the ice-core depth was always higher than that of the underlying water integrated over an equivalent depth (Table 1). At 3 stations, even more ATP was found in the 1 m ice layer than in the 100 m water column beneath.

ATP concentrations in relation to viable and total bacterial counts are presented in Figs. 2 & 4. The low ATP concentrations of all water and most upper ice-core samples correspond with viable rather than with total bacterial counts. If ATP is compared with calculated bacterial ATP, all water, some upper ice-core samples, and the samples of ice core 52 226 ('predominantly congelation ice') differed from the remaining ice samples (Fig. 6). In the former cases, calculated bacterial ATP exceeded measured total ATP, indicating that the conversion factor is much too high. As the

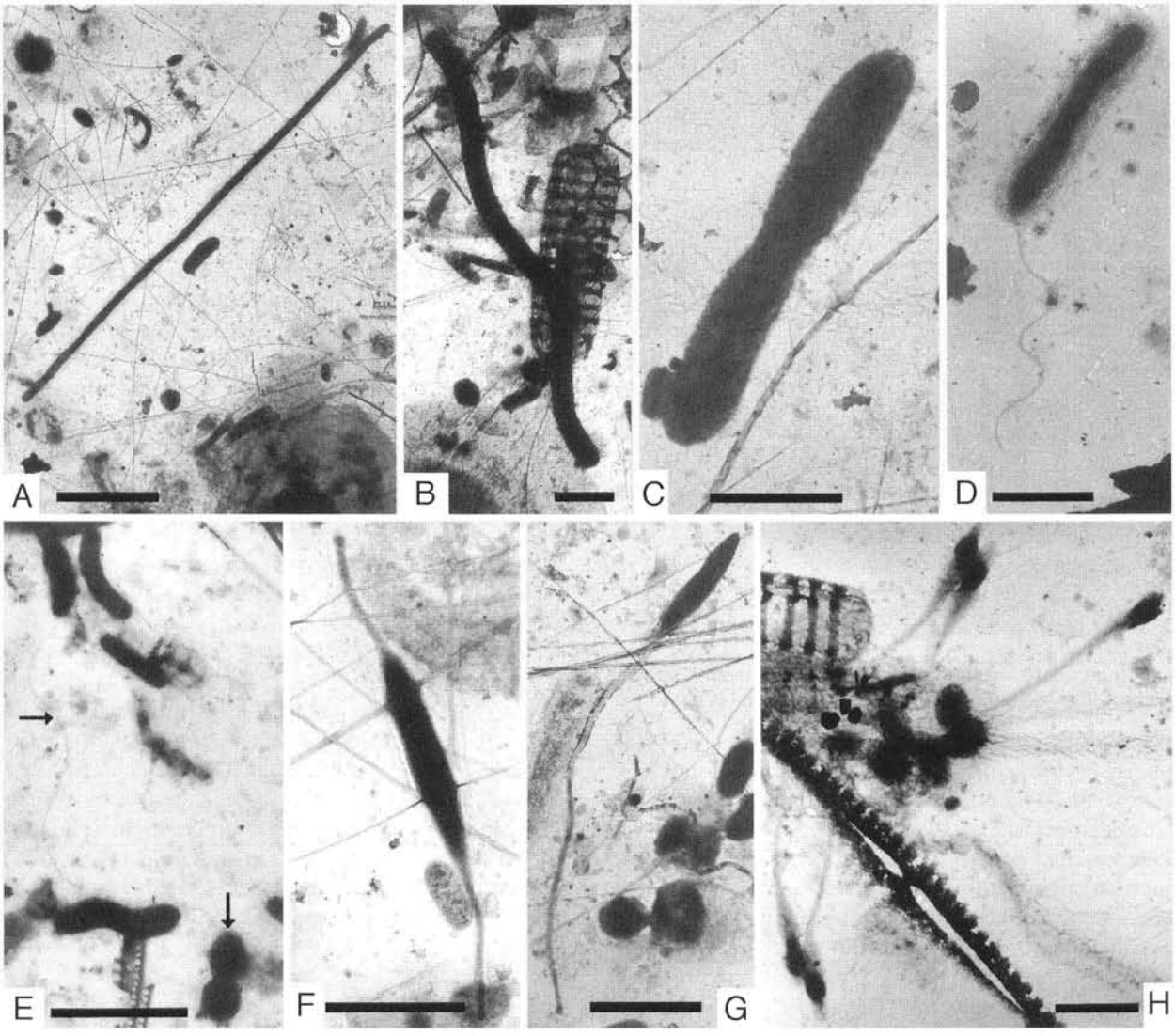

Fig. 5. Transmission electron micrographs of bacteria in sea-ice microbial communities (Stn 09/297). (A, B) Long bacterial cells, (C) thick, dividing bacterium, (D) flagellated bacterium with slime sheath, (E) differently shaped bacteria, among others a flagellated and a dividing bacterium, (F, G, H) bacteria with appendages, (H) appendaged bacteria attached on algal detritus. Scale bar = 5 µm in (A) and 2 µm in (B) to (H)

factor applied was derived from growing cultures (Karl 1980), a poor physiological state of these bacterial assemblages has to be assumed.

The other ice-core samples yielded bacterial ATP values which constituted between 7 and 70% (on average 34%) of the total ATP (Fig. 6). The conversion factor is appropriate here as indicated by the correspondence between calculated bacterial ATP, size-fractionated ATP, and size-class pattern. The measured total ATP of the >0.2 to 3 µm size fraction, for instance, makes up a similar percentage of the calculated bacterial ATP as is the case with the biovolume of the small size classes (<1.33 μm^3) and the total bacterial biovolume (Figs. 3 & 6). Small deviations occur due to the relatively high abundance of small appendaged bacteria attached to algal debris (Fig. 5H).

