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Seasonal and Spatial Variability of Bacterial and Archaeal Assemblages in the Coastal Waters near Anvers Island, Antarctica

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A previous report of high levels of members of the domain *Archaea* in Antarctic coastal waters prompted us to investigate the ecology of Antarctic planktonic prokaryotes. rRNA hybridization techniques and denaturing gradient gel electrophoresis (DGGE) analysis of the bacterial V3 region were used to study variation in Antarctic picoplankton assemblages. In Anvers Island nearshore waters during late winter to early spring, the amounts of archaeal rRNA ranged from 17.1 to 3.6% of the total picoplankton rRNA in 1996 and from 16.0 to 1.0% of the total rRNA in 1995. Offshore in the Palmer Basin, the levels of archaeal rRNA throughout the water column were higher (average, 24% of the total rRNA) during the same period in 1996. The archaeal rRNA levels in nearshore waters followed a highly seasonal pattern and markedly decreased during the austral summer at two stations. There was a significant negative correlation between archaeal rRNA levels and phytoplankton levels (as inferred from chlorophyll *a* concentrations) in nearshore surface waters during the early spring of 1995 and during an 8-month period in 1996 and 1997. In situ hybridization experiments revealed that 5 to 14% of DAPI (4′,6-diamidino-2-phenylindole)-stained cells were archaeal, corresponding to 0.9×10^4 to 2.7×10^4 archaeal cells per ml, in late winter 1996 samples. Analysis of bacterial ribosomal DNA fragments by DGGE revealed that the assemblage composition may reflect changes in water column stability, depth, or season. The data indicate that changes in Antarctic seasons are accompanied by significant shifts in the species composition of bacterioplankton assemblages and by large decreases in the relative proportion of archaeal rRNA in the nearshore water column.

Until recently (2, 9, 33), most research on bacterioplankton in Antarctic seas has centered on integrating the bulk properties of entire assemblages, as opposed to the compositions and variabilities of the assemblages. Previous studies have focused on biomass determinations (1, 5), productivity and activity measurements (3, 28), and the coupling of primary production and secondary production (27). Antarctic Peninsula waters have long been recognized as biologically productive and important, and this recognition has resulted in the establishment of a long-term ecological research site at Palmer Station (48). This region is sensitive to climate change and variations in the extent and duration of seasonal sea ice. Changes in zooplankton (salps are present in high numbers during low-ice years [47]) and in phytoplankton (compositional shifts from diatom- to chrysophyte-dominated assemblages [35]) have been observed with temperature changes of less than 1°C. Little is known about the effect that seasonal changes have on the bacterioplankton community, although it is evident that the bacterioplankton represent the most significant amount of biomass in this region (17) and that the roles of these organisms in biogeochemical cycling of carbon, nitrogen, and sulfur are critical to the functioning of the Antarctic ecosystem.

The waters of the Antarctic Peninsula are characterized by extremely wide variations in sea ice cover and solar irradiation. There are concurrent seasonal peaks in photosynthetic biomass and production (primarily diatoms and prymnesiophytes [26, 35]) and associated grazer populations (predominantly krill [47]). Bacterial biomass has been reported to fluctuate with the season (6, 22) and to remain constant in the midst of variations in primary production (1, 27). Significant turnover in the community compositions of planktonic assemblages due to the variable nature of the environment may also occur. Moline (34) and Moline and Prezelin (35) investigated the phytoplankton assemblage composition of the Palmer Station region and its relationship to water column stability and sea ice cover. These authors observed a predictable successional pattern in the phytoplankton community between early spring and summer. Similar investigations of bacterioplankton dynamics have been limited to studies of bacterial abundance (4) since it has been difficult to assess assemblage dynamics and composition due to the limitations of cultivability and species identification. However, molecular ecological approaches have made detection of compositional changes and variability in prokaryotic assemblages feasible.

A recent question in Antarctic microbiology involves the presence and significance of planktonic archaea, which have been reported to constitute significant fractions of Antarctic picoplankton assemblages (9). Planktonic archaea were originally detected in marine environments by using rRNA hybridization and cloning and sequencing approaches (8, 18). The planktonic archaea appear to be abundant, as judged by their rRNA and DNA distributions, are vertically stratified in the Santa Barbara Channel (31, 38), and are distributed in the deep sea in both the Atlantic and Pacific basins (19). The Antarctic ecosystem provides a unique study site for planktonic archaea because of its extremely low temperatures and dramatic seasonal variation.

The goals of this study were (i) to obtain more detailed information concerning planktonic archaeal assemblages with respect to their abundance and temporal and spatial distributions and (ii) to assess bacterial diversity and variability in relation to hydrographic and biological parameters. Microscopic enumeration of picoplankton constituents, chlorophyll *a*

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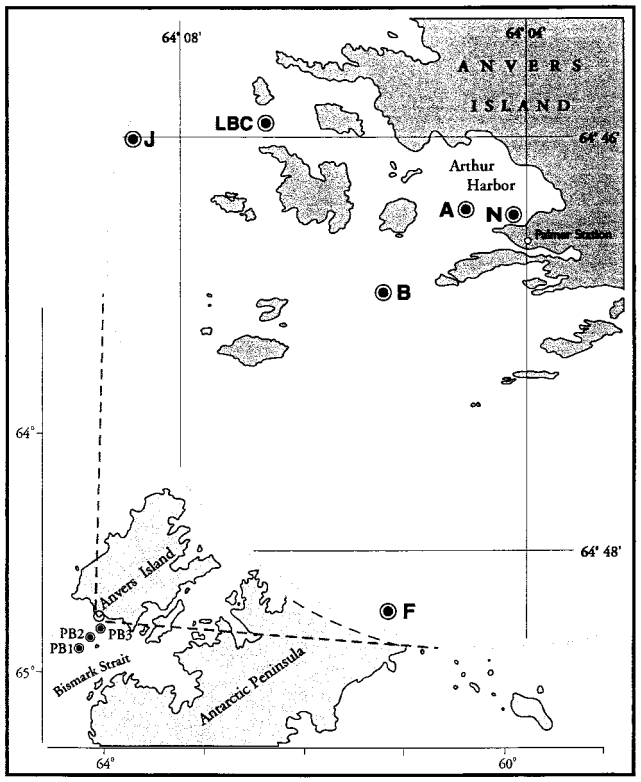


FIG. 1. Sampling stations in the Anvers Island coastal region of the Antarctic Peninsula.

determinations, and hydrographic measurements were used in combination with rRNA-targeted oligonucleotide hybridization to whole cells and nucleic acid extracts (37, 50) and denaturing gradient gel electrophoretic (DGGE) analysis of PCR-amplified ribosomal DNA (rDNA) fragments (14, 37, 39, 52). In this paper we present the data from a study of the coastal and offshore regions of Anvers Island. A parallel study of Gerlache Strait waters was performed simultaneously in 1996 (33).

MATERIALS AND METHODS

Sample collection and processing. Coastal waters off Anvers Island in the Antarctic Peninsula region were sampled for 2 consecutive years (1995 and 1996) in the late austral winter and early spring. Additional samples were collected between November 1996 and April 1997 at station B and between November 1996 and September 1997 at station N. Seawater samples were collected from the nearshore waters of Arthur Harbor (stations A, B, LBC, and N), the coastal waters of the Bismark Strait (stations F and J), and deeper offshore waters of the Palmer Basin (stations PB1, PB2, and PB3) (Fig. 1). Table 1 shows the sample collection schedule and associated biological and physical data for most stations. Samples were obtained at all of the Palmer Basin stations on 6 and 29 September 1996 and at station PB1 on 26 October 1996. The majority of the seawater samples were collected with subsurface pumps rigged with 0.5-in-diameter polyvinyl chloride tubing and rechargeable 12-V direct-current batteries. The Palmer Basin and station B samples obtained between November and April were collected by using Go Flo bottles (General Oceanics). Station N seawater samples were collected from the Palmer Station seawater intake system (the intake was located 100 m offshore at a depth of approximately 20 m). Extensive sea ice coverage prevented boating in the 1995 season and limited sampling to stations that could be reliably and safely reached. All 1995 samples were collected from the sea ice surface through holes drilled in the ice. Seawater samples (50-liter carboys) were hauled back to Palmer Station on sleds by cross-country skiing prior to filtration. Large volumes (20 to 100 liters) were collected from depths of 3 and 40 m (or 50 m in 1996) at most sites. The sea ice during the 1996 season was not as extensive as the sea ice during the 1995 season, and occasional open-water conditions allowed samples to be collected with Zodiac Mark V boats.

