

## Predominance of $\beta$ -proteobacteria in summer melt pools on Arctic pack ice

Robin Brinkmeyer<sup>1</sup>

Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, D-27570, Bremerhaven, Germany

Frank-Oliver Glöckner

Max-Planck Institute for Marine Microbiology, Celsiusstrasse 1, D-28359 Bremen, Germany

Elisabeth Helmke

Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, D-27570, Bremerhaven, Germany

Rudolf Amann

Max-Planck Institute for Marine Microbiology, Celsiusstrasse 1, D-28359 Bremen, Germany

### Abstract

The diversity and community structure of bacteria in melt pools on Arctic pack ice floes were dominated by  $\beta$ -proteobacteria. Thirty-five percent of the pure cultures isolated in 1997 from pack ice floes north of Svalbard and in the Fram Strait were from the  $\beta$ -proteobacteria group. Within this group, there were only two phylotypes clustering within the widespread Beta I cluster, also known as the Comamonadaceae clade. One phylotype, most closely related to *Aquaspirillum arcticum* (96.0–97.3% identical), was frequent among cultures isolated from 10 melt pools. A 16S ribosomal RNA (rRNA) gene clone library, constructed from a melt pool that was sampled 2 yr later in the Fram Strait, was also dominated by  $\beta$ -proteobacteria, in particular the same recurrent isolate phylotype designated “MP-BetaI”. Fluorescence in situ hybridization of 20 melt pools corroborated the cultivation and cloning data.  $\beta$ -Proteobacteria were the most abundant bacterial group, constituting ~49% of the bacteria that were stained by 4′6-diamidino-2-phenylindole (DAPI).  $\alpha$ - and  $\gamma$ -proteobacteria accounted for only 2% each, the *Cytophaga-Flavobacterium* group accounted for 9%, and the *Actinobacteria* spp. accounted for 9%. Approximately 63% of the  $\beta$ -proteobacterial fraction that was found in the melt pools was determined with a newly developed probe to be the recurrent  $\beta$ -proteobacterial MP-BetaI phylotypes, indicating that it is particularly adapted for success in this extreme environment.

During summer months in the Arctic, increasing solar radiation rapidly warms and melts the snow cover on the perennial pack ice. Melted snow and ice form shallow (10–50 cm deep) freshwater pools that can cover as much as 50% of the surface area of ice floes (Fetterer and Untersteiner 1998). The temporary pools occur in early June when melting starts and freeze solid in late August or early September with the onset of winter (Fetterer and Untersteiner 1998).

Living conditions for biota (i.e., bacteria, microalgae, and protozoans) (Melnikov 1997; Carstens 2001) in the melt pools are harsh. Temperatures hover around 0°C. Growth

substrates are highly limiting. The melt pools are oligotrophic to ultraoligotrophic, having very low chlorophyll *a* (Chl *a*) (0.08  $\mu\text{g L}^{-1}$ ), dissolved organic carbon (DOC; <1 mg C L<sup>-1</sup>), and inorganic nutrient concentrations (Wickham and Carstens 1998). The 24 h of sunlight or “white nights” of the Arctic summer subject organisms to high ultraviolet B (UVB) radiation exposure (Wickham and Carstens 1998). Moreover, the lack of shading, low DOC (e.g., minimal UV filtration; Gibson et al. 2001), and reflection of UVB waves back from the icy bottom in the shallow pools intensify exposure. Wickham and Carstens (1998) examined the effects of UVB radiation on melt pool organisms and found that the bacteria were the least vulnerable of the groups tested, suggesting a resistance.

Studies of bacteria in pack ice have focused mainly on the interior ice matrix. Highly active and robust microbial communities in the brine channels are reflective of the concentrated and labile dissolved organic matter derived from ice algae (Smith and Clement 1990; Grossmann and Dieckmann 1994; Helmke and Weyland 1995; Amon et al. 2001). Molecular characterizations have shown several phylotypes that are unique to the sea ice habitat or polar seas. The most dominant groups are *Colwellia* spp., *Glaciecola* spp., *Octadecabacter* spp., and *Polaribacter* spp., which fall within bacterial lineages generally associated with marine algae (Bowman et al. 1997; Staley and Gosink 1999; Brown and Bowman 2001; Junge et al. 2002; Brinkmeyer et al. 2003).

<sup>1</sup> Corresponding author (brinkmer@tamug.edu). Present address: Texas A&M University at Galveston, 5007 Avenue U, Galveston, Texas 77550.

### Acknowledgments

We thank the captains and crews of the RV *Polarstern* cruises ARKXVIII/2, ARKXV/1, and ARKXVI/2 and the Wasserthal Helicopter service for logistical support. K. Berlitz, M. Wanger, J. Jokiel, and K. Reuter assisted with sampling. We are grateful to J. Jürgens for maintenance of the isolate cultures and to G. Kattner and M. Stürken for nutrient analyses. The constructive comments from two anonymous reviewers improved an earlier version of this manuscript. This research was supported by the German Ministry of Education and Science (BMBF 03F0233B), the Alfred Wegener Institute for Polar and Marine Research Foundation, and the Max Planck Society.

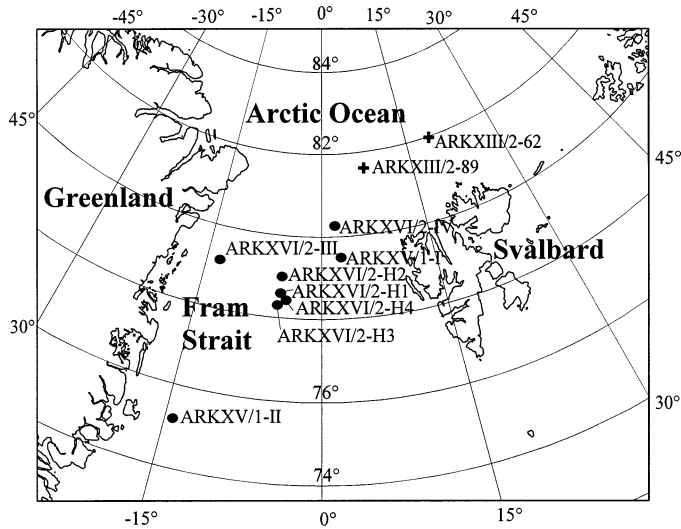


Fig. 1. Sampling stations in the Arctic Ocean and Fram Strait. Crosses represent sampling stations for isolate cultures, and filled circles represent sampling stations for environmental samples. Maps were generated with PanMap software ([www.pangaea.de](http://www.pangaea.de)).