Viable counts

The number of culturable heterotrophic bacteria obtained with different culture approaches is given in Table 2. The MPN method yielded estimates of viable bacterial numbers on average 6 times higher than the spread plate technique and 60 times higher than the membrane filter method. Maximum viable counts were recorded on Chitin agar 100%SW whereas low numbers of CFUs developed on Chitin agar 300%SW.

Table 1. Bacterial biomass (based on direct microscopic biovolume estimates) and total particulate ATP of sea-ice and seawater samples. nd: not determined

Ice core no.	Total ice-core depth (m)	Bacterial biomass (mg C m^{-2})			Height of water column accomodating bacterial biomass equivalent to that of the overlying sea ice (m)	Total particulate ATP (µg m^{-2})			Height of water column accomodating total particulate ATP equivalent to that of the overlying sea ice (m)
		Sea ice	Sea water			Sea ice	Sea water		
			(Surface water integrated over ice-core depth)	(Integrated over 100 m water depth)			(Surface water integrated over ice-core depth)	(Integrated over 100 m water depth)	
52 202	0.76	1.7	2.8	222	0.8	47.2	0.8	104	45.4
52 205	0.62	2.0	1.6	195	1.0	8.8	4.4	328	2.7
52 207	0.92	51.0	nd	220[a]	23.1[a]	551.2	nd	248[b]	222.2[b]
52 214	0.84	6.5	3.1	221	2.9	142.0	3.6	256	55.5
52 221	0.60	0.5	2.1	219	0.2	11.6	3.6	300	3.8
52 226	0.52	0.5	1.0	180	0.3	2.4	0.8	152	1.6
52 231	1.01	69.0	nd	220[a]	31.3[a]	377.6	nd	248[b]	152.3[b]
52 232	0.99	157.0	3.6	223	70.4	3312.0	3.6	240	1380.0
52 248	0.48	1.2	2.9	283	0.4	37.2	10.0	1560	2.4

[a]Calculation based on an averaged bacterial biomass of 2.2 mg C m^{-3}
[b]Calculation based on an averaged total particulate ATP concentration of 2.48 µg m^{-3}

Extremely few bacterial colonies were obtained on freshwater medium (Chitin agar 0%SW), indicating salt requirements of the bacterial ice consortia tested. On the other hand, these bacteria were able to survive short-term salinity reductions. Experiments with 4 different ice cores demonstrated that sea ice melted without any addition of seawater (final salinity 5 to 10‰) yielded similar viable counts on Chitin agar 100%SW as did sea-ice samples melted in an equal volume of seawater (final salinity 20 to 23‰).

A comparison between viable and total bacterial counts revealed that a large number of bacteria from consolidated ice floes were able to propagate on solid agar and especially in liquid medium (Table 2). While the ratio of viable to total bacterial counts of natural samples is in general 1% or less, the proportion of reproducing bacteria clearly exceeded 1% in the majority of sea-ice samples. Some middle and near-bottom ice-core samples had more than 10%, and 1 MPN series even 87%. However, some upper ice layers, one bottom ice sample as well as nilas ice had percentages below 1% (Table 2). Young pancake and grease ice displayed ratios even less than 0.1% (Table 2, Fig. 4).

The water samples beneath the consolidated ice floes yielded about 0.3% viable relative to total bacterial counts. Towards the ice-edge area, the ratios decreased and at last attained values of almost zero (Table 2). Thus, the already low proportions of culturable bacteria in the young grease and pancake ice exceeded those in the surrounding water (Table 2, Fig. 4).

Temperature tolerance

A collection of strains isolated from the various habitats were examined for temperature adaptation. Psychrophilic as well as facultatively psychrophilic growth responses were obtained. The psychrophilic and facultatively psychrophilic bacteria differed clearly in their temperature maxima and optima, whereas their temperature minima were quite similar. About 80% of both psychrophiles and facultative psychrophiles were able to grow down to –5°C (Fig. 7b) with insignificantly different generation times (Fig. 8). In spite of this marked tolerance towards low temperatures in both bacterial groups, clear differences became evident in their distribution (Fig.7a). More than 90% of the isolates from consolidated sea ice showed a psychrophilic/stenothermic growth response. Only a negligible portion of isolates (9%) grew beyond 30°C. Sixty percent of these facultatively psychrophilic sea-ice isolates originated from upper ice-core sections.

Newly formed grease ice and young pancake ice (about 2 cm thick) were colonized exclusively by facultatively psychrophilic bacteria. An older pancake ice floe (about 15 cm thick, Stn 09/400) as well as the ice core 52 248 sampled near the ice edge were already occupied by a marked proportion of psychrophilic bacteria, indicating a successive increase of psychrophiles with the age of ice. Like young sea ice, the water beneath the consolidated ice harboured almost exclusively facultative psychrophiles. At lower latitudes (south of 65° S) a slight increase of psychrophiles in the water was observed (Fig. 7a).