Most of the seawater samples collected were filtered in environmental rooms (temperature, −1.5 to −0.5°C) at Palmer Station; the exceptions were samples collected in the Palmer Basin, which were processed on board the RV *Polar Duke*. Seawater was processed in two ways. (i) In 1995, cells were collected by pressure filtration (<5 lb/in^2). The cells were prefiltered through 142-mm-diameter, 0.8-μm-pore-size membrane filters (Supor [Gelman]) and were collected on 0.2-μm-pore-size filters (diameter, 142 mm; Supor [Gelman] or Durapore, [Millipore]). These filters were folded in half and frozen at −70°C until nucleic acid extraction. (ii) In both 1995 and 1996, cells were collected by peristaltic pumping (49). Seawater was prefiltered through 47-mm-diameter type GF/A filters (nominal pore size, 1.6 μm; Whatman) to screen out larger eukaryotes and particulate matter, and the <1.6-μm fraction was collected with 0.22-μm-pore-size Sterivex filters (Durapore [Millipore]). The filter units were filled with 1.8 ml of lysis buffer (40 mM EDTA, 50 mM Tris-HCl, 0.75 M sucrose), sealed, and frozen at −70°C until nucleic acid extraction.

Supplementary biological and physical data (i.e., picoplankton abundance, temperature, and salinity) were collected when possible. Seawater subsamples were preserved at 4°C in 1% (final concentration) glutaraldehyde and 3.7% (final concentration) formaldehyde for enumeration and in situ hybridization, respectively. Prokaryotic cell counts were determined by filtering 5 to 15 ml of seawater onto 0.2-μm-pore-size black polycarbonate filters (Poretics Corp.), staining the filters with DAPI (4′,6-diamidino-2-phenylindole) (5 μg/ml), and enumerating prokaryotes by epifluorescence microscopy (44) for almost all samples. Typically, 10 fields containing more than 30 cells per field were counted. Heterotrophic nanoflagellates and photoautotrophic nanoflagellate (detected under blue light irradiation) counts were determined for a limited number of samples by staining with DAPI and counting by epifluorescence microscopy. Chlorophyll *a* concentrations were determined for most samples in order to estimate phytoplankton levels. Samples (0.2 to 0.5 liter) were collected on 25-mm-diameter type GF/F filters (Whatman) in 1995 and on 25-mm-diameter, 0.45-μm-pore-size nylon filters (Cole-Parmer) in 1996. Chlorophyll *a* was extracted in 90% acetone in the dark for at least 24 h at −20°C, and fluorescence was determined with a Turner model 10-AU digital fluorometer calibrated with chlorophyll *a* (Sigma) by using standard procedures (41).

Hydrographic measurements were recorded with a conductivity temperature, and depth sensor (Surveyor 3; Hydrolab Instruments) at selected nearshore stations and were recorded at all of the offshore stations in the Palmer Basin with a Seabird SBE-9 profiler. In addition, the salinities of the nearshore samples were determined with a Guidine model 8410 salinometer calibrated with standard seawater.

Nucleic acid extraction. The extraction procedure used for all Sterivex-filtered samples was a modified version of the procedure described by Sommerville et al. (49). Freshly prepared lysozyme (1 mg/ml) was added to filter units containing lysis buffer, and the units were incubated at 37°C for 30 min. Then 1% sodium dodecyl sulfate (SDS) and freshly prepared proteinase K (0.5 mg/ml) were added to the filter units, and they were incubated at 55°C for 2 h. Lysates were removed from the filter units with sterile 3-ml syringes, and the filter units were each rinsed with 1 ml of lysis buffer and incubated for 15 min. The rinse buffer and lysates were pooled. Crude lysates were extracted once with phenol-chloroform-isoamyl alcohol (25:24:1, pH 8.0) and once with chloroform-isoamyl alcohol (24:1). The nucleic acids in the aqueous phase were concentrated and washed with TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) in microconcentrators (Centricon 100; Amicon), and the preparations were reduced to final volumes of 100 to 200 μl. For nucleic acid extraction of 142-mm-diameter filters a similar protocol was used. Frozen filters were broken into small pieces, and the cells were lysed and extracted as described above except that they were in 15-ml sterile conical tubes. The DNA concentration of each sample was fluorometrically quantified by the Hoechst dye assay (as modified by Hoeffer Scientific [42]) with a fluorometer (model TKO; Hoeffer Scientific). The extraction yields were in the range reported previously in other studies, and the average yield was 0.37 μg of DNA per liter of seawater (n = 121).

Quantitative oligonucleotide hybridization. The relative levels of eucaryal, bacterial, and archaeal rRNAs were determined by oligonucleotide probe hybridization as described previously (31). Nucleic acids were first immobilized on nylon membranes (Hybond-N; Amersham) by using a slot blot apparatus. Each membrane contained three or four unknowns in a six- to eightfold dilution series, starting with 50 ng in the first dilution. Control rRNAs were applied in a fourfold dilution series. Six to seven replicate blots were prepared, and each blot was hybridized with a different probe. The oligonucleotide probe database designations and sequences of the probes used in this study are as follows: S-*-Univ-1390-a-A-18 (GAC GGG CGG TGT GTA CAA), S-*-Univ-1392-a-A-18 (ACG GGC GGT GTG TRC), S-D-Euk-1209-a-A-16 (GGG CAT CAC AGA CCT G), S-D-Arch-0915-a-A-20 (GTG CTC CCC CGC CAA TTC CT), S-D-Bact-0338-a-A-18 (GCT GCC TCC CGT AGG AGT), S-O-Cenar-0554-a-A-20 (TTA GGC CCA ATA ATC MTC CT), and S-O-ArGII-0554-a-A-20 (TTA GGC CCA ATA AAA KCG AC). The following rRNA and rDNA controls were used: *Saccharomyces cerevisiae*, *Escherichia coli* or *Shewanella putrefaciens*, *Haloferax volcanii*, and *Cenarchaeum symbiosum* (a sponge-associated group I archaeon [45]). Plasmids containing crenarchaeaotal group I or euryarchaeaotal group II 16S rDNA gene fragments were included in most experiments (n = 41). For the station B and station N temporal series, rRNA transcripts of the group I and II archaea were used for all samples collected after November 1996. The rRNA transcripts

TABLE 1. Summary of sample collection dates and related biological and hydrographic data

Station	Date (day-mo-yr)	Prokaryote concn (10^5 cells/ml)		Chlorophyll *a* concn (μg/liter)		Salinity (ppt)		Temp (°C)	
		Surface sample[a]	Deep sample	Surface sample	Deep sample	Surface sample	Deep sample	Surface sample	Deep sample
A	22-Sep.-95	1.54		0.10					
	28-Sep.-95	1.62		0.11		33.953			
	5-Oct.-95	1.79	1.73	0.15	0.07	33.827	34.072		
	13-Oct.-95	1.54		0.32		33.621		−1.28	−1.43
	24-Oct.-95	2.17	1.90	0.43	0.33	33.663	33.901		
	1-Nov.-95			0.60					
	4-Sep.-96	1.77	1.88	0.13	0.13				
	8-Sep.-96	1.57						−1.82	−1.84
	13-Sep.-96	1.70	1.50	0.15	0.19			−1.84	−1.89
	19-Sep.-96	2.13	1.88	0.18	0.18			−1.95	−1.95
	29-Sep.-96	1.05				33.840			
	5-Oct.-96	2.19	2.05	0.19	0.19	33.819			
	16-Oct.-96	1.92		0.19		33.840		−1.89	−1.77
LBC	1-Oct.-95	1.80	1.67	0.22	0.05	33.817	34.058		
	18-Oct.-95	1.94	1.73	0.46	0.09	33.606	34.090	−1.59	−1.03
	25-Oct.-95	2.45	2.09	0.63	0.11				
	30-Oct.-95	2.00	1.55	0.70	0.08	33.517	34.157	−1.64	−0.72
	4-Sep.-96	1.74	2.07	0.14	0.14				
	13-Sep.-96	1.81	1.92	0.18	0.26			−1.80	−1.87
	5-Oct.-96	1.96	2.19	0.23	0.23				
	16-Oct.-96	1.94	1.77	0.22	0.16	33.796	33.968	−1.88	−1.92
B	19-Aug.-96								
	13-Sep.-96	1.72	1.88	0.21	0.19				
	16-Oct.-96	2.05	1.89	0.19	0.11	33.840	33.996	−1.86	−1.77
	30-Nov.-96								
	28-Dec.-96			0.77	0.16				
	18-Jan.-97			0.95	0.44				
	4-Feb.-97								
	19-Feb.-97			1.12	0.23				
	28-Feb.-97			1.54					
	14-Mar.-97								
	3-Apr.-97								
F	13-Sep.-96	1.45	1.64	0.19	0.20			−1.93	−1.98
	17-Oct.-96	1.38	1.40	0.19	0.11	33.741	34.032		
J	13-Sep.-96	1.72	1.94	0.21	0.19			−2.00	−1.96
	17-Oct.-96	1.57	1.51	0.20	0.12	33.759	33.960	−1.79	−1.52

