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Abundance and distribution of planktonic *Archaea* and *Bacteria* in the waters west of the Antarctic Peninsula

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Abstract

Polyribonucleotide probes targeting planktonic archaeal (Group I and II) and bacterial rRNA revealed that *Archaea* comprised a significant fraction of total prokaryote cell abundance in the marine waters west of the Antarctic Peninsula. Determinations of *Archaea* and *Bacteria* cell abundances were made during two research cruises to the Palmer Long-Term Ecological Research region during the austral winter and summer of 1999. During the austral summer, surface water abundances of Group I (GI) *Archaea* were generally low, averaging 4.7×10^3 cells ml^{-1} and accounting for 1% of the total picoplankton assemblage. The abundance of GI *Archaea* increased significantly with depth, averaging 2.1×10^4 cells ml^{-1} and comprising 9–39% of the total picoplankton abundance in the meso- (150–1,000 m) and bathypelagic (1,000–3,500 m) circumpolar deep water (CDW). Relative to summertime distributions, GI cells were more evenly distributed throughout the water column during the winter, averaging 10% of the picoplankton in the surface waters and 13% in the CDW. Surface water GI abundance increased 44% between the summer and winter, coincident with a fivefold decrease in GI abundance in the deeper waters. The abundance of Group II (GII) *Archaea* was persistently $<2\%$ of the total picoplankton throughout the water column in both summer and winter. Bacterial abundance was greatest in the upper water column (0–100 m) during the summer, averaging 3.9×10^5 cells ml^{-1} and comprised 89% of the total picoplankton assemblage. Generally, GI *Archaea* varied seasonally in the deeper waters, whereas bacterial abundance varied more in the upper waters. The observed variability in bacterial and archaeal abundance suggests that these two groups of marine picoplankton are dynamic components of Southern Ocean microbial food webs.

Marine picoplankton are arguably the most poorly understood members of the marine plankton community, mainly because of poor cultivation success. Continuing development of cultivation (Connon and Giovannoni 2002; Rappé et al. 2002) and cultivation-independent (Olsen et al. 1986; DeLong et al. 1989; Amann et al. 1990) approaches to studying marine picoplankton are providing information on the phylogenetic diversity of marine plankton. Among other significant findings, studies have revealed that marine picoplankton communities include all three domains of life: *Bacteria, Archaea,* and *Eukarya* (Giovannoni et al. 1990; DeLong 1992; López-Garcia et al. 2001*c*).

The ecological roles and biogeochemical importance of planktonic *Archaea* are not well understood, but *Archaea*

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appear to constitute a numerically significant component of plankton biomass (DeLong 1992; Fuhrman et al. 1992; Karner et al. 2001). The domain *Archaea* encompasses two kingdoms: the Crenarchaeota and the Euryarchaeota (Woese 1987; Barnes et al. 1996). Phylogenetic analyses of planktonic *Archaea* indicate that marine *Archaea* generally group within the Crenarchaeota and Euryarchaeota lineages. Notable members of the Crenarchaeota include the hyperthermophiles that thrive in environments >70°C (Barnes et al. 1996; Moyer et al. 1998) and the psycrophilic sponge symbiont *Cenarchaeum symbiosium* (Preston et al. 1996). The Euryarchaeota include the anaerobic methanogens and extreme halophiles. Generally, Group I (GI) *Archaea,* members of the Crenarchaeota, appear to be the most abundant archaeal group in the oceans (DeLong et al. 1998). Crenarchaeota typically appear in greatest relative abundance in deep neritic waters and in the meso- (150–1,000 m) and bathypelagic depth strata (1,000–3,500 m) of oceanic systems (DeLong et al. 1999; Massana et al. 2000; Karner et al. 2001). The Group II (GII) marine *Archaea* are members of the Euryarchaeota. In the marine plankton, Euryarchaeota tend to show greatest relative abundance in the surface waters of pelagic and coastal environments (Murray et al. 1999*a*; Massana et al. 2000). The Group III (GIII) *Archaea,* also members of the Euryarchaeota, are distantly related to the order Thermoplasmales (Fuhrman and Davis 1997; Massana et al. 2000). GIII *Archaea* appear most prevalent in subeuphotic waters and are less widely distributed and abundant than either the GI or GII *Archaea* (López-García et al. 2001*a*,*b*). Finally, a recent study has revealed a fourth group of planktonic *Archaea* (GIV), distantly related to the extremely halophilic Euryarchaeota, found in the deep-ocean waters of the Antarctic Polar Front region (López-Garcia et al. 2001*a*,*b*).