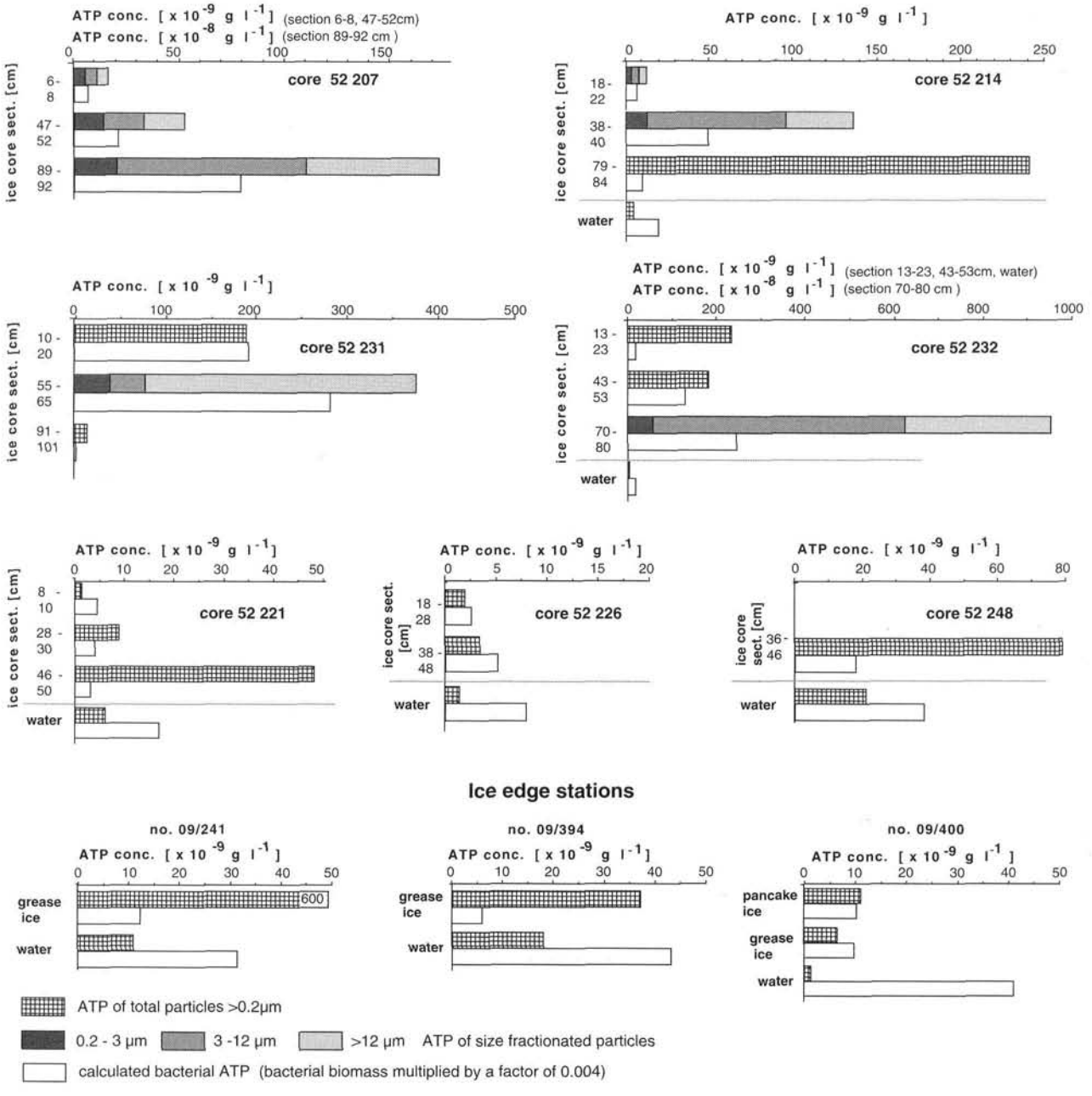

Fig. 6. Total ATP of ice-core sections, young ice, and underlying water in relation to calculated bacterial ATP. Note different scales on ordinates

Turnover of monomeric substrates and secondary production

To evaluate the influence of sea-ice preparation on the turnover times of monomeric substances, different ice treatments were tested (Table 3). The methods applied yielded turnover times which differed by more than 1 order of magnitude. These variations were particularly due to problems with the diffusion of the tracer towards the sites of heterotrophic activities (soaking of sea ice cubes, Method 1) as well as to the small salinity tolerance spectrum of the sea-ice bacteria (melting without seawater addition, Method 4). Satisfactory replicates and short turnover times were obtained with Methods 2 and 3. Method 2 was routinely employed since a homogeneous distribution of the tracer is achieved; concurrently, sea-ice structure and thereby salinity conditions are maintained to a satisfactory degree.

Table 2. Viable counts of ice edge samples, consolidated sea ice, and underlying water. SW: seawater content; MPN: most probable number; nd: not determined

Ice core no.	Ice class or core section	Chitin agar 100 %SW		Chitin agar 300 %SW	Chitin agar 0 %SW	ZoBell broth 100 %SW	
		Viable counts (ml^{-1})	Viable/total counts (%)	Viable counts (ml^{-1})		MPN (ml^{-1})	MPN/total counts (%)
Exterior ice edge zone							
09/394	Grease ice	0.6	0.001	nd	nd	36	0.09
	Water	0	0	nd	nd	0.9	0.0004
09/400	Grease ice	33	0.05	nd	0	316	0.46
	Old pancake floe	2773	3.70	nd	0	3750	5.0
	Water	0	0	nd	0	4	0.002
09/423	Pancake ice	35.6	0.02	1	1	16	0.01
	Water	0.3	0	2	2	5	0.003
Beginning of interior ice zone							
52 202	8–10 cm	20	0.08	0	0	nd	nd
	71–73 cm	750	2.20	0	0	nd	nd
	Water	30	0.04	40	0	nd	nd
52 205	8–10 cm	30	0.23	nd	nd	nd	nd
	28–30 cm	1630	6.79	nd	nd	nd	nd
	60–65 cm	60	0.32	nd	nd	nd	nd
	Water	0	0	nd	nd	nd	nd
52 248	36–46 cm	714	1.86	2	22	6000	12.4
	Water	190	0.12	290	20	190	0.2
Consolidated ice zone							
52 207	6–8 cm	2090	8.30	30	0	nd	nd
	47–52 cm	53800	15.60	31800	0	nd	nd
	89–92 cm	322000	14.30	16800	nd	1900000	84.4
52 210	30–40 cm	350	nd	nd	nd	nd	nd
	Water	50	nd	nd	nd	nd	nd
52 214	18–22 cm	1163	3.25	120	0	1900	5.3
	38–40 cm	57466	81.80	3900	0	nd	nd
	69–71 cm	50000	40.90	230	0	nd	nd
	79–84 cm	14300	29.01	nd	nd	43000	87.2
	Water	270	0.35	nd	nd	nd	nd
52 221	8–10 cm	50	0.29	0	0	150	0.9
	28–30 cm	760	5.67	0	0	500	3.5
	46–50 cm	1685	12.90	10	0	9000	69.0
	Water	130	0.30	90	20	nd	nd
52 226	18–28 cm	118	0.80	nd	nd	nd	nd
	38–48 cm	190	1.32	nd	nd	nd	nd
	Water	170	0.20	nd	nd	nd	nd
52 231	10–20 cm	nd	nd	6965	41	nd	nd
	55–65 cm	145555	28.00	4236	5	102000	19.3
	91–101cm	1176	12.50	20	0	nd	nd
52 232	13–23 cm	23575	35.00	6351	0	43000	65.2
	43–53 cm	33407	20.20	6858	22	110000	68.7
	70–80 cm	816477	4.56	26013	0	1041000	5.8
	Water	220	0.29	nd	nd	nd	nd
52 239	10–20 cm	1086	3.10	nd	nd	nd	nd
	30–40 cm	6432	5.50	nd	nd	nd	nd
	60–70 cm	1006	1.58	nd	nd	nd	nd
52 244	17–27 cm	1483	8.60	40	10	10000	58.1
	53–63 cm	14584	7.00	0	0	100000	46.5
	73–83 cm	7322	8.96	46	1	38000	45.6