[a] Surface samples were collected at a depth of 3 m, and deep samples were collected at a depth of 40 m in 1995 and at a depth of 50 m in 1996.

were prepared from environmental 16S rRNA clones (33) SB95-57 (group I) and SB95-77 (group II) as described by Poltz and Cavanaugh (43). Environmental samples and rRNA controls were denatured with 0.5% glutaraldehyde–50 mM Na_2PO_4 for 10 min at room temperature. Plasmid controls were denatured with 0.5 N NaOH–1.5 N NaCl or were boiled for 10 min in 15 μl of water and placed directly on ice. Nucleic acids were UV cross-linked to the membranes (Stratalinker; Stratagene) and then prehybridized at 45°C for 30 min in 10 ml of hybridization buffer (0.9 M NaCl, 50 mM Na_2PO_4, 5 mM EDTA, 0.5% SDS, 10× Denhardt's solution, 0.5 mg of polyadenosine per ml). 16S rRNA probes end labeled with ^{32}P were added after prehybridization. Hybridizations were performed overnight at 45°C, and then the blots were washed first in 1× SET–1% SDS buffer (1× SET contains 150 mM NaCl, 20 mM Tris-HCl [pH 7.8] and 2 mM disodium EDTA) for 30 min at room temperature and then in prewarmed wash buffer for 30 min at the high-stringency wash temperature for each probe (31). The hybridization signal of each dried membrane was quantified with a radioanalytic gas proportional counter (Ambis; Scanalytics).

Data processing. The relative contributions of eucaryal, bacterial, and archaeal rRNAs to each picoplankton sample were determined by calculating a slope (counts per minute of probe bound per unit of rRNA) for each domain-specific probe. The slopes were multiplied by a probe-specific correction factor (determined by dividing the slope for the universal probe bound to control rRNA by the slope for the domain-specific probe bound to the same control rRNA) to correct for any differences in probe binding efficiency and specific activity. The hybridization signal (HS) was defined as the corrected domain-specific slope divided by the slope of the universal probe bound to the same sample. HS values are expressed below as percentages.

The archaeal group-specific rRNA signal (for groups I and II) was calculated in the manner described above, but the correction factor was generated from the slope for the archaeal probe bound to the group rDNA or rRNA transcript divided by the slope for the group-specific probe bound to the same template. The group-specific HS is reported below as a percentage of the archaeal slope (HS_{arch}) for the group I probe.

Universal probe 1392 was used in all 1995 experiments. Both universal probe 1392 and universal probe 1390 were used in analyses of all 1996 samples, since in a recent report Zheng et al. (55) suggested that universal probe 1392 may overestimate archaeal abundance. For simplicity, the universal probes to which the data were normalized are indicated by subscripts; the HS obtained with universal probes 1390 and 1392 are designated HS_{1390} and HS_{1392}, respectively.

In situ hybridization. In situ hybridization experiments were performed by filtering 10-ml aliquots of formalin-fixed seawater samples onto 25-mm-diameter, 0.22-μm-pore-size polycarbonate filters. Teflon-coated slides with 7-mm wells (Cell Line Associates, Inc.) were coated with a gelatin solution [0.1% gelatin, 0.01% $KCr(SO_4)_2$]. Three microliters of sterile water was added to each well of each slide. Filters were placed upside down over each well and removed once they had dried (23). The slides were then dehydrated in an ethanol series (50, 75, and 100% ethanol, 2 min each). The hybridization procedure, wash conditions, and probe specificity have been described previously by Preston et al. (45). The slides were then counterstained with DAPI (1 μg/ml) for 5 min at room temperature, washed for 10 min at room temperature in 1× SET, rinsed in water, and air dried. The slides were mounted in Citifluor AF1 and viewed by epifluorescence microscopy (Zeiss standard 25 microscope). Between 5 and 10 fields per well and between one and four wells were counted in each experiment. For each field, counts were obtained with tetramethyl rhodamine isocyanate, fluorescein isothiocyanate (FITC), and DAPI filter sets.

Hybridizations were performed with the following probe mixtures: a mixture of four Texas Red (Molecular Probes)-labeled oligonucleotide probes specific for the group I archaea, designated TR-GI-4-mix (S-O-Cenar-0131-a-A-20 [TCC CGT CCA TAG GTT AGG]; S-O-Cenar-0538-a-A-20 [TCC TGA CCA CTT GAG GTG], S-O-Cenar-0554-a-A-20, and S-O-Cenar-0655-a-A-20 [GTA CCG TCT ACY TCT CCC ACT CC]) (31, 45); and a mixture of two FITC-labeled

oligonucleotide probes specific for bacteria, designated FITC-bact-2-mix (S-D-Bact-0338-a-A-18 [50] and S-D-Bact-0927-a-A-17 [20]). In several experiments, we included a negative control in which a 50-fold excess (250 ng/μl) of an unlabeled marine crenarchaeotal probe mixture was added to TR-GI-4-mix at its standard concentration (5 ng/μl). Experiments were conducted simultaneously with both TR-GI-4-mix and FITC-bact-2-mix, which tested for specificity and potential cross-reactivity.

PCR-DGGE. PCR-DGGE was used to assess the variation in bacterial planktonic assemblages. The method used has been described in detail previously (36, 37). Briefly, 10 to 20 ng of nucleic acid extract per 100-μl PCR mixture was amplified with primers GC358f and 517r (37), which are specific to the V3 domain of the *Bacteria*. The PCR conditions were the same as those described previously (37), except that (i) *Taq* DNA polymerase (Fisher-Biotech) was used, (ii) the reaction buffer contained 50 mM KCl, 10 mM Tris (pH 8.3), 2 mM $MgCl_2$ 0.01% gelatin, and 0.05% Nonidet P-40, and (iii) 26 cycles were used for amplification. Blank controls were included with each set of PCR mixtures. All samples and controls were evaluated by agarose gel electrophoresis after amplification.

DGGE ribotype profile analysis was performed with 8% polyacrylamide gels with a 40 to 70% denaturing gradient as previously described (36). PCR mixtures were precipitated with ethanol and resuspended in 8 μl of sterile water, 1 μl of which was quantified with the fluorometer by the Hoechst dye method referred to above. Attempts were made to use equal amounts (approximately 500 ng) of amplified product on each gel in order to allow careful discrimination of variation between samples. Bacterial ribotype profile analysis gels were electrophoresed for 15 h at 75 V. Gels were stained by using ethidium bromide and 1× TAE (40 mM Tris base, 20 mM sodium acetate, 1 mM EDTA) for 10 to 15 min and then destained for 20 to 30 min in water. Gels were documented by Polaroid photography. Polaroid photographs were scanned, and the gels were analyzed by using Quantity One (PDI) software. The numbers of bands (ribotypes) per lane were determined, the ribotypes were compared across all lanes, and common ribotypes were determined. Pairwise similarity values were calculated by using Sorenson's index: $C_s = 2j/(a + b)$, where j is the number of common ribotypes in samples A and B, a is the number of ribotypes in sample A, and b is the number of ribotypes in sample B (30, 37). A similarity value of 1 indicates that ribotype profiles (band patterns) are identical, and a similarity value of 0 indicates that no bands are shared. The similarity values are numerical representations of pairwise levels of similarity in band patterns. It should be noted that there is not necessarily a one-to-one correspondence between the number of bands and the number of unique sequences or bacterial species. Pairwise similarity matrices were constructed for all gels in order to make comparisons between samples.