For several decades, the Arctic melt pools were assumed to be mostly devoid of life; however, the observations of Wickham and Carstens (1998) and a comprehensive inventory of melt pool biota (Carstens 2001) indicate a classical microbial loop. Our goals were to determine the types of bacteria that could live in such an extreme, growth-inhibiting, and highly ephemeral habitat. To our knowledge, this study presents the first look at bacterial diversity and community composition in melt pools on Arctic pack ice.

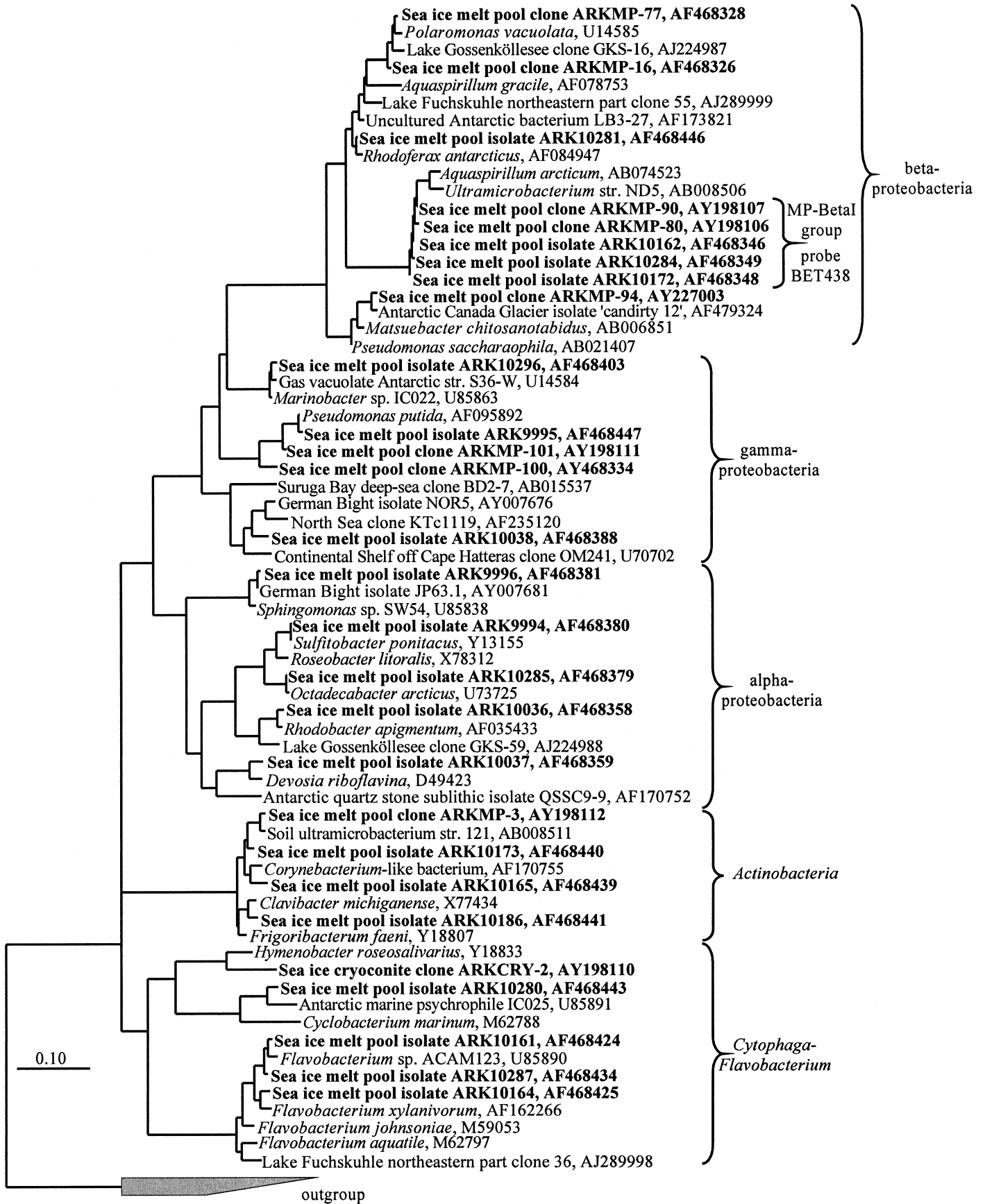
## Materials and methods

Bacteria were isolated from melt pools during the RV *Polarstern* cruise ARKXIII/2 (June/July 1997) north of Svålbard (Sta. ARKXIII/2-62 [81°90'N/19°46'E] and ARKXIII/2-89 [81°54'N/7°57'E]; Fig. 1), and pure cultures were obtained from colonies that developed on solid medium as described previously by Helmke and Weyland (1995). For clone library and fluorescence in situ hybridization (FISH) analyses, melt pools, including a cryoconite hole (accumulations of sediment and/or dust on the floe surface that melt 1- to 2-cm-wide depressions into the ice), were sampled during subsequent RV *Polarstern* cruises ARKXV/1 (June/July 1999) and ARKXVI/2 (July/August 2000) in the Fram Strait (Fig. 1). Careful attention was paid to maintain sterile conditions during sampling and processing. Temperature, salinity, and pH in the melt pools were measured in situ on the ice floes. Samples were processed immediately on the ship

in a cold room. Aliquots (up to 500 ml) from some samples were collected onto polycarbonate filters (pore size = 0.2  $\mu\text{m}$ ) and stored at  $-80^{\circ}\text{C}$  for later extraction and analysis of nucleic acids for the purpose of characterizing the diversity of the 16S ribosomal (rRNA) gene. For community structure analysis, sample aliquots were fixed with paraformaldehyde (final concentration = 2–4% [v/v]), immobilized on polycarbonate filters (pore size = 0.2  $\mu\text{m}$ ), and then rinsed with 3 ml each of phosphate-buffered saline and distilled water. Air-dried filters were stored at  $-20^{\circ}\text{C}$  until analysis with FISH. Total count preservations were fixed with formalin (final concentration = 3% [v/v]) and stored at  $2^{\circ}\text{C}$  for up to 2 months before enumeration. Aliquots for Chl *a* were filtered (GF/F; Whatman) under dimmed lighting and frozen at  $-20^{\circ}\text{C}$  for later measurement using a standard protocol (Arar and Collins 1992). Additional aliquots for the determination of inorganic nutrients were fixed with  $\text{HgCl}_2$  (0.1% final concentration) and stored at  $4^{\circ}\text{C}$  until analysis with an autoanalyzer (Technicon System II) according to Kattner and Becker (1991).