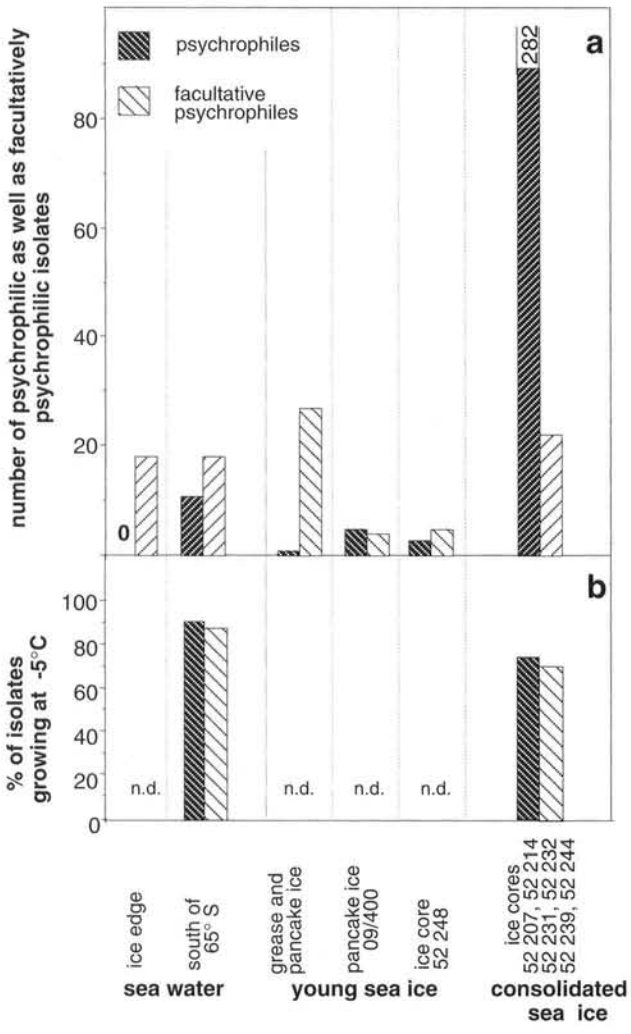

Fig. 7. (a) Portions of psychrophilic and facultatively psychrophilic strains of the isolates from different environments. (b) Percentages of psychrophilic and facultatively psychrophilic strains growing at –5°C

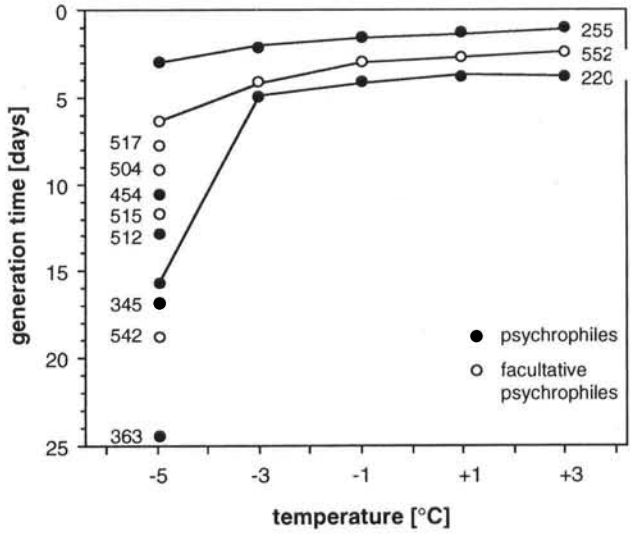

Fig. 8. Generation times of various psychrophilic and facultatively psychrophilic isolates from sea ice (bacterial strain nos. 220, 255, 345, 363,454, 512, 542 from grease ice, 552 pancake ice) and water (strain nos. 504, 515, 517) in the very low temperature range at high substrate concentrations