RESULTS

Nearshore early spring conditions in 1995 and 1996. Sea ice and ice melt conditions in 1995 contributed to stabilization of the water column, which allowed the water column to become stratified at station LBC by the end of the sampling period (late October) (Table 1). The 1996 field season (1 August to 26 October) was quite different. Unstable ice conditions prevented collection of samples until 4 September. The average monthly wind speeds in September and October were higher in 1996 (16 and 14 knots, respectively) than in 1995 (13 and 11 knots, respectively), and the sea ice conditions were more variable in 1996 than in 1995. Between most sample dates in 1996 the region was covered by broken pack ice. Hydrographically and biologically, the water column was well-mixed throughout the 1996 late winter-early spring sampling season.

Bacterioplankton levels did not vary significantly between or within stations in either year (Table 1). The numbers of organisms ranged from 1.5×10^5 to 2.2×10^5 cells/ml in 1995 and from 1.0×10^5 to 2.2×10^5 cells/ml in 1996. The nanoflagellate counts obtained in September 1996 at stations A and LBC indicated that the concentrations of photoautotrophic nanoflagellates were quite low and variable, ranging from 346 to 2,080 cells/ml at the surface and from 596 to 1,261 cells/ml at a depth of 40 m. The concentrations of heterotrophic nanoflagellates were also low and ranged from 190 to 370 cells/ml at the surface and from 67 to 729 cells/ml at a depth of 40 m.

The chlorophyll *a* concentrations in the late winter-early spring seasons in 1995 and 1996 differed (Fig. 2), which probably was a result of the differences in sea ice cover. The sea ice

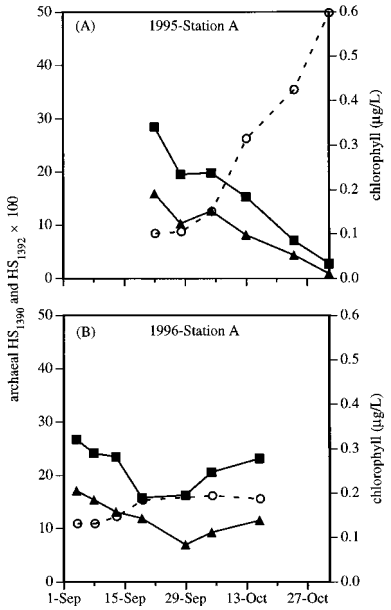


FIG. 2. Variation in archaeal rRNA levels at Arthur Harbor station A. Samples were collected at a depth of 3 m. An rRNA hybridization analysis was performed with the 0.8- to 0.2-μm seawater fraction in 1995 (A) and with the 1.6- to 0.2-μm seawater fraction in 1996 (B). Symbols: ■, archaeal HS_{1392}; ▲, archaeal HS_{1390}; ○, chlorophyll *a* concentration in unfiltered seawater.

in 1995 had a substantial diatom assemblage associated with the bottom layers (the bottom 5 to 10 cm) of ice. The chlorophyll concentrations in 1995 ranged from 0.21 to 0.70 μg/liter between 1 and 30 October at station LBC and were typically three- to sevenfold higher at the surface than at depth as a result of the combined effects of sea ice melt and stratification in the water column. In the 1996 early spring season, there was less sea ice and the chlorophyll *a* concentrations were lower, ranging from 0.14 to 0.22 μg/liter at station LBC; during this time the chlorophyll was uniformly distributed throughout the water column, and the concentrations at the surface were nearly equivalent to those at depth. At all stations sampled in 1996 (stations A, LBC, B, F, and J) there was no spatial variation in chlorophyll *a* levels throughout the early spring season. Seawater samples collected throughout the summer into the early fall of 1996 and 1997 at station B revealed that the chlorophyll *a* concentrations were as high as 1.54 μg/liter in late February (Fig. 3).

rRNA oligonucleotide hybridization experiments. The utility of normalizing rRNA probe hybridization values to a universal probe has been established previously (50), and the approach was recently refined (55). Figure 4 shows the relationship between the archaeal HS values obtained in experiments in which both universal probes were used and indicates that universal probe 1392 overestimated the archaeal signal compared to universal probe 1390 by a factor of almost 2. For consistency and comparison purposes the 1995 archaeal HS_{1392} values were numerically converted (by using the linear relationship $Y = 0.58X - 0.49$; $r^2 = 0.90$; $n = 113$) so that all of the rRNA data were normalized to the universal probe 1390 signal unless indicated otherwise.

Evidence from our experiments suggested that there was little variation in the correction factor ([amount of archaeal probe bound/unit of control RNA]/[amount of group I probe bound/unit of control RNA]) for the group I probe, since the average correction factors for *C. symbiosum* rRNA were 1.46

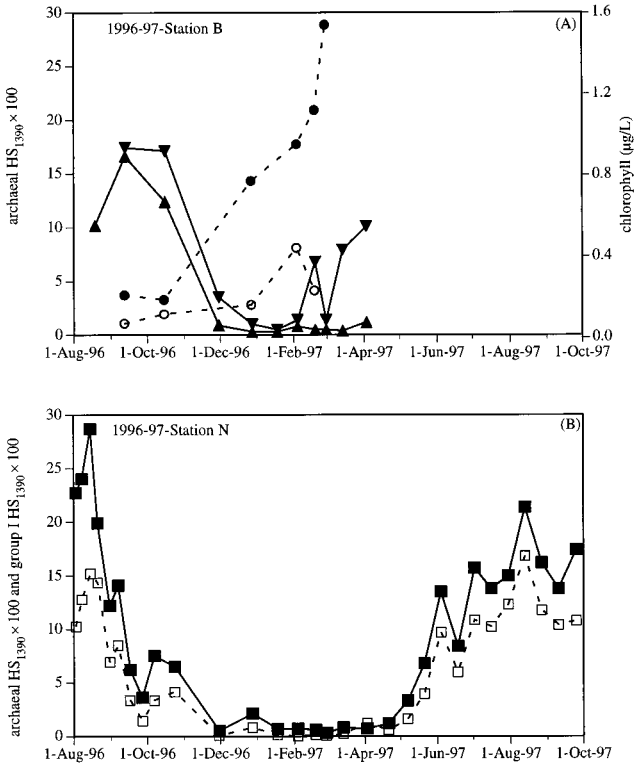


FIG. 3. Temporal changes in archaeal HS in the nearshore waters off Anvers Island in 1996 and 1997. (A) Station B archaeal HS_{1390} at the surface (▲) and at a depth of 50 m (▼) and the corresponding chlorophyll *a* concentrations at the surface (●) and at a depth of 50 m (○). (B) Annual variation in archaeal HS_{1390} (■) and group I HS_{1390} (□) at station N.

(standard deviation, 0.47; $n = 59$) and 1.38 (standard deviation, 0.62; $n = 41$) for the group I rDNA and 1.15 (standard deviation, 0.31; $n = 18$) for the group I rRNA transcript. On the other hand, there were differences in the nucleic acid hybridization properties of the group II probe, since the rDNA correction factor was 1.31 (standard deviation, 0.77; $n = 41$) and the rRNA transcript correction factor was 4.57 (standard deviation, 2.65; $n = 18$). Sauer and Raskin (46) have reported that there may still be biases associated with RNA transcript controls compared with native RNA, possibly due to modified bases in native rRNA, that may affect oligonucleotide hybrid stability.