Genomic DNA from pure cultures and environmental samples was extracted by means of a 3% cetyltrimethylammonium bromide procedure (Doyle and Doyle 1990) with additional proteinase K, lysozyme, and lysostaphin preincubations. The efficiency of the extractions of environmental samples was controlled by a microscopic examination of filters before and after the processing. Between 98% and 100% of the bacterial cells that were stained by 4'-diamidino-2-phenylindole (DAPI) were effectively lysed. An additional purification step using the WIZARD DNA Clean up System (Promega) was necessary to remove potential polymerase chain reaction (PCR) inhibitors that were coextracted from samples. Nearly full-length 16S rRNA gene sequences were amplified from isolates and environmental nucleic acid extracts ( $\sim 100$  ng) by hotstart PCR with an automated thermal cycler (Eppendorf) using the bacterial-specific primers 8*f* and 1542*r* (for isolates) and 8*f* and 1492*r* (for environmental samples). PCR products were purified using the QIAquick Purification Kit (Qiagen), and 16S rRNA gene clone libraries were constructed with the pGEM-T-Easy vector system (Promega). Diversity among the isolates and clone libraries was characterized with amplified ribosomal DNA (rDNA) restriction analysis (ARDRA; Massol-Deya et al. 1999) using two restriction endonucleases, *Hae*III and *Rsa*I (Promega). Coverage values (Mullins et al. 1995) that were  $>75\%$  indicated that most of the actual diversity was represented by our 16S rRNA clone libraries. A saturation of phylotypes calculated with rarefaction (Analytic Rarefaction 1.3: <http://www.uga.edu/~strata/software/index.html>) also indicated that further screening of clones with ARDRA would not reveal additional phylotypes.

Fig. 2. Phylogenetic tree representing bacterial diversity in Arctic pack ice melt pools. Isolates were obtained in 1997 during the RV *Polarstern* cruise leg ARKXIII/2, and 16S rRNA gene clones were recovered from a melt pool (ARKXV/1-11.3) sampled 2 yr later. The tree was reconstructed using maximum-likelihood (FastDNAmI) analysis. The scale bar indicates 10% estimated sequence divergence. Isolate strain ("ARK") and 16S rRNA gene clone ("ARKMP" and "ARKCRY") sequences are in bold type. Brackets indicate taxonomic groups and the target group for probe BET438.





Three potential chimeras, detected by the CHECK\_CHIMERA software of the Ribosomal Database Project (Maidak et al. 1999) and by an examination of canonical base pairing between the 5' and 3' ends of sequences using the software package ARB (<http://www.arb-home.de>), were excluded from the phylogenetic analyses. Sequence data were analyzed with ARB (database release from June 2002, 31,385 sequences), and phylogenetic trees were reconstructed using maximum-likelihood analysis. Only 16S rRNA sequences containing at least 1,200 bases were used for tree reconstruction. Alignment positions at which <30% (1,441 valid columns) of the sequences selected for initial tree reconstruction (154) had the same residues were excluded from the calculations to prevent uncertain alignments within highly variable positions of the 16S rRNA. Tree topologies were tested by performing distance matrix and parsimony analysis including different filter sets.

Total bacterial counts were determined with epifluorescence microscopy of acridine orange-stained cells (Hobbie et al. 1977). The standard set of oligonucleotide probes specific for domains and large taxonomic groups (NON338, EUB338, ALF968, BET42a, GAM42a, CF319a, HGC69a, PLA46, and ARCH915) were applied with FISH to examine the community structure of bacteria in melt pools. An additional probe, BET438 (5'...CCG TTT CTT CCC CGA CAA...3'), specific for a recurring  $\beta$ -proteobacterial phylotype ("MP-BetaI"), was developed according to the method of Stahl and Amann (1991). Hybridization specificity was achieved with the addition of a 20% formamide solution to the hybridization buffer. FISH analysis with CY3-labeled oligonucleotide probes (final concentration = 5 ng  $\mu\text{l}^{-1}$ ; Interactiva) was conducted according to Glöckner et al. (1996). All probe-specific cell counts are presented as the percentage of cells visualized by DAPI. The mean abundances and standard deviations were calculated from counts of 20–30 randomly chosen fields on each filter section (~500 probe-hybridized cells). The cell counts were corrected for background signal by subtracting counts obtained using the NON338 probe. Medians and 95% confidence intervals were determined from pooled data sets.

To test the temperature sensitivity of environmental melt pool samples (cruise ARKXV/1), aliquots were incubated at 1°C and 22°C for 24 h, and uptake of  $^3\text{H}$ -leucine (spec. activity 141 Ci mmol $^{-1}$ , Amersham) was measured by liquid scintillation methods (Kirchman 1993).

The almost full-length (>1,400 bases) 16S rRNA gene sequences generated in this study were deposited in GenBank under the following accession numbers: isolates ARK10150, AF468344; ARK10157, AF468345; ARK10162, AF468346; ARK10170, AF468347; ARK10172, AF468348; ARK10284, AF468349; ARK10299, AF468350; ARK10036, AF468358; ARK10037, AF468359; ARK10285, AF468379; ARK9994, AF468380; ARK9996, AF468381; ARK10038, AF468388; ARK10296, AF468403; ARK10159, AF468423; ARK10161, AF468424; ARK10164, AF468425; ARK10280, AF468433; ARK10287, AF468434; ARK10165, AF468439; ARK10173, AF468440; ARK10186, AF468441; ARK10190, AF468442; ARK10281, AF468446; ARK9995, AF468447; and ARK10167, AY227004, along with 16S

rRNA gene clones AF468322 to AF468342 and AY198105 to AY198112 and AY227003.

## Results and discussion

The diameter of the melt pools sampled during the ARKXV/1 and ARKXVI/2 cruises varied from 0.5 to 10 m, with most pools having depths of 25–30 cm. The physical parameters, inorganic nutrients, and Chl *a* varied only slightly from pool to pool (Table 1). The temperature in the melt pools ranged from 0.1°C to 0.4°C. The cryoconite hole, filled with sediments, was slightly warmer at 0.8°C. Salinity ranged from 0 to 3, and pH values were slightly acidic at 5.5, reflective of snow (Carstens 2001). The melt pools could be described as oligotrophic (Wetzel 2001) from the low inorganic N and P concentrations (rarely exceeding 0.1  $\mu\text{mol L}^{-1}$ ) and the Chl *a* concentrations that ranged from 0.01 to 0.52  $\mu\text{g L}^{-1}$ .