The leucine and glucose turnover data of various sea-ice and water samples are compiled in Table 4. Since respiration was not considered, an underestimation of about 30% for ^{14}C-glucose turnover (Gocke 1976) and about 10% for ^{3}H-leucine (Gocke 1976, Kuparinen & Tamminen 1982) has to be taken into account. The different pack-ice communities assimilated glucose and leucine at –2°C. Turnover times ranged from a few days to 3 mo in densely populated sea-ice material and extended to about 1 yr in sparsely inhabited ice-core samples. The data correlated closely with viable as well as total counts (Table 4) (correlation factors are in the range of $r^2 = 0.76$ for leucine and $r^2 = 0.81$ for glucose). The water beneath the ice showed very low heterotrophic activities, whereas water from an ice free area south of the Antarctic Convergence (west of Bouvet Island, samples were taken before entering the ice area) had leucine turnover times (at 2°C) similar to densely populated ice-core sections (Table 4). Heterotrophic activity associated with sea-ice algae were tested by means of antibiotic inhibition experiments. The prokaryotic inhibitor chloramphenicol strongly affected leucine assimilation, whereas the eukaryotic inhibitor cycloheximide caused negligible effects (Table 4). Both antibiotics are specific protein-synthesis inhibitors, therefore, a similar clear inhibition

Table 3. Influence of different sea-ice preparations on the turnover time of ^{3}H-leucine. nd: not determined

Method of preparing ice sample	Leucine turnover time (d) Brash ice 09/297	Ice core 52223	Ice core 52226	Ice core 52231	Ice core 52232
(1) Crushed ice	141	9980	2138	1179	472
(2) Crushed ice suspended in seawater	nd	332	356	84	9.5
(3) Ice melted in seawater	15.5	399	658	nd	nd
(4) Ice melted without added seawater	224	8317	1425	3305	nd

Table 4. Turnover times of ^{3}H-leucine and ^{14}C-glucose in ice core and water samples. nd: not determined

Sample	Turnover time (d)						Bacterial counts ($\times 10^3$ ml^{-1})	
	Leucine	Leucine + chloramphenicol	Leucine + cycloheximide	Glucose	Glucose + chloramphenicol	Glucose + cycloheximide	Total	Viable
Brown ice floe:								
Stn 09/297	15.5[a]	nd	nd	14.4[b]			15000	nd
Ice core section:								
52 223, near bottom	332	nd	nd	nd			18	0.15
52 226, near bottom	356	nd	nd	nd			15	0.19
52 231, middle	84	531	94	70			550	145.55
52 232, near bottom	9.5	237	12	2.5	5.0	2.9	2410	816.47
52 238, near bottom	430	nd	nd	173			59	nd
52 244, top	476	1919	609	3990			51	1.48
middle	86	1427	135	315			210	14.58
near bottom	78	4328	119	179			81	7.32
Water beneath ice core:								
52 202	1166	nd	nd	nd			72	0.03
52 238	2366	nd	nd	1001			100	0.05
Surface water off Bouvet Island:	79	1234	107	nd			nd	nd

	Bacterial cell production ($\times 10^{10}$ cells m^{-3} d^{-1})	Bacterial carbon production (mg C m^{-3} d^{-1})		
Ice core section:				
52 214, near bottom	2.3	0.2	122	50.00
52 232, near bottom	13.6	2.7	2410	816.47
52 244, middle	3.8	0.3	210	14.58

[a]Ice melted in seawater
[b]Crushed ice without added seawater

response after an 18 h incubation could not be expected with glucose assimilation. This was reduced to about 50% by chloramphenicol, but contrary to results of Smith & Clement (1990), only to about 14% by cycloheximide. A significant contribution of eucaryotes to heterotrophic processes in winter sea ice is thus unlikely.

Incorporation of tritiated thymidine into cold TCA precipitate was used to estimate bacterial secondary production. Only densely populated sections of 3 different ice cores were analyzed. The calculated bacterial cell and carbon production ranged from 2.3 to 13.6 $\times 10^{10}$ cells m^{-3} d^{-1} and from 0.2 to 2.7 mg C m^{-3} d^{-1}, respectively (Table 4).

Extracellular enzyme activities

The potential to decompose particulate organic matter (POM) was investigated in sea-ice and water samples by means of fluorogenic model substrates. Activities of extracellular proteases, phosphatases, and α– and β–glucosidases could be shown at 1°C with all sea-ice samples tested (Table 5). Sea ice with low bacterial concentrations (core no. 53 210; 52 221) displayed relatively low extracellular activities, whereas the coloured ice section of core no. 52 244 exhibited very short hydrolysis times and high maximal hydrolysis rates.

The limit of the sensitive fluorescence method was reached with water samples from beneath the sea ice (Tables 5 & 6), again indicating a severe reduction of biological activity in the water column beneath sea ice during winter.

The leu-MCA-protease as well as the MUF-β–glucosidase activities correlated closely with viable counts (Table 6). Corresponding with results from temperate waters (Hoppe 1983), MUF-phosphatase activities did not correlate with viable counts. These enzymes are also exceptional with respect to their temperature response. Temperature optima of MUF-phospatases were in the range of 50 to 60°C (Fig. 9) whereas leu-MCA-proteases and MUF-α– and β–glucosidases displayed optima at about 30°C.