The level of archaeal rRNA was lower on average in the 1995 late winter-early spring season (14.3%) than in the 1996 season (24.4%) (Table 2). The majority of the archaeal signal appeared to be attributable to the crenarchaeotal group I archaea (Table 2), except for samples collected in the summer, when the archaeal HS was very low. Figure 2 shows the variation in archaeal rRNA levels over the periods when station A surface samples were collected (deep data are not shown since the station was shallow, 45 m, and well-mixed for the periods sampled). The archaeal HS_{1390} decreased from 16.0 to 1.0% over the sampling period in 1995, and there was a much smaller net change (from 17.1 to 11.5%) for the same period in 1996. The 1995 data suggested that there was a negative correlation between the archaeal HS values and phytoplankton levels (as inferred from chlorophyll concentrations) at station A ($P < 0.005$) and at station LBC ($P < 0.05$), where the archaeal HS_{1392} in the surface waters decreased from 15.2 to 3.4% (the archaeal HS_{1390} was 8.3 to 1.5% when it was converted) (Fig. 4). There was no decrease in the archaeal rRNA signal between 1 and 30 October 1995 in the station LBC deep samples (archaeal HS_{1392}, 15.8 to 19.7%; converted archaeal HS_{1390}, 8.6 to 10.9%). The 1996 data for station A, where the chlorophyll concentrations were quite low (≤0.22 μg/liter) and the archaeal HS_{1390} were ≥11% during the same period, were consistent with this relationship. The 1996 station LBC data indicated that there was a slight decrease in archaeal rRNA levels in the surface waters (Table 3). Spatially, there was little variation in the archaeal HS_{1390} at all stations sampled on 13 September and 16 or 17 October 1996 (station A, 12.3% ± 1.1%; station B, 15.9% ± 2.3%; station LBC, 12.1% ± 3.3%; station F, 19.1% ± 4.1%; station J, 16.4% ± 1.2% [$n = 4$]), which supported the hydrographic data that indicated that there was thorough mixing due to high winds.

A more detailed investigation of temporal variation was conducted at station B throughout the 1996-1997 austral summer and at station N throughout a full annual cycle. This study allowed us to investigate the archaeal assemblage during the most biologically productive time of year in Antarctica and revealed a pattern of remarkable seasonality in the archaeal rRNA HS. This was characterized by relatively high archaeal HS in the austral winter and early spring, followed by nearly complete disappearance of archaeal rRNA throughout the summer and early fall. The archaeal HS declined dramatically throughout the water column as the season progressed from winter to spring (Table 2 and Fig. 3). By 30 November the archaeal HS_{1390} was 0.9% at the surface, 3.5% at a depth of 50 m at station B, and 0.5% at station N. The station B archaeal HS at a depth of 50 m remained extremely low until 14 March (with the exception of an archaeal HS of 6.8% on 19 February) and did not recover by the last sampling date (3 April) in the surface waters. At station N, the archaeal HS increased to 6.8% by 21 May and to a maximum value of 28.7% in the austral winter. There was a negative correlation between the archaeal HS and chlorophyll concentration at both depths at station B through the 1996-1997 season ($P < 0.05$). Interestingly, when the chlorophyll concentration dropped to 0.23 μg/liter on 19 February 1997 at a depth of 50 m, the archaeal HS_{1390} increased to 6.8%. One of the two archaeal groups, group I, appeared to dominate the plankton in all of the sur-

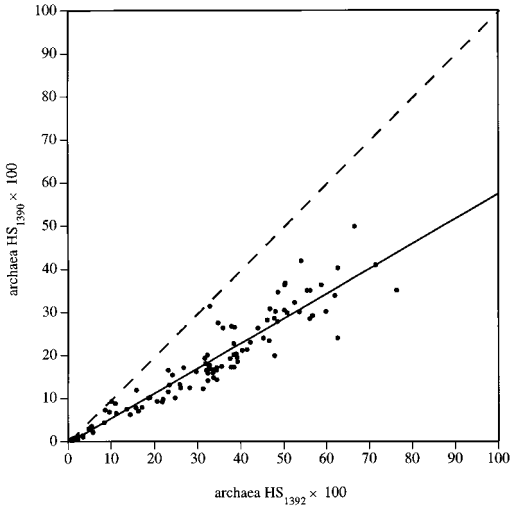


FIG. 4. Linear regression showing the relationship between archaeal rRNA HS normalized to universal probes 1390 and 1392 for all late winter-early spring samples in which both universal probes were used. The dashed line represents the 1:1 relationship. $Y = 0.58X - 0.49$; $r^2 = 0.90$; $n = 113$.

TABLE 2. Summary of archaeal and group-specific rRNA hybridization data

Season[a]	Sample dates	Sampling stations	Archaeal HS_{1390} (%)	Group I HS_{arch} (%)	n[b]
LWES	22 Sep.–1 Nov. 1995	A, LBC	14.3 ± 7.7[c]	61.9 ± 18.5[c]	12
LWES	4 Sep.–16 Oct. 1996	A, B, LBC, F, J, N	24.4 ± 11.6	58.3 ± 17.3	31
LWES	6 Sep.–26 Oct. 1996	PB1, PB2, PB3	25.0 ± 8.2	61.1 ± 18.6	43
S	30 Nov. 1996–28 Feb. 1997	B, N	2.3 ± 2.4	30.6 ± 22.3	17
F	14 Mar.–21 May 1997	B, N	5.8 ± 7.1	70.5 ± 46.2	9
W	3 Aug.–31 Aug. 1996 and 4 Jun.–27 Aug. 1997	B, N	17.7 ± 5.5	65.4 ± 12.2	13

[a] LWES, late winter-early spring; S, summer; F, fall; W, winter.
[b] Number of samples.
[c] Average ± standard deviation.

face samples and all but one of the 50-m samples at station B (data not shown). A similar pattern was observed for the samples collected at station N, where the group I HS_{arch} accounted for an average of 57.1% ± 25.8% (n = 30) of the archaeal signal. The relative proportion of the group II HS_{arch} became significant (>0.3) only when the archaeal HS_{1390} was less than 1.0% (data not shown).

In situ hybridization. The absolute numbers of archaea in the Antarctic coastal waters (stations A and LBC in 1996) were estimated by in situ hybridization. We used four Texas Red-labeled probes specific for group I archaea and two fluorescein-labeled probes specific for the *Bacteria*. The TR-GI-4-mix probe hybridized to a population of cells with morphology similar to the morphology of the crenarchaeal symbiont of a marine sponge (45). The cells were slightly curved with distinct cellular regions that stained differentially with the DAPI and rRNA probes. Simultaneous hybridization with the TR-GI-4-mix and FITC-bact-2-mix probes showed that each probe hybridized to a different population of cells. No Texas Red-labeled archaeal cells were observed when hybridizations were performed with a 50-fold excess of unlabeled GI-4-mix probe. The archaeal concentrations ranged from 0.9×10^4 to 2.7×10^4 cells/ml (Table 3). A slight increase in the number of archaea was seen in the surface samples from both stations A and LBC over the 6-week period when samples were examined. In 14 of 16 samples more than 10% of the cells were dividing (as determined by the presence of cells with three or more stained intracellular regions [45]), and in 4 of 16 samples more than 20% of the cells were dividing. Between 40 and 80% of the DAPI-stained cells specifically hybridized with the *Bacteria*-specific probes. The fluorescently labeled archaeal and bacterial cells accounted for 48 to 83% of the DAPI-stained cells.

Offshore hydrography and archaeal distribution. Offshore profile data are shown in Fig. 5. Other profile data obtained at different stations (stations PB2 and PB3) showed similar trends. The bacterioplankton concentration was highest at the surface and then decreased (to 10^5 cells/ml) at depths below 100 m. The chlorophyll *a* and flagellate concentrations peaked at a depth of 50 m and then declined dramatically at depth. Overall, the archaeal HS were high (Table 2) at all depths in the Palmer Basin. On 6 September the highest subsurface archaeal HS_{1390} was at a depth of 50 m; on 26 October the highest subsurface value was at a depth of 100 m, perhaps as a result of the disappearance of the upper mixed layer. The 6 September and 26 October profiles revealed that there were marked differences at 500 and 1,200 m in the archaeal rRNA levels (the difference in archaeal HS_{1390} was more than 15%) compared to the other depths where similar rRNA levels were found.