Sequencing of 28 pure cultures, isolated from 10 melt pools at four ice floe stations during the 1997 ARKXIII/2 cruise, showed 14 phylotypes dispersed among the  $\alpha$ -,  $\beta$ -, and  $\gamma$ -proteobacteria, the *Cytophaga-Flavobacterium* (C/F) group, and the gram-positive *Actinobacteria* spp. The closest relatives to the isolates were cultivated previously from sea ice, marine sediments, or terrestrial soils. Thirty-five percent of the isolates were identified as  $\beta$ -proteobacteria; however, diversity within this group was limited to only two phylotypes that clustered within the globally distributed Beta I cluster (Glöckner et al. 2000), also referred to as the Comamonadaceae clade (Zwart et al. 1998; Urbach et al. 2001). The first phylotype represented by ARK10281 is most closely related to *Rhodospirillum antarcticum* (98.7% identical). The second phylotype, most closely related to the *Aquaspirillum arcticum* (96.0–97.3% identical) that was isolated from arctic sediment covered by 1 m of snow and ice in Resolute, Northwest Territories, Canada (Butler et al. 1989), occurred frequently within the cultivatable bacterial fraction in the melt pools, prompting us to question if the abundance of this phylotype was an artifact of our cultivation methods. To answer this question, we went back to the Arctic Ocean 2 and 3 yr later and sampled several additional melt pools to examine the diversity of environmental 16S rRNA genes and the in situ composition of the bacterial communities.

ARDRA screening and sequencing of 16S rRNA gene clones ( $n = 83$ ) recovered from a sea ice melt pool, sampled in 1999 (ARKXV/1-11.3), also showed a predominance of  $\beta$ -proteobacteria (recognizable as "ARKMP" in Fig. 2). Diversity within the clone library appeared to be limited to only a few clades of previously cultivated bacteria. Two predominantly freshwater clusters (Beta I and CFB II), first characterized in Adirondack lakes (Hiorns et al. 1997) and recovered worldwide from freshwater ecosystems (Zwart et al. 1998; Glöckner et al. 2000), including an Arctic lake (Bahr et al. 1996), were detected by our clone library (Fig. 2). Surprisingly, 83.1% of the 16S rRNA gene clones were most closely related to the second dominant  $\beta$ -proteobacterial isolate phylotype and formed a stable subgroup within the Beta I cluster that we designated MP-BetaI. These clones were 98.5–99.3% identical to pure cultures within the same

Table 1. Physicochemical parameters and inorganic nutrients in melt pools, a cryoconite hole, and snow. Samples were collected in 1999 (RV *Polarstern* cruise leg "ARKXXV/1") and in 2000 (RV *Polarstern* cruise leg "ARKXXVI/2").

| Sample                    | Latitude/<br>longitude | Temp.<br>(°C) | Salinity | pH  | SiO <sub>4</sub><br>(µmol L <sup>-1</sup> ) | PO <sub>4</sub><br>(µmol L <sup>-1</sup> ) | NO <sub>2</sub><br>(µmol L <sup>-1</sup> ) | NO <sub>3</sub><br>(µmol L <sup>-1</sup> ) | Chl α<br>(µg L <sup>-1</sup> ) |
|---------------------------|------------------------|---------------|----------|-----|---------------------------------------------|--------------------------------------------|--------------------------------------------|--------------------------------------------|--------------------------------|
| Pool ARKXXV/1-I.1         | 79°50'N/2°57'E         | 0.1           | 0        | 5.5 | 0.35                                        | 0.02                                       | 0.06                                       | 0.01                                       | 0.01                           |
| Pool ARKXXV/1-I.2         | 79°50'N/2°57'E         | 0.1           | 0        | 5.5 | 0.41                                        | 0.03                                       | 0.03                                       | 0.03                                       | 0.11                           |
| Pool ARKXXV/1-I.3         | 79°50'N/2°57'E         | 0.1           | 0        | 5.5 | 0.16                                        | 0.05                                       | 0.02                                       | 0.07                                       | 0.16                           |
| Pool ARKXXV/1-I.6         | 79°50'N/2°57'E         | 0.1           | 0        | 5.5 | 0.53                                        | 0.05                                       | 0.03                                       | 0.02                                       | 0.34                           |
| Pool ARKXXV/1-I.7         | 79°50'N/2°57'E         | 0.1           | 0        | 5.5 | 0.43                                        | 0.04                                       | 0.03                                       | 0.13                                       | 0.23                           |
| Pool ARKXXV/1-I.8         | 79°50'N/2°57'E         | 0.1           | 0        | 5.5 | 0.24                                        | 0.01                                       | 0.03                                       | 0.02                                       | 0.26                           |
| Pool ARKXXV/1-II.1        | 75°20'N/14°23'W        | 0.1           | 0        | 5.5 | 1.13                                        | 0.05                                       | 0.02                                       | 0.07                                       | 0.30                           |
| Pool ARKXXV/1-II.2        | 75°20'N/14°23'W        | 0.1           | 0        | 5.5 | 0.02                                        | 0.04                                       | 0.02                                       | 0.01                                       | 0.12                           |
| Pool ARKXXV/1-II.3        | 75°20'N/14°23'W        | 0.1           | 0        | 5.5 | 0.01                                        | 0.05                                       | 0.02                                       | 0.11                                       | 0.11                           |
| Pool ARKXXV/2-H1.3        | 78°59'N/5°14'W         | 0.1           | 0        | 5.5 | 0.13                                        | 0.00                                       | 0.03                                       | 0.15                                       | 0.01                           |
| Pool ARKXXV/2-H1.6        | 78°59'N/5°14'W         | 0.1           | 0        | 5.5 | 0.01                                        | 0.01                                       | 0.01                                       | 0.05                                       | 0.01                           |
| Pool ARKXXV/2-H2.1        | 79°00'N/5°12'W         | 0.1           | 1        | 5.5 | 0.01                                        | 0.01                                       | 0.02                                       | 0.00                                       | 0.15                           |
| Pool ARKXXV/2-H2.2        | 79°00'N/5°12'W         | 0.1           | 1        | 5.5 | 0.01                                        | 0.03                                       | 0.02                                       | 0.51                                       | 0.16                           |
| Pool ARKXXV/2-H3.1        | 78°30'N/5°30'W         | 0.1           | 0        | 5.5 | 0.04                                        | 0.01                                       | 0.01                                       | 0.00                                       | 0.22                           |
| Pool ARKXXV/2-H3.2        | 78°30'N/5°30'W         | 0.1           | 0        | 5.5 | 0.01                                        | 0.01                                       | 0.01                                       | 0.00                                       | 0.16                           |
| Pool ARKXXV/2-H4.1        | 78°47'N/4°44'W         | 0.1           | 3        | 5.5 | 0.10                                        | 0.03                                       | 0.04                                       | 0.04                                       | 0.01                           |
| Pool ARKXXV/2-H4.2        | 78°47'N/4°44'W         | 0.4           | 3        | 5.5 | 0.01                                        | 0.04                                       | 0.02                                       | 0.04                                       | 0.01                           |
| Pool ARKXXV/2-III.2       | 79°18'N/13°36'W        | 0.1           | 0        | 5.5 | 0.01                                        | 0.02                                       | 0.02                                       | 0.00                                       | 0.06                           |
| Pool ARKXXV/2-III.4       | 79°18'N/13°36'W        | 0.1           | 0        | 5.5 | 0.01                                        | 0.01                                       | 0.02                                       | 0.00                                       | 0.03                           |
| Pool ARKXXV/2-IV.1        | 80°32'N/1°54'E         | 0.1           | 0        | 5.5 | 1.55                                        | 0.97                                       | 0.04                                       | 0.17                                       | 0.52                           |
| Cryoconite ARKXXV/1-I.cry | 79°50'N/2°57'E         | 0.8           | 0        | 5.5 | 0.01                                        | 0.01                                       | 0.02                                       | 0.01                                       | 0.05                           |