Table 5. Maximum velocity of hydrolysis (V_{max}, pmol l^{-1} min^{-1}) and hydrolysis times (d; according to Hoppe et al. 1988) of extracellular enzymes of different sea-ice core samples assayed by means of fluorogenic substrate analogues at 1°C. nd: not determined. See 'Methods; Extracellular enzyme activity' for enzyme names in full

Ice core Section (cm)	Viable counts (ml^{-1})	Leu-MCA V_{max}	Leu-MCA Hydrol. time	MUF-phos. V_{max}	MUF-phos. Hydrol. time	MUF-α-gluc. V_{max}	MUF-α-gluc. Hydrol. time	MUF-β-gluc. V_{max}	MUF-β-gluc. Hydrol. time
52 210									
30–40	350	23.4	636	3.6	204	nd	nd	nd	nd
Water	50	7.2	14300	1.5	812	nd	nd	nd	nd
52 221									
46–50	1685	9.7	586	6.8	1023	nd	nd	nd	nd
52 244									
17–27	1483	162.2	43	51.6	258	nd	nd	nd	nd
53–63	14584	1058.5	1.6	135.1	57	41.9	211	44.8	392

Table 6. Hydrolysis rates of extracellular enzymes of different sections of 2 sea-ice cores as well as of the water beneath the ice related to viable counts (Chitin agar 100%SW, 1°C). The extracellular enzymatic activities of core 52244 were determined at 1°C with substrate concentrations of 12.9 µmol l^{-1} leu-MCA and 12.4 µmol l^{-1} MUF-phosphate. The ice material of the poorly colonized ice core 52221 was assayed for extracellular enzymatic activities at 20°C with substrate concentrations of 8.6 µmol l^{-1} leu-MCA, 8.3 µmol l^{-1} MUF-phosphate, and 16.7 µmol l^{-1} MUF-β-glucoside. r^2: correlation between hydrolysis rates and viable counts of bacteria. nd: not determined

Ice core Section (cm)	Viable counts (ml^{-1})	Hydrolysis rates (pmol l^{-1} min^{-1}) Leu-MCA	MUF-phos.	MUF-β-gluc.
52 221				
8–10	50	1.6	1.3	0.35
28–30	760	3.7	11.5	0.84
46–50	1685	8.0	11.0	1.66
Water	130	2.5	15.8	0.28
		$r^2 = 0.97$	$r^2 = 0.06$	$r^2 = 0.98$
52 244				
17–27	1483	89.6	38.6	nd
53–63	14584	851.1	54.9	nd
Water	70	0.5	1.3	nd
		$r^2 = 0.99$	$r^2 = 0.63$	

DISCUSSION

Bacterial processes within the sea ice during winter were expected to be severely inhibited by low temperatures and corresponding high brine salinities. However, a clear temperature-induced limitation of bacterial development was only evident in the upper ice layer where (dependent on snow cover) average temperatures of −7 to −9°C and a minimum of −15°C (Bartsch 1989) were recorded. The low bacterial concentrations and the poor viability found here contrast with the well developed and abundant surface assemblages observed during spring and summer (Garrison & Buck 1989) as well as with the highly productive melt pond communities recorded during autumn (Kottmeier & Sullivan 1990). Significant inhibition effects were not obvious in middle and lower ice-core zones, where temperatures ranged between −2.5 (bottom) and −5.5°C (middle) (Weissenberger 1992). Maximal cell concentrations and microbial activities were determined in the lower part of the middle ice layers in sparsely as well as densely populated ice cores. In contrast to the major part of the sea ice the comparatively warmer water (>−1.9°C) beneath the protecting ice cover was biologically impoverished. This is indicated by ATP concentrations, which were about 1 order of magnitude less (Fig. 6) than ATP concentrations reported in the surface water from other parts of the Southern Ocean during summer and autumn (Hodson et al. 1981, Hanson & Lowery 1985, Vosjan et al. 1987), as well as by extremely low chlorophyll *a* (chl *a*) values (in the range of 0.03 to 0.08 µg l^{-1}; Bartsch 1989, Scharek et al. 1994). Heterotrophic activities (Table 4) were also at least 1 order of magnitude less compared to those determined in the marginal ice zone of the northwestern Weddell Sea during spring and autumn (Cota et al. 1990, Sullivan et al. 1990) but were similar to the very low values measured during summer beneath a consolidated ice cover in McMurdo Sound (Hodson et al. 1981). Viable bacterial counts (Table 2) were correspondingly low. These, however, contrasted with relatively high total bacterial abundances as well as bacterial biomass (Fig. 2, Table 1) which resembled values determined during spring and autumn in the marginal pack-ice zone of the western Weddell Sea (Kottmeier & Sullivan 1990). In particular, the comparison of total and bacterial ATP values (Fig. 6) revealed that the high

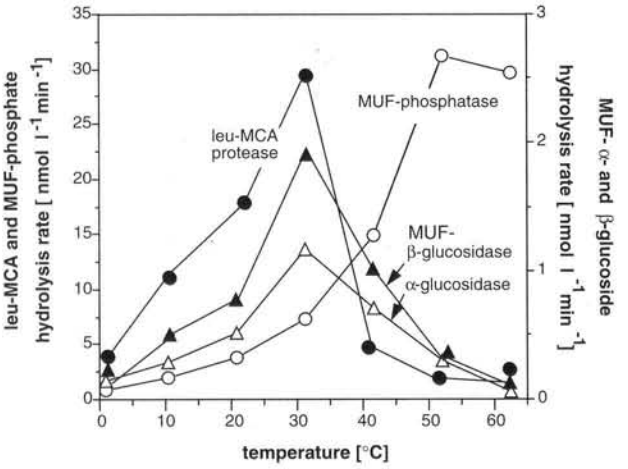

Fig. 9. Effect of temperature on the activity of extracellular enzymes of brown ice floe material (Stn 09/297) assayed by means of the dissolved methylumbelliferyl derivatives leu-MCA (8.63 µmol l^{-1}), MUF-phosphate (8.32 µmol l^{-1}), MUF-α–D-glucopyranoside (16.7 µmol l^{-1}) and MUF-β–D-glucopyranoside (16.7 µmol l^{-1})

bacterial standing stock comprised largely starving and moribund bacterial cells which persist, presumably, due to a paucity of bacterivores. The bacterial biomass would have been misjudged if the AODC method, which fails to discriminate between active, starving, and dead cells, had been solely employed.