TABLE 3. rRNA hybridization data and whole-cell counts determined by using in situ rRNA probes specific for the group I archaea and bacteria at stations A and LBC

Station	Date (day-mo-yr)	Sample[a]	rRNA hybridization data (%)			In situ hybridization data				
			Archaeal HS_{1392}	Archaeal HS_{1390}	Group I HS_{1390}	% Group I cells[b]	Group I concn (10^4 cells/ml)	% Dividing group I cells[b]	% Bacterial cells[b]	Bacterial concn (10^4 cells/ml)
A	4-Sep.-96	S	26.8	17.1	8.7	8.5 (1.9)[c]	1.5	15.7	50.7	9.0
	4-Sep.-96	D				7.5	1.3	18.9	47.1	7.9
	13-Sep.-96	S	23.5	13.1	6.2	10.4 (0.5)	1.7	17.7	50.9	8.5
	13-Sep.-96	D				8.3	1.3	11.8	39.7	6.0
	5-Oct.-96	S	20.6	9.3	3.6	6.0 (1.9)	1.3	12.7	53.3	11.7
	5-Oct.-96	D				6.8 (2.0)	1.4	8.1	42.8	9.9
	17-Oct.-96	S	23.2	11.5	4.4	10.9 (3.1)	2.3	27.4	49.1	10.3
	17-Oct.-96	D				13.3 (7.5)	2.7	13.6	42.6	8.7
LBC	4-Sep.-96	S	31.9	18.1	10.2	5.5 (2.5)	0.9	12.4	56.8	9.7
	4-Sep.-96	D	29.7	16.2	8.1	12.6 (7.7)	2.6	22.4	54.8	11.3
	13-Sep.-96	S	32.1	16.5	10.3	5.2 (2.1)	0.9	11.1	79.3	14.3
	13-Sep.-96	D	22.0	9.8	4.9	9.6 (6.2)	1.7	2.0	62.9	11.4
	5-Oct.-96	S	11.0	8.8	3.6	8.4 (0.1)	1.7	18.9	62.2	12.2
	5-Oct.-96	D	8.6	7.3	2.9	7.9 (3.1)	1.7	22.2	58.5	12.8
	16-Oct.-96	S	10.1	9.3	4.2	14.5 (3.2)	2.2	20.1	57.5	12.0
	16-Oct.-96	D	26.1	12.4	9.0	11.8 (3.1)	2.1	13.4	59.9	10.6

[a] S, surface sample (depth, 3 m); D, deep sample (depth 50 m).
[b] Fraction of all DAPI-stained cells that were stained in situ.
[c] The values in parentheses are standard deviations.

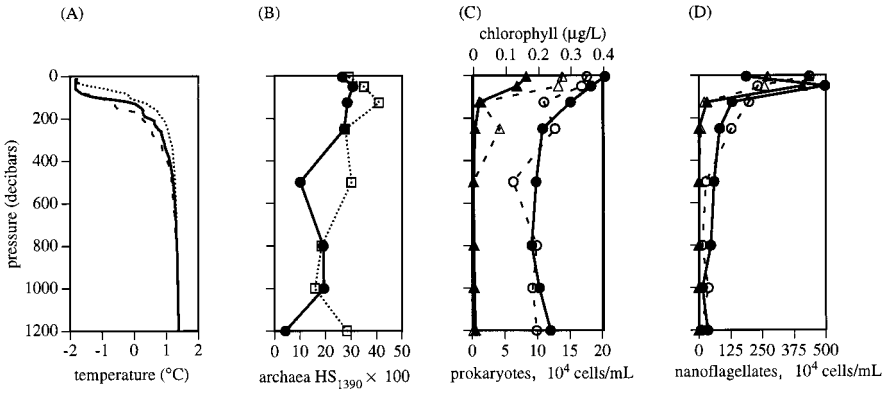


FIG. 5. Hydrographic, biological, and molecular data corresponding to depth profiles at station PB1. (A) Temperature profiles for 6 September (solid line), 29 September (dashed line), and 26 September (dotted line). (B) Archaeal HS_{1390} on 6 September (●) and 26 September (□). (C) Prokaryote concentrations, as determined by enumeration of DAPI-stained cells, on 6 September (●) and 29 September (○) and chlorophyll *a* concentrations on 6 September (▲) and 29 September (△). (D) Levels of phototrophic nanoflagellates on 6 September (●) and 29 September (○) and levels of heterotrophic nanoflagellates on 6 September (▲) and 29 September (△).

Bacterial ribotype profile analysis. All samples collected in the 1995 and 1996 field seasons were assayed by DGGE. Analysis of the V3 region of bacterial rDNA fragments on denaturing gradient gels was used to examine the variability of coastal Antarctic bacterioplankton assemblages over temporal, spatial, and vertical scales (Fig. 6). The assemblages appeared to be quite stable in the early spring and changed substantially through the summer and with depth.

A comparison of samples collected in 1993, 1995, and 1996 in early spring revealed that there was minimal variation in the bacterial assemblage ribotypes (Fig. 6a, lanes 1, 2, and 10). The levels of similarity for the 1993 ribotype profile were 0.83 and 0.82 compared to samples collected in 1995 and 1996, respectively. The samples used in lanes 2 through 4 and 10 were analyzed with a second gel to ensure that there was a fair comparison since the amounts used in the Fig. 6A gel were small. There was more variation between the 3- and 40-m samples in 1995; the average similarity value for the data for the four dates was 0.76, compared to the average similarity value of 0.95 for the 3- and 50-m samples in 1996, corroborating the finding that stratification occurred in 1995 and mixing occurred in 1996 in the nearshore water column.

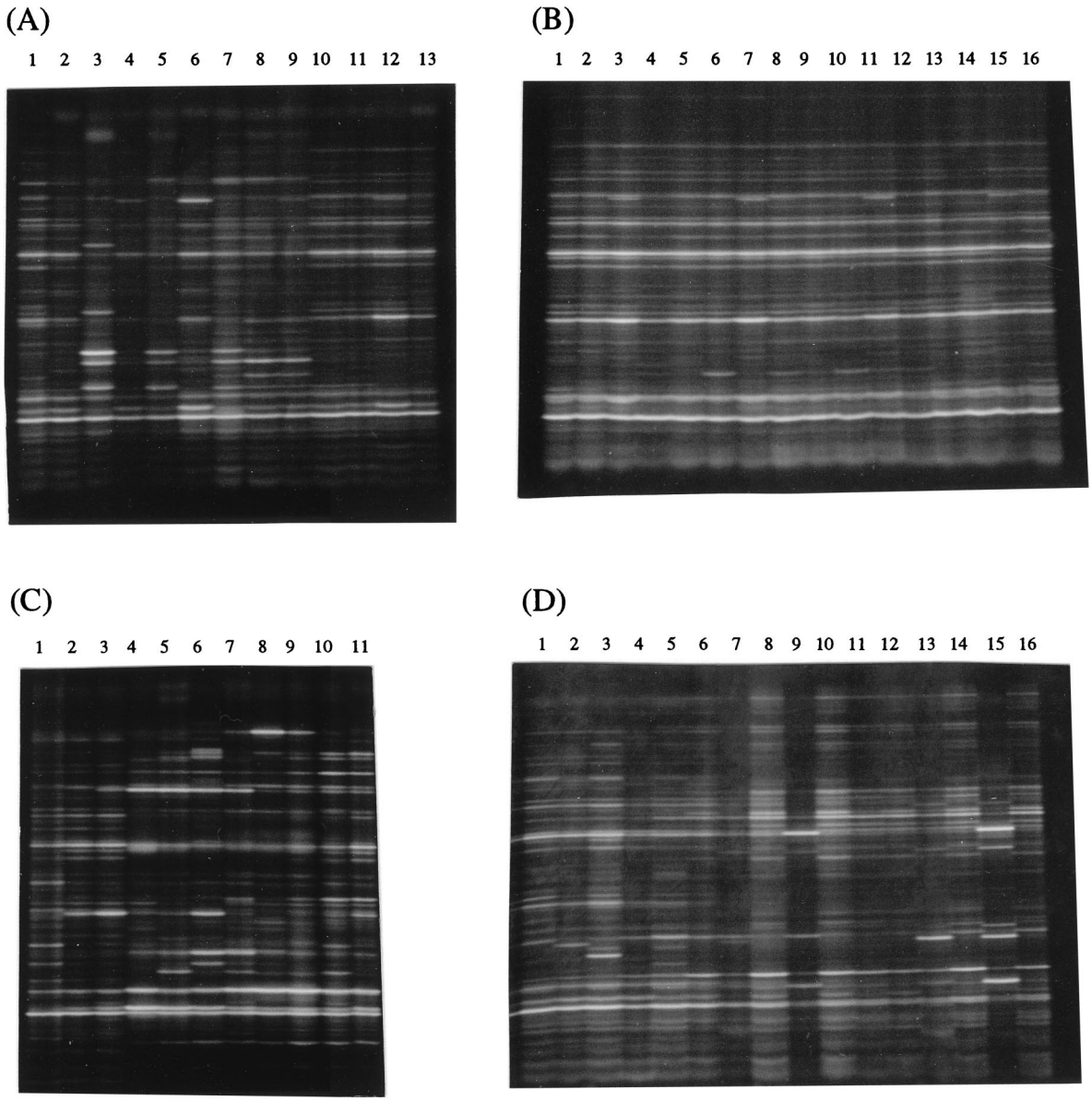
There were no obvious spatial differences in bacterial assemblage composition in the early spring of 1996 (Fig. 6B), when 3- and 50-m samples from stations LBC, B, F, and J (area, 3 square nautical miles) were compared on two dates. The differences in the ribotype profiles were consistent throughout all samples for the same depth or date. These data also indicate that the water was well-mixed due to high winds in September and October. The reproducibility of the technique is shown by these data, since all of the samples were collected and processed independently yet gave the same profile and even similar band intensities. If band intensity is representative of the natural abundance of a sequence in a nucleic acid extract (and in plankton), then 5 of the 31 bands obtained were dominant (5 bands exhibited >5% of the total reflective density of all of the bands). However, several factors, including rRNA copy number, PCR cycling parameters (>16 cycles), and template bias (12, 15, 51), may affect band intensity.