Table 2. Total cell counts and bacterial community composition in melt pools and a cryoconite hole on Arctic pack ice floes determined by FISH. Samples were collected in 1999 (RV *Polarstern* cruise leg "ARKXXV/1") and in 2000 (RV *Polarstern* cruise leg "ARKXXVI/2").

| Sample                     | Total cell counts<br>( $10^4$ cells $ml^{-1}$ )<br>[mean $\pm$ SD)] | Fraction (%) of total cells (mean $\pm$ SD) detected with probe* |           |            |            |           |            |            |     |         |  |
|----------------------------|---------------------------------------------------------------------|------------------------------------------------------------------|-----------|------------|------------|-----------|------------|------------|-----|---------|--|
|                            |                                                                     | EUB338                                                           | ALF968    | BET42a     | BET438     | GAM42a    | CF319a     | HGC69a     | PLA | ARCH915 |  |
| Pool ARKXXV/1-I.1†         | 6.63 $\pm$ 0.02                                                     | 86 $\pm$ 9                                                       | 3 $\pm$ 2 | 60 $\pm$ 6 | 43 $\pm$ 6 | 2 $\pm$ 1 | 13 $\pm$ 9 | 7 $\pm$ 2  | <1  | <1      |  |
| Pool ARKXXV/1-I.2†         | 9.85 $\pm$ 0.41                                                     | 83 $\pm$ 4                                                       | 3 $\pm$ 2 | 59 $\pm$ 9 | 48 $\pm$ 7 | 2 $\pm$ 1 | 10 $\pm$ 3 | 6 $\pm$ 4  | <1  | <1      |  |
| Pool ARKXXV/1-I.3          | 6.47 $\pm$ 0.03                                                     | 82 $\pm$ 9                                                       | 5 $\pm$ 2 | 47 $\pm$ 6 | 19 $\pm$ 4 | 5 $\pm$ 1 | 7 $\pm$ 3  | 14 $\pm$ 2 | <1  | <1      |  |
| Pool ARKXXV/1-I.6          | 5.91 $\pm$ 0.04                                                     | 78 $\pm$ 7                                                       | 5 $\pm$ 2 | 67 $\pm$ 2 | 46 $\pm$ 3 | <1        | 1 $\pm$ 1  | 6 $\pm$ 3  | <1  | <1      |  |
| Pool ARKXXV/1-I.7          | 5.66 $\pm$ 0.76                                                     | 72 $\pm$ 6                                                       | <1        | 57 $\pm$ 8 | 38 $\pm$ 5 | <1        | <1         | 8 $\pm$ 4  | <1  | <1      |  |
| Pool ARKXXV/1-I.8          | 6.65 $\pm$ 0.06                                                     | 78 $\pm$ 10                                                      | 1 $\pm$ 1 | 69 $\pm$ 7 | 43 $\pm$ 6 | <1        | 2 $\pm$ 1  | 6 $\pm$ 4  | <1  | <1      |  |
| Pool ARKXXV/1-II.1         | 8.84 $\pm$ 0.35                                                     | 83 $\pm$ 7                                                       | <1        | 74 $\pm$ 3 | 45 $\pm$ 5 | <1        | <1         | 5 $\pm$ 2  | <1  | <1      |  |
| Pool ARKXXV/1-II.2†        | 9.44 $\pm$ 0.05                                                     | 90 $\pm$ 4                                                       | 9 $\pm$ 3 | 51 $\pm$ 4 | 37 $\pm$ 3 | 3 $\pm$ 1 | 8 $\pm$ 2  | 9 $\pm$ 2  | <1  | <1      |  |
| Pool ARKXXV/1-II.3         | 9.87 $\pm$ 0.06                                                     | 88 $\pm$ 4                                                       | 1 $\pm$ 1 | 59 $\pm$ 6 | 41 $\pm$ 4 | 3 $\pm$ 1 | 8 $\pm$ 2  | 6 $\pm$ 1  | <1  | <1      |  |
| Pool ARKXXV/1-II.6         | 9.63 $\pm$ 0.50                                                     | 94 $\pm$ 3                                                       | 5 $\pm$ 3 | 50 $\pm$ 4 | 29 $\pm$ 6 | 6 $\pm$ 1 | 4 $\pm$ 2  | 14 $\pm$ 6 | <1  | <1      |  |
| Pool ARKXXV/1/2-H1.3       | 6.36 $\pm$ 0.06                                                     | 75 $\pm$ 9                                                       | <1        | 42 $\pm$ 3 | 22 $\pm$ 5 | <1        | 9 $\pm$ 2  | 18 $\pm$ 3 | <1  | <1      |  |
| Pool ARKXXV/1/2-H2.1†      | 7.85 $\pm$ 0.09                                                     | 79 $\pm$ 8                                                       | 8 $\pm$ 5 | 5 $\pm$ 4  | 1 $\pm$ 1  | <1        | 46 $\pm$ 8 | 5 $\pm$ 3  | <1  | <1      |  |
| Pool ARKXXV/1/2-H2.2†      | 3.71 $\pm$ 0.08                                                     | 65 $\pm$ 8                                                       | 3 $\pm$ 2 | 6 $\pm$ 3  | <1         | 5 $\pm$ 1 | 43 $\pm$ 7 | 6 $\pm$ 2  | <1  | <1      |  |
| Pool ARKXXV/1/2-H3.1       | 7.93 $\pm$ 0.09                                                     | 85 $\pm$ 4                                                       | 2 $\pm$ 1 | 45 $\pm$ 5 | 28 $\pm$ 6 | 7 $\pm$ 4 | 7 $\pm$ 3  | 7 $\pm$ 1  | <1  | <1      |  |
| Pool ARKXXV/1/2-H3.2       | 6.55 $\pm$ 0.26                                                     | 86 $\pm$ 4                                                       | 6 $\pm$ 2 | 61 $\pm$ 4 | 38 $\pm$ 4 | <1        | 9 $\pm$ 1  | 2 $\pm$ 1  | <1  | <1      |  |
| Pool ARKXXV/1/2-H4.1†      | 8.13 $\pm$ 0.31                                                     | 84 $\pm$ 5                                                       | <1        | 43 $\pm$ 2 | 26 $\pm$ 4 | <1        | 21 $\pm$ 6 | 20 $\pm$ 1 | <1  | <1      |  |
| Pool ARKXXV/1/2-H4.2‡      | 2.48 $\pm$ 0.06                                                     | 46 $\pm$ 5                                                       | <1        | 22 $\pm$ 3 | 16 $\pm$ 1 | 3 $\pm$ 1 | 12 $\pm$ 2 | <1         | <1  | <1      |  |
| Pool ARKXXV/1/2-III.2†     | 6.44 $\pm$ 0.04                                                     | 79 $\pm$ 7                                                       | <1        | 27 $\pm$ 6 | 15 $\pm$ 4 | 6 $\pm$ 2 | 33 $\pm$ 7 | <1         | <1  | <1      |  |
| Pool ARKXXV/1/2-III.4‡     | 4.26 $\pm$ 0.05                                                     | 56 $\pm$ 7                                                       | <1        | 17 $\pm$ 4 | 13 $\pm$ 6 | <1        | 8 $\pm$ 4  | <1         | <1  | <1      |  |
| Pool ARKXXV/1/2-IV.1†      | 7.64 $\pm$ 0.26                                                     | 91 $\pm$ 8                                                       | 2 $\pm$ 1 | 35 $\pm$ 6 | 19 $\pm$ 4 | 7 $\pm$ 1 | 19 $\pm$ 7 | 8 $\pm$ 1  | <1  | <1      |  |
| Cryoconite ARKXXV/1-I.cry† | 5.90 $\pm$ 0.20                                                     | 86 $\pm$ 6                                                       | 3 $\pm$ 1 | 3 $\pm$ 1  | 1 $\pm$ 1  | <1        | 73 $\pm$ 4 | 2 $\pm$ 1  | <1  | <1      |  |