The water beneath fast ice (Kottmeier & Sullivan 1987, Rivkin et al. 1989, Satoh et al. 1989, Matsuda et al. 1990) is also reported to have very little plankton in winter. On the other hand, considerable primary as well as secondary production was observed during this season underneath an unconsolidated pack-ice cover in the Bellingshausen Sea (Kottmeier & Sullivan 1987). Obviously, the biological impoverishment of the water column in winter depends to a high degree on the consolidation of ice cover which limits light.

Annual sea-ice communities are recruited every year from seawater. While the initial stock of organisms in the sea ice depends on the abundance and composition of the microbial assemblages within the water column at the time and location of ice formation, the further fate of the sea-ice biota is strongly controlled by the course of the ice-floe development. Even adjacent floes may have evolved completely differently due to dynamic processes such as drifting, divergence, and rafting. In order to understand the development of the sea-ice biota, knowledge about the history of the floe studied is important. Systematic glaciological studies during the cruise ANT V/2 revealed that ice texture is indicative of the history of sea-ice formation and evolution (Lange et. al. 1989, 1990). A close connection between genetic ice classes and biological features became obvious in this study as well as elsewhere (Spindler et al. 1990, Dieckmann et al. 1991a, b). Thus, conclusions on the dynamics of the different members of the sea-ice community become possible in spite of the patchy horizontal distribution of microorganisms.

Similarities between the biological features of the poor winter water and of the sea ice were only found with the 'predominantly congelation ice' which develops in calm water of leads.

Most of the winter ice is formed in the turbulent water of the marginal ice zone with relatively high chl *a* concentrations. Physical concentration mechanisms such as 'harvesting' and 'scavenging' play an important role in inoculation of this 'predominantly frazil ice' (Ackley 1982, Garrison et al. 1983, 1989, Ackley et al. 1987). During ANT V/2, foraminifers as well as chl *a* concentrations in 'predominantly frazil ice' exceeded those in the underlying water. However, the further development of algae as well as foraminifers appeared to be limited in this ice category since algal nutrient concentrations did not change significantly and foraminiferal concentrations decreased towards the continent (Spindler et al. 1990, Dieckmann et al. 1991a). A physical enrichment of total bacterial cells in the sea ice of the ice edge was not encountered (Fig. 4), although, at a low level, viable bacterial counts in the ice exceeded those in the associated water (Fig. 4). This phenomenon may be explained by a selective harvesting of viable bacteria due to their attachment to larger nutritious particles such as algae or detritus. Results of ice formation experiments (Grossmann & Gleitz 1993) corroborate this speculation, as bacteria were found to be enriched only in the presence of specific algae.

Even if viable bacteria may be preferentially enclosed in sea ice, the ratios of viable to total bacteria are strikingly low in these young sea-ice samples as well as in the adjacent water of the ice edge area (Table 2). The bulk of bacteria is obviously strongly affected by initial ice formation. A clear metabolic reduction during ice formation processes was also observed in simulated ice formation experiments (Grossmann & Gleitz 1993) as well as in an ice edge study during late autumn (Grossmann & Dieckmann 1994). However, in the present study the increase of culturable and psychrophilic bacteria with advancing age of ice presents evidence for a new formation of specific bacterial communities in sea ice. Correspondingly, Grossmann & Dieckmann (1994) found an increase of leucine uptake from young to older sea ice.

The new organization of bacterial communities in the 'predominantly frazil ice' is obviously supported by the relatively high concentrations of POM harvested during initial ice formation at the ice edge. In contrast, bacterial assemblages did not develop in the likewise

young 'predominantly congelation ice' which is formed in the impoverished water of leads. These observations correspond with the findings of Pomeroy et al. (1991) as well as Wiebe et al. (1992) that bacterial reproduction in the minimum temperature range depends strongly on higher substrate concentrations.

The densely populated ice floes belonged to the 'mixed ice class' and the 'mainly frazil ice class' which were already formed during autumn or the season before and occurred more frequently towards the continent. Chl *a* concentrations were high, and former and/or ongoing phototrophic activity was indicated by low silicate concentrations and sometimes complete exhaustion of algal nutrients (Dieckmann et al. 1991a). Bacterial counts and biomass reached also maximal values (Figs. 2 & 5, Table 1). By means of a variety of methods it is substantiated that the bacterial accumulations within these ice floes do not solely derive from a passive physical incorporation of autumnal bacterial blooms, but could be traced back to selective bacterial growth within the ice. Floes of these 2 ice classes turned out to be sites of concentrated heterotrophic activity during the entire midwinter period. They are, in comparison to the 2 other genetic ice classes discussed, of great biological significance in the annual pack ice of the Weddell Sea during midwinter.

A mean bacterial production of 6.6×10^{10} cells $m^{-3} d^{-1}$ equivalent to 1.06 mg C $m^{-3} d^{-1}$ was calculated for the densely colonized ice layers in these floes (Table 4). This is up to 5 times less than the bacterial production determined in the dispersed winter ice of the Bellingshausen Sea (Kottmeier & Sullivan 1987). Nevertheless, both winter estimates are within the broad range of spring and summer values determined in the water column and marginal ice zone of different regions of the Southern Ocean and in the bottom ice sections of the fast ice of McMurdo Sound (compiled by Kottmeier et al. 1987, Cota et al. 1990; ranges from undetectable to 24 mg C $m^{-3} d^{-1}$). Only autumnal melt pond communities (1094 mg C $m^{-3} d^{-1}$; Kottmeier & Sullivan 1990) clearly surpass this range.