Striking differences were observed in the bacterial component of the planktonic assemblage on a longer temporal scale. Figure 6C shows the temporal variation in the bacterial assemblage V3 region between 19 August 1996 and 3 April 1997 at a single sampling site (station B). The similarity matrix for this gel is shown in Table 4. Temporal variation was seen in the number of common bands and the calculated similarity values when the 19 August ribotype profile was compared with all of the other profiles in a pairwise fashion. The turnover in ribotype composition was shown by the decrease in pairwise similarity values when late winter-early spring (19 August and 13 September) profiles (C_s = 0.85) and winter and summer (19 August and 4 February) profiles (C_s = 0.29) were compared; later, the similarity value increased to 0.66 (19 August 1996 compared to 3 April 1997). We predict that the similarity values would probably continue to increase to values seen in late winter, as was the case for the station LBC samples (C_s = 0.82 between 1995 and 1996). The same pattern was also evident from the decrease in ribotypic richness (number of bands) between early spring and summer (the number of bands decreased from 32 in early spring to an average of 23 in summer) and during the transition of summer to fall (the number of bands increased to 32 in April).

The profiles obtained at depths of up to 1,200 m in Palmer Basin revealed depth-related compositional changes (Fig. 6D). Decreases in the levels of similarity of the ribotype patterns were observed when surface samples were compared by the pairwise similarity method with samples from greater depths. The samples analyzed in Fig. 6D, lanes 7, 9, and 15 (6 September; station PB1; 250, 500, and 1,200 m), were electrophoresed in a second gel since the amounts loaded appeared to be small. The station PB1 500- and 1,200-m samples produced ribotype profiles identical to those in Fig. 6D, while the bands from the station PB1 250-m sample that appeared light increased in intensity, resulting in a similarity to the 26 October station PB1 250-m sample of 0.77. The highest similarity values were obtained for the upper 50 m for both sample dates, while the lowest similarity values for each depth profile were observed when surface and 1,200-m ribotype profiles were compared; in these cases similarity values were 0.26 and 0.57 for the samples collected on 6 and 29 September, respectively. Substantial differences in ribotype composition were observed when the data for the two sample dates were compared, most notably at depths of 500 and 1,200 m.

DISCUSSION

Antarctic planktonic archaea. In general, our data confirm and extend the results of a previous report which showed that



(A)

Lane	Stn	S/D	Date
1	B	S	13 Sep 93
2	LBC	S	1 Oct 95
3	LBC	D	1 Oct 95
4	LBC	S	18 Oct 95
5	LBC	D	18 Oct 95
6	LBC	S	25 Oct 95
7	LBC	D	25 Oct 95
8	LBC	S	30 Oct 95
9	LBC	D	30 Oct 95
10	LBC	S	4 Sep 96
11	LBC	D	4 Sep 96
12	LBC	S	16 Oct 96
13	LBC	D	16 Oct 96

(B)

Lane	Stn	S/D	Date
1	LBC	S	13 Sep 96
2	LBC	D	13 Sep 96
3	LBC	S	16 Oct 96
4	LBC	D	16 Oct 96
5	B	S	13 Sep 96
6	B	D	13 Sep 96
7	B	S	16 Oct 96
8	B	D	16 Oct 96
9	F	S	13 Sep 96
10	F	D	13 Sep 96
11	F	S	17 Oct 96
12	F	D	17 Oct 96
13	J	S	13 Sep 96
14	J	D	13 Sep 96
15	J	S	17 Oct 96
16	J	D	17 Oct 96

(C)

Lane	Stn	S/D	Date
1	B	S	19 Aug 96
2	B	S	13 Sep 96
3	B	S	16 Oct 96
4	B	S	30 Nov 96
5	B	S	28 Dec 96
6	B	S	18 Jan 97
7	B	S	4 Feb 97
8	B	S	19 Feb 97
9	B	S	28 Feb 97
10	B	S	14 Mar 97
11	B	S	3 Apr 97

(D)

Lane	Stn	m	Date
1	PB1	5	6 Sep 96
2	PB1	5	26 Oct 96
3	PB1	50	6 Sep 96
4	PB1	50	26 Oct 96
5	PB1	100	6 Sep 96
6	PB1	125	26 Oct 96
7	PB1	250	6 Sep 96
8	PB1	250	26 Oct 96
9	PB1	500	6 Sep 96
10	PB1	500	26 Oct 96
11	PB1	800	6 Sep 96
12	PB1	800	26 Oct 96
13	PB1	1000	6 Sep 96
14	PB1	1000	26 Oct 96
15	PB1	1200	6 Sep 96
16	PB1	1200	26 Oct 96

TABLE 4. Ribotype profile similarity matrix comparing temporal variation at station B[a]

Date (day-mo-yr)	No. of common bands or similarity value[b]										
	19-Aug.-96	13-Sep.-96	16-Oct.-96	30-Nov.-96	28-Dec.-96	18-Jan.-97	4-Feb.-97	19-Feb.-97	28-Feb.-97	14-Mar.-97	3-Apr.-97
19-Aug.-96		26	24	14	14	11	8	13	17	18	20
13-Sep.-96	0.85		29	16	16	15	14	16	19	21	23
16-Oct.-96	0.80	0.92		18	17	17	14	16	18	22	23
30-Nov.-96	0.55	0.59	0.68		18	16	17	17	18	19	20
28-Dec.-96	0.51	0.55	0.60	0.75		20	18	19	23	23	23
18-Jan.-97	0.40	0.52	0.60	0.67	0.77		17	17	21	22	23
4-Feb.-97	0.29	0.48	0.49	0.71	0.69	0.65		19	21	23	19
19-Feb.-97	0.49	0.57	0.58	0.74	0.76	0.68	0.76		23	23	21
28-Feb.-97	0.59	0.62	0.60	0.71	0.84	0.76	0.76	0.87		28	25
14-Mar.-97	0.58	0.65	0.69	0.69	0.78	0.75	0.78	0.81	0.90		28
3-Apr.-97	0.66	0.72	0.73	0.74	0.79	0.79	0.66	0.75	0.82	0.86	

[a] See Fig. 6C.
[b] The numbers on the upper right are numbers of common bands, and the values on the lower left are similarity values (C_s).

archaea are abundant in the late winter and early spring in the Antarctic Peninsula region (9). The Antarctic planktonic archaeal assemblage appeared to be dominated by group I crenarchaeota both in the Gerlache Strait (33) and in the Anvers Island coastal region. The group II euryarchaeota seemed to be a minor fraction, although a relative increase in the group II rRNA in March and April suggests that these organisms may be more significant at that time of year. The data available at this point (probe data, rDNA sequence data, and data from colony hybridizations with 16S rDNA libraries) suggest that groups I and II are the predominant archaeal types in Antarctic plankton (9, 10, 33). The levels of planktonic group I archaea determined by in situ hybridization techniques suggested that archaeal cells account for approximately 10% of all DAPI-stained cells. The direct cell count data are in general agreement with the rRNA hybridization data and suggest that the rRNA hybridization data can be a good indicator of cellular abundance. The observation that a significant portion of the archaeal cells were dividing implies that the cells were active, suggesting that the planktonic archaea are locally active in Antarctic coastal surface waters.