\* Numbers have been corrected by subtracting NON338 counts.

† Sample contained sediment.

‡ Covered with a 2- to 3-cm ice layer.

MP-BetaI subgroup. Two other  $\beta$ -proteobacterial phylotypes within the Beta I cluster, ARKMP82/94 (98.4% identical to the Antarctic Canada Glacier bacterium [“candirty12”]) and ARKMP16/77 (97.2–98.1% identical to *Polaromonas vacuolata*), made up 6.0% of the clone library. The rest of the 16S rRNA gene clone phylotypes (11.8%) could be assigned to the  $\gamma$ -proteobacteria, the *Actinobacteria* spp., and the C/F group. All clones recovered from a sea ice cryoconite hole ( $n = 50$ ; “ARKCRY”) sampled during the same 1999 cruise were affiliated with *Hymenobacter* spp. (Fig. 2).

Total bacterial counts in the cryoconite hole (ARKXV/1-I.cry) and the snow sample (ARKXVI/2-H3.SA) were  $5.90$  and  $9.83 \times 10^4$  cells  $\text{ml}^{-1}$ . In the pools, total counts ranged from  $2.48$  to  $9.87 \times 10^4$  cells  $\text{ml}^{-1}$  (Table 2), which is slightly below the ranges reported previously for oligotrophic systems (Glöckner et al. 1999).

Using FISH methods with the EUB338 probe, between 46% and 94% (median = 82%; CI = 78–85%) of the bacteria from the pools ( $n = 20$ ) were detected (Table 2). Only 0.5% (median; CI = 0–3%) of nonspecifically stained cells hybridized with the probe NON338. No *Archaea* spp. were detected in the pools; however, nonspecific hybridization of probe ARCH915 with cells that also hybridized with the probe EUB338 and the probe CF319a (specific for the C/F group) was observed in the ARKXVI/2-IV.1 sample. Greater than 50% of DAPI-stained cells in most of the pools (median = 76%; CI = 69–79%) were further assignable with probes targeting larger phylogenetic groups specific for bacteria (Table 2). However, FISH-detection yields of DAPI-stained cells were lowest in two of the pools (ARKXVI/2-H4.2 and ARKXVI/2-III.4) that were beginning to freeze and already covered with a 3- to 5-cm layer of ice. Like other freshwater habitats (Méthé et al. 1998; Pernthaler et al. 1998; Glöckner et al. 1999),  $\beta$ -proteobacteria constituted the largest fraction of the bacterial community.  $\beta$ -Proteobacteria detected with probe BET42a were most abundant (median = 49%; CI = 37–59%) in all of the pools, with the exception of two sediment-laden samples in which the C/F group dominated. The  $\beta$ -proteobacterial phylotype MP-BetaI recurrent among our isolates and in the ARKMP clone library appeared to constitute most (from 20% up to 81%) of the  $\beta$ -proteobacterial fraction in the pools, corroborating the cultivation and clone data. Probe BET438, designed specifically for the MP-BetaI group, hybridized with 1–48% of DAPI-stained cells, having a median detection rate of 31% (CI = 19–38%). Less than 5% of the DAPI bacterial counts were identified as  $\alpha$ -proteobacteria (median = 2%; CI = 0.8–5%), and even fewer were identified as  $\gamma$ -proteobacteria (median = 2%; CI = 0.9–3%). Detection rates of the C/F group were highest in pools containing sediments (median = 9%; CI = 7–13%). The C/F group also dominated the bacterial fraction within the cryoconite hole (ARKXI/2-I.cry; Table 2), accounting for 73% of the DAPI bacterial count. Gram-positive *Actinobacteria* spp. were also abundant in many of the pools (median = 9%; CI = 7–13%), sometimes making up >10% of the bacterial fraction. *Planctomycetales*, which are typical and widespread members of freshwater and marine ecosystems (Glöckner et al. 1999), were also detected in two pools.