A rough estimate of the magnitude of secondary production in the annual pack ice of the Weddell Sea during winter was obtained by a conservative calculation based on a 20 cm thick, densely populated, middle ice layer of the 'mainly frazil ice' and 'mixed ice' (these account for 26.2 % and 19 % respectively of the sea ice in the study area, after Lange et al. 1989). The comparatively poorly colonized 'predominantly frazil ice' and 'predominantly congelation ice' were neglected. A bacterial production of 4.05×10^{10} g C was calculated for a 3 mo winter period in the 4.7×10^6 km^2 (Zwally et al. 1983) large study area which comprises 24 % of the total area of the Antarctic sea-ice cover. The mean bacterial carbon production would amount to 95.6 µg C $m^{-2} d^{-1}$ and would account for 43 % of the primary production of interior ice algal communities in the Antarctic Ocean (0.016×10^{14} g C yr^{-1} per 20×10^6 km^2; Legendre et al. 1992). This significant bacterial production as well as the relatively high proportion of bacterial ATP to total ATP (on average 34 %) indicates a substantial role of the heterotrophic bacterial component within the microbial sea-ice community during winter.

Productivity measurements in the unconsolidated pack ice of the Bellinghausen Sea (Kottmeier & Sullivan 1987) as well as in the fast ice and water of the McMurdo Sound (Rivkin et al. 1989) also revealed that bacterial production amount to a substantial fraction of primary production during winter. An increasing importance of heterotrophy during winter can be explained by light limitation. Kottmeier & Sullivan (1988) further found that bacterial production is favoured in comparison to primary production in the low temperature range. In addition, lysis of sea-ice algae, induced by nutrient exhaustion, will provide bacteria with increased amounts of organic nutrients, among others macromolecules. These, however, have to undergo extracellular enzymatic decomposition prior to incorporation as dissolved substances by bacteria. POM hydrolysing processes have not yet been studied in sea ice. Leu-MCA hydrolysis times were surprisingly short in densely populated ice cores (Tables 5 & 6). These values were comparable with data determined in eutrophic water samples of a temperate region (Hoppe 1983, Hoppe et al. 1988). POM-degradation, therefore, does not appear to be a limiting step in recycling organic carbon in densely populated sea-ice samples, even if a definite evaluation is difficult due to the uncertain analogous character of the fluorogenic substrates to natural substances (Hoppe 1983, Hoppe et al. 1988, Helmke & Weyland 1991). However, a pronounced hydrolysis potential for natural polymers could also be demonstrated with a collection of 326 bacterial sea-ice isolates (authors' unpubl. results).

The leu-MCA-proteases and MUF-α– and β–glucosidases of sea ice had relatively low temperature optima and maxima (Fig. 9) compared to extracellular enzymes of Antarctic sediments (Helmke & Weyland 1991). The sea-ice enzymes resembled those formed by psychrophilic bacteria which have optima and maxima 10 to 20°C lower than enzymes produced by mesophilic bacteria (Helmke & Weyland 1991). Water samples from the North Sea which were populated by mesophilic bacteria showed extracellular enzyme reactions similar to those from sea ice. However, the V_{max} values in the low temperature range were on average higher with the sea-ice samples (Helmke & Weyland 1991). The similarities between extracellular enzymes of temperate and cold habitats underline the

generally broad tolerance spectrum of extracellular enzymes and corroborate the assumption that degradation of particulate matter is less controlled by a specific temperature adaptation of the extracellular enzymes per se than by the formation of the enzymes which is governed by the adaptation of the producers (Helmke & Weyland 1986).

The bacterial sea-ice and seawater assemblages differed clearly in their temperature-adaptive strategies. Bacteria isolated from consolidated sea ice responded preponderantly stenothermic/ psychrophilic whereas those from water and young ice behaved eurythermic/facultatively psychrophilic. Since the percentage of culturable bacteria was extraordinarily high in older sea-ice samples, psychrophily has to be considered as the general adaptation concept in well-developed bacterial sea-ice assemblages. The proportion of culturable bacteria in the water beneath the ice as well as in young ice was 1% or less. Thus, the facultative psychrophiles studied might be less representative. A dominance of facultative psychrophiles (psychrotrophs) in sea-ice-associated water was also found in the western Weddell Sea during summer (Delille 1992). On the other hand, a strong partitioning between water and sea-ice communities, as found in the present study, was not encountered during a cruise in autumn where, in still unconsolidated pack ice of the eastern Weddell Sea, psychrophilic bacteria predominated in sea ice as well as in plankton-rich water (Helmke unpubl. obs.). This observation as well as the insignificantly different generation times of psychrophiles and facultative psychrophiles in the very low temperature range (Fig. 8) indicate that low temperatures alone could not account for the selective enrichment of psychrophiles. Salinity also cannot be taken into account as the relevant controlling factor since psychrophiles have a smaller salinity tolerance spectrum than facultative psychrophiles (authors' unpubl. results, partly indicated in this study by the viable count data as well as the heterotrophic activity data). Beside sea ice and plankton-rich Antarctic water, shallow Antarctic sediments also harbour bacterial communities predominated by psychrophilic bacteria (Helmke & Weyland 1991) but with a different taxonomical structure (authors' unpubl. results). The 3 habitats are characterized by low temperatures as well as higher levels of organic matter mostly associated with increased solid surfaces. The selective enrichment of psychrophiles are obviously determined by the combined effect of these ecofactors. Competition experiments in continuous cultures at varying temperature and substrate conditions with psychrophilic and facultatively psychrophilic isolates from the North Sea (Harder & Veldkamp 1971) corroborate our assumption based on observations in the field.

The present study gives evidence that temperature is generally not the major controlling factor for the development of active or inactive bacterial communities in the surface layer of the eastern Weddell Sea during midwinter. Sea ice in comparison to seawater turned out to be the preferred habitat of bacteria in spite of the exceptionally low temperatures and high salinities. Apparently, due to an improved availability of energy/ nutrient sources in sea ice, unique bacterial communities develop and propagate. Their contribution to the bacterial productivity in the Weddell Sea during winter is obviously substantial.

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