Temporal dynamics. A marked decrease in the relative percentage of archaeal rRNA in the coastal waters of Anvers Island occurred at the onset of the austral spring. The possibility that planktonic archaea are outcompeted for resources by other microbes associated with austral summer conditions might be supported by the observed negative correlation ($P < 0.005$ and $P < 0.05$) of archaeal rRNA levels and chlorophyll *a* concentrations. Archaea are often characterized by the "extreme" niches that they occupy, habitats which appear to be less intense with respect to biological competition. Perhaps this scenario holds for Antarctic planktonic archaea, which appear to dominate in the austral winter and early spring when competitive pressures for resources are probably at a minimum. Alternatively, the planktonic archaea may respond to increases in solar irradiance, since decreases in archaeal rRNA levels also occur with increases in day length, as observed in the 1995 data obtained at stations A and LBC, where the archaeal rRNA levels remained high at depth while the surface archaeal rRNA was depleted. However, the extended 1996 temporal results obtained for station B provide less convincing data that light regulation occurs, since the archaeal rRNA levels were extremely low throughout the summer in both the surface and 50-m samples.

DGGE analyses were useful for studying the temporal variation in assemblage composition. The ribotype profile analysis of bacterial V3 fragments revealed little variation between the resident late winter bacterioplankton assemblages in different years. On short time scales (days to weeks), the late winter assemblages appeared to be quite stable, although stratified conditions allowed changes in composition to develop, as observed at station LBC in 1995 (Fig. 6A). The bacterial ribotype profiles determined on longer time scales indicated that seasonal changes occurred; similarity values (relative to late winter data) changed dramatically through the summer and then rose again at the onset of fall.

The hybridization data revealed that major changes in archaeal rRNA levels occurred throughout the austral summer, and the DGGE data revealed that major changes in bacterial assemblage composition occurred in the same period. These data provide a picture of a dynamic prokaryotic assemblage which varies with time coincident with seasonal changes in the Antarctic Peninsula region. Heterotrophic bacterioplankton probably respond to changes in carbon source quantity and quality, which presumably also vary temporally in the Antarctic environment. Moline and Prezelin (35) observed successional patterns in the phytoplankton assemblage which could have direct effects on the dissolved and particulate carbon sources available for remineralizing.

Spatial variability and environmental gradients. The variation in the bacterioplankton assemblage over small horizontal distances in the Anvers Island region was minimal, as shown by

FIG. 6. Ribotype profile analysis of bacterial V3 fragments by DGGE. (A) Comparison of bacterial assemblages obtained in 1993, 1995, and 1996 and short-time series analysis of surface assemblages (depth, 3 m) and deep assemblages (depth, 40 m in 1995 and 50 m in 1996) obtained in the late winter-early spring sampling period. (B) Spatial analysis of ribotype compositions at four stations, stations LBC, B, F, and J, sampled at the surface and depth on two dates in late winter-early spring. (C) Temporal analysis of variability in bacterial ribotype compositions at station B between 19 August 1996 and 3 April 1997. (D) Vertical profile of bacterial ribotype compositions in the Palmer Basin on two dates in late winter-early spring. Abbreviations: S, surface sample (depth, 3 m); D, sample obtained at a depth of 40 or 50 m; Stn, station.

the lack of variation in the rRNA signals or V3 rDNA ribotype profile signatures at five stations covering a 3-nautical mile square on two different dates in 1996. This is consistent with the hydrography, which indicated that there was little hydrographic variability over the sample dates. Spatial variation in the bacterioplankton assemblages may occur in this region at other times due to intrusions of circumpolar deep water (25, 35), glacial melting, or influences from phytoplankton or grazer patches (notably krill), which could affect local water column characteristics.

Microbial assemblage composition varied along physical and chemical gradients. Variability in natural microbial assemblages has been demonstrated previously with DGGE along temperature gradients (13, 15), estuarine salinity gradients (37), and oxic-anoxic interfaces (11, 40, 52). Differences in bacterial ribotype profiles and in archaeal rRNA HS between surface and deep samples in the nearshore waters were observed under stratified conditions. In contrast, identical ribotype profiles and archaeal rRNA signals were often observed during periods when shallow and deep layers were mixed. On greater scales, compositional differences in bacterial ribotype patterns obtained from vertical profiles in the Palmer Basin were readily detectable (Fig. 6D). Other workers (16, 21, 31, 54) have reported evidence that stratification of prokaryotic types occurs in the Pacific and Atlantic oceans.

The archaea appear to reside in a variety of locales, both nearshore surface waters and offshore deep waters, which have the signature of Antarctic circumpolar water (temperature, >1°C). We detected high levels of archaeal rRNA throughout the water column, and archaeal rRNA maxima were observed in the upper 100 m for all Palmer Basin samples.

Bacterial ribotype profiles appear to reflect hydrography. A high level of similarity in the ribotype composition of bacteria (C_s = 0.91) was found within the upper mixed layer on 6 September 1996 in the Palmer Basin. Large differences in ribotype composition were evident between the 6 September 1996 profile, which indicated that there was a larger gradient of stratification among the ribotypes at depths below 50 m, and the 26 October 1996 profile, in which the compositions were more similar (C_s = 0.26, compared with C_s = 0.57 for samples obtained between 5 and 1,200 m). Notable differences in bacterial ribotype profiles between the two dates were observed at depths of 500 and 1,200 m, which coincides with the finding that there were temporal differences in the archaeal rRNA HS at the same depths (Fig. 5). Perhaps late winter profiles represent resident assemblages present in winter water (24), which are replaced in October by advective processes, as the sea ice ablates. Although in situ changes in composition are imaginable over the sampling period, we suspect that advective processes occurred instead, since activity is so low at the depths investigated. The activity at 250 m estimated by leucine incorporation was 0.15 pmol/liter/h, or 2.3% of the activity measured for the surface water on 6 September at station PB3 (32). Substantial changes in composition over the 7-week time period examined appear to be unlikely. Thus, the rRNA and DGGE data are complimentary since the rRNA hybridization data indicate archaeal levels and potential regions of activity, the DGGE data show ribotype variation in bacterial assemblage composition on a vertical gradient, and both types of data reveal biological changes coincident with changes in water mass properties.

Although we demonstrated patterns of temporal variation in the archaeal assemblage, many questions remain unanswered regarding the physiological lifestyles of the archaea. These organisms appear to be subject to biological competition with the onset of spring and to increases in seasonal primary and secondary production. The timing of potential competition for NH_4 stocks between autotrophic and heterotrophic assemblages (53) coincided with the apparent decline in archaeal rRNA levels in surface waters. The high levels of archaea in the austral winter and early spring suggest that if these organisms are heterotrophic, they could be key remineralizers of recalcitrant carbon stocks and regenerate NH_4 over winter. If the archaea were poor competitors with low rates of metabolic turnover, their levels could also be drastically affected by grazers in the spring and summer if the microbial loop becomes more active, although the significance of the microbial loop in Antarctic waters remains controversial (1, 22). Large variations in microbial activities in the Southern Ocean are thought to result from the great spatial and temporal heterogeneity of the Antarctic environment (29). An alternative hypothesis is that the archaea are chemoautotrophs that grow slowly but are not affected by the low-carbon conditions prevalent in the austral winter.

The dynamics within the water column are mediated by a combination of physical factors (water mass qualities) and biological factors (changes in dissolved organic carbon and particulate organic carbon). Unlike the phytoplankton, prokaryotes are present in high numbers year round and, especially in Antarctic surface waters, are susceptible to dramatic environmental fluctuations. This great environmental variability is reflected by the dynamic shifts evident in Antarctic planktonic archaeal and bacterial assemblages.

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