During cruise ARKXV/1, we measured the  $^3\text{H}$ -leucine uptake rates in samples from three melt pools (II.1, II.2, and

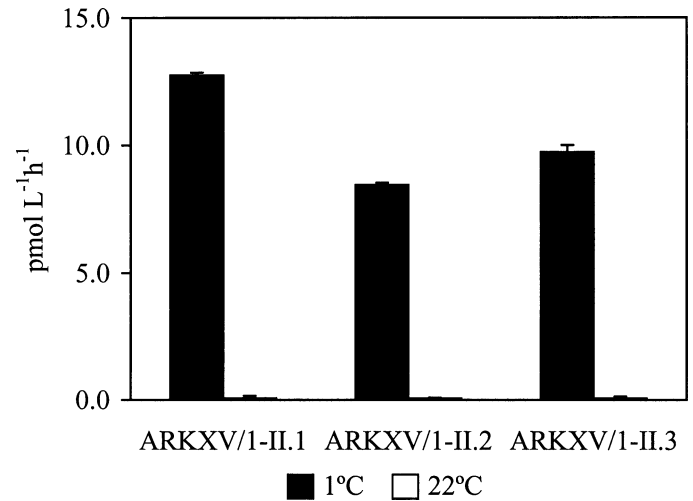


Fig. 3. Bacterial  $^3\text{H}$ -leucine uptake in melt pools ARKXV/1.11.1–11.3 that were incubated for 12 h at 1°C and 22°C.

II.3) that were incubated for 12 h at 1°C and 22°C (Fig. 3). Uptake rates ranged from 8.4 to 12.7 pmol  $^3\text{H}$ -leucine  $\text{L}^{-1} \text{h}^{-1}$  in samples incubated at 1°C but were markedly reduced to <0.07 pmol  $^3\text{H}$ -leucine  $\text{L}^{-1} \text{h}^{-1}$  at 22°C, suggesting a physiological adaptation for cold temperatures in the melt pool community. The 1°C rates are within the range observed among highly active bacterial communities in the ice and snow covers of high mountain lakes (Felip et al. 1995; Alfreider et al. 1996).  $\beta$ -Proteobacteria made up 51–74% of the DAPI bacterial counts in these melt pool samples, with >60% of these as the MP-BetaI subgroup. These activity data and the repeated detection of the MP-BetaI group over a span of 4 yr indicate that these bacteria are an important and stable member of the melt pool bacterial community.

The predominance of  $\beta$ -proteobacteria in the Arctic pack ice melt pools is consistent with the paradigm established by Méthé et al. (1998) that  $\beta$ -proteobacteria (excluding the marine ammonia-oxidizers) are physiologically predisposed to and characteristic of freshwater habitats. However, the MP-BetaI phylotype was detected in interior samples from some of the same pack ice floes having salinities as high as 50‰ (Brinkmeyer et al. 2003). FISH-determined abundances of  $\beta$ -proteobacteria in these samples were low (<10%), whereas  $\alpha$ - and  $\gamma$ -proteobacteria, dominant in marine habitats (Glöckner et al. 1999), accounted for ~75% of the EUB338-detectable fraction. Downward percolation of meltwater from the surface of ice floes and brine drainage, which dramatically reduces salinities in the upper meters of Arctic multiyear floes (Fetterer and Untersteiner 1998), could transport members of the melt pool community into the sea ice brine channel system, which would readily explain the presence of  $\beta$ -proteobacteria in interior samples. Alternatively, as suggested by Melnikov (1997), upward vertical displacement of the internal sea ice matrix exposes sea ice bacteria, microalgae, and even protozoans to the ice floe surface. The presence of interior sea ice phylotypes (*Octadecabacter arcticus*, *Marinobacter* IC022, and *Flavobacterium* sp. ACAM123) in the melt ponds lends support to the latter scenario. In either case, the distributional abundances of bac-



teria in Arctic pack ice floes also appear to be in line with those in the estuarine environment, where  $\beta$ -proteobacteria are most abundant in freshwater zones and  $\alpha$ - and  $\gamma$ -proteobacteria are predominant in more saline zones (Bouvier and del Giorgio 2002). It is remarkable that in sea ice, despite the semisolid structure and extreme cold temperatures, the distribution of bacteria reflects that observed in other aquatic environments.

Whether the MP-Beta1 bacteria are characteristic only of Arctic pack ice melt pools remains to be determined through broader 16S rRNA gene surveys of polar regions. However, their predominance in the melt pools stimulates the intriguing question of their adaptation or survival mechanisms for this extremely harsh environment.

## References

- ALFREIDER, A., J. PERNTHALER, R. AMANN, B. SATTLER, F. O. GLÖCKNER, A. WILLE, AND R. PSENNER. 1996. Appl. Environ. Microbiol. **62**: 2138–2144.
- AMON, R. M. W., H.-P. FITZNER, AND R. BENNER. 2001. Linkages among the bioreactivity, chemical composition, and diagenetic state of marine dissolved organic matter. *Limnol. Oceanogr.* **46**: 287–297.
- ARAR, E. J., AND G. B. COLLINS. 1992. Method 445.0. In vitro determination of chlorophyll a and pheophytin a in marine and freshwater phytoplankton by fluorescence. Version 1.1 Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency. Turner Designs.
- BAHR, M., J. E. HOBIE, AND M. L. SOGIN. 1996. Bacterial diversity in an arctic lake: A freshwater SAR11 cluster. *Aquat. Microb. Ecol.* **11**: 271–277.
- BOUVIER, T. C., AND P. A. DEL GIORGIO. 2002. Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. *Limnol. Oceanogr.* **47**: 453–470.
- BOWMAN, J. P., S. A. MCCAMMON, M. V. BROWN, D. S. NICHOLS, AND T. A. MCMEEKIN. 1997. Diversity and association of psychrophilic bacteria in Antarctic sea ice. *Appl. Environ. Microbiol.* **63**: 3068–3078.
- BRINKMEYER, R., K. KNITTEL, J. JÜRGENS, H. WEYLAND, R. AMANN, AND E. HELMKE. 2003. Diversity and community structure of bacterial communities in Arctic versus Antarctic sea ice. *Appl. Environ. Microbiol.* **69**: 6610–6619.
- BROWN, M. V., AND J. P. BOWMAN. 2001. A molecular phylogenetic survey of sea-ice microbial communities (SIMCO). *FEMS Microb. Ecol.* **35**: 267–275.
- BUTLER, B. J., K. L. MCCALLUM, AND W. E. INNISS. 1989. Characterization of *Aquaspirillum arcticum* sp. nov., a new psychrophilic bacterium. *Syst. Appl. Microbiol.* **12**: 263–266.
- CARSTENS, M. 2001. Zur Ökologie von Schmelzwassertümpeln auf arktischem Meeres-Charakteristika, saisonale Dynamik und Vergleich mit anderen aquatischen Lebensräumen polarer Regionen. Ph.D. thesis, Christian Albrecht Univ.
- DOYLE, J. J., AND J. L. DOYLE. 1990. Isolation of plant DNA from fresh tissue. *Focus* **12**: 13–15.
- FELIP, M., B. SATTLER, R. PSENNER, AND J. CATALAN. 1995. Highly active microbial communities in the ice and snow cover of high mountain lakes. *Appl. Environ. Microbiol.* **61**: 2394–2401.
- FETTERER, F., AND N. UNTERSTEINER. 1998. Observations of melt ponds on Arctic sea ice. *J. Geophys. Res.* **103**: 24821–24835.
- GIBSON, J. A. E., W. F. VINCENT, B. NIEKE, AND R. PIENITZ. 2001. Control of biological exposure to UV radiation in the Arctic Ocean: Comparison of the roles of ozone and riverine dissolved organic matter. *Arctic* **53**: 372–382.
- GLÖCKNER, F. O., R. AMANN, A. ALFREIDER, J. PERNTHALER, R. PSENNER, K. TREBESLUS, AND K.-H. SCHLEIFER. 1996. An *in situ* hybridization protocol for detection and identification of planktonic bacteria. *Syst. Appl. Microbiol.* **19**: 403–406.
- , B. M. FUCHS, AND R. AMANN. 1999. Bacterioplankton compositions of lakes and oceans: A first comparison based on fluorescence *in situ* hybridization. *Appl. Environ. Microbiol.* **65**: 3721–3726.
- , E. ZAICHKOV, N. BELKOVA, L. DENISSOVA, J. PERNTHALER, A. PERNTHALER, AND R. AMANN. 2000. Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of *Actinobacteria*. *Appl. Environ. Microbiol.* **66**: 5053–5065.
- GROSSMANN, S., AND G. DIECKMANN. 1994. Bacterial standing stock, activity, and carbon production during formation and growth of sea ice in the Weddell Sea, Antarctica. *Appl. Environ. Microbiol.* **60**: 2746–2753.
- HELMKE, E., AND H. WEYLAND. 1995. Bacteria in sea ice and underlying water of the eastern Weddell Sea in midwinter. *Mar. Ecol. Prog. Ser.* **117**: 269–287.
- HIORNS, W. D., B. A. METHÉ, S. A. NIERZWICKI-BAUER, AND J. P. ZEHR. 1997. Bacterial diversity in Adirondack mountain lakes as revealed by 16S rRNA gene sequences. *Appl. Environ. Microbiol.* **63**: 2957–2960.
- HOBIE, J. E., R. J. DALEY, AND S. JASPER. 1977. Use of Nucleopore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.* **33**: 1225–1228.
- JUNGE, K., F. IMHOFF, J. T. STALEY, AND J. W. DEMING. 2002. Phylogenetic diversity of numerically important Arctic sea-ice bacteria cultured at subzero temperature. *Microb. Ecol.* **43**: 315–328.
- KATTNER, G., AND H. BECKER. 1991. Nutrients and organic nitrogenous compounds in the marginal ice zone of the Fram Strait. *J. Mar. Syst.* **2**: 385–394.
- KIRCHMAN, D. L. 1993. Leucine incorporation as a measure of biomass production by heterotrophic bacteria, p. 509–512. *In* P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole [eds.], *Handbook of methods in aquatic microbial ecology*. Lewis.
- MAIDAK, B. L., AND OTHERS. 1999. A new version of the RDP (Ribosomal Database Project). *Nucleic Acids Res.* **27**: 171–173.
- MASSOL-DEYA, A. A., D. A. ODELSON, R. F. HICKEY, AND J. M. TIEDJE. 1999. Bacterial community fingerprinting of amplified 16S and 23S ribosomal DNA gene sequences and restriction endonuclease analysis (ARDRA), p. 3.3.2/1–8. *In* A. D. L. Akkermans, J. D. Van Elsas, and F. J. De Bruijn [eds.], *Molecular microbial ecology manual*. Kluwer.
- MELNIKOV, I. A. 1997. The Arctic sea ice ecosystem. Gordon and Breach Science.
- METHÉ, B. A., W. D. HIORNS, AND J. P. ZEHR. 1998. Contrasts between marine and freshwater bacterial community composition: Analyses of communities in Lake George and six other Adirondack lakes. *Limnol. Oceanogr.* **43**: 368–374.
- MULLINS, T. D., T. B. BRITSHGI, R. L. KREST, AND S. J. GIOVANNONI. 1995. Genetic comparisons reveal the same unknown bacterial lineages in Atlantic and Pacific bacterioplankton communities. *Limnol. Oceanogr.* **40**: 148–158.
- PERNTHALER, J., F. O. GLÖCKNER, S. UNTERHOLZNER, A. ALFREIDER, R. PSENNER, AND R. AMANN. 1998. Seasonal community and population dynamics of pelagic *Bacteria* and *Archaea* in a high mountain lake. *Appl. Environ. Microbiol.* **64**: 4299–4306.
- SMITH, R. E., AND P. CLEMENT. 1990. Heterotrophic activity and



- bacterial productivity in assemblages of microbes from sea ice in the high Arctic. *Polar Biol.* **10**: 351–357.
- STAHL, D. A., AND R. AMANN. 1991. Development and application of nucleic acid probes, p. 205–248. *In* E. Stackebrandt and M. Goodfellow [eds.], *Nucleic acid techniques in bacterial systematics*. Wiley.
- STALEY, J. T., AND J. J. GOSINK. 1999. Poles apart: Biodiversity and biogeography of sea ice bacteria. *Annu. Rev. Microbiol.* **53**: 189–215.
- URBACH, E., K. L. VERGIN, L. YOUNG, A. MORSE, G. L. LARSON, AND S. J. GIOVANNONI. 2001. Unusual bacterioplankton community structure in ultra-oligotrophic Crater Lake. *Limnol. Oceanogr.* **46**: 557–572.
- WETZEL, R. G. 2001. *Limnology: Lake and river ecosystems*, 3rd ed. Academic.
- WICKHAM, S., AND M. CARSTENS. 1998. Effects of ultraviolet-B radiation on two arctic microbial food webs. *Aquat. Microb. Ecol.* **16**: 163–171.
- ZWART, G., AND OTHERS. 1998. Nearly identical 16S rRNA sequences recovered from lakes in North America and Europe indicate the existence of clades of globally distributed freshwater bacteria. *System Appl. Microbiol.* **21**: 546–556.

*Received: 21 May 2003*  
*Accepted: 9 December 2003*  
*Amended: 11 February 2004*