Molecular biology has provided new tools for the study of picoplankton. Single-cell fluorescent in situ hybridization (FISH) with rRNA-targeted probes has provided a method of directly quantifying the abundance of planktonic *Archaea* and *Bacteria* in various aquatic ecosystems (Amann et al. 1990; Fuhrman and Ouverney 1998; DeLong et al. 1999; Karner et al. 2001). FISH allows identification of whole, intact, individual picoplankton cells, enabling direct microscopic enumeration and quantification of the phylogenetic composition of the picoplankton assemblages. However, applications of oligonucleotide rRNA FISH (oligoFISH) to marine picoplankton assemblages have been hampered by the inability to quantify more than ~80% of the picoplankton population in any given sample (Glöckner et al. 1999), and at certain times of year and at depth, the oligoFISH method seems to perform quite poorly (Pernthaler et al. 2002).

In an effort to alleviate some of the methodological problems associated with oligoFISH enumeration of planktonic *Archaea,* a method based on multiply labeled 16S rRNA polyribonucleotide probes (Trebesius et al. 1994) was adapted for identification of marine *Archaea* and *Bacteria* (DeLong et al. 1999). Initial studies using the polyribonucleotide rRNA probes indicate that many of the limitations associated with the oligoFISH methods had been eliminated (DeLong et al. 1999; Karner et al. 2001; Pernthaler et al. 2002). Moreover, these investigations confirmed that Crenarchaeota and Euryarchaeota were important contributors to prokaryote abundance in marine plankton ecosystems (DeLong et al. 1999; Karner et al. 2001).

Studies describing the distributions of marine *Archaea* indicate that they might be ubiquitous components of the perennially cold waters of both coastal and oceanic ecosystems within the Southern Ocean (DeLong et al. 1994; Murray et al. 1998, 1999*b*; Massana et al. 1998, 2000; López-Garcia et al. 2001*a*,*b*). Initial observations in the Southern Ocean found that archaeal ribonucleic acids accounted for 16–34% of the bulk community rRNA obtained from surface seawater in the nearshore waters off Anvers Island, Antarctica, in the late austral winter (DeLong et al. 1994). Subsequent studies in this region confirmed the presence of *Archaea* and noted an apparent seasonality in the relative amount of archaeal rRNA in the surface waters (Murray et al. 1998; Massana et al. 1998).

As participants in the Palmer Long-Term Ecological Research (LTER) program, we examined the distribution of planktonic *Archaea* and *Bacteria* in seawaters west of the Antarctic Peninsula during both the austral winter and summer. Use of polyribonucelotide FISH (polyFISH) enabled us to observe the spatial and temporal variability in the phylogenetic structure of the picoplankton assemblage in Antarctic waters. In this study, we present the first comprehensive observations of the abundance of planktonic *Archaea* in the waters off the Antarctic Peninsula and describe the vertical and temporal differences in archaeal cell abundance. Our results indicate that the abundance of planktonic Crenarchaeota and *Bacteria* are both temporally and spatially variable in the Antarctic marine environment.

Methods

Study region and sampling—Samples for identification of planktonic *Archaea* and *Bacteria* were collected in the Palmer LTER sampling grid off the Antarctic Peninsula. The Palmer LTER sampling grid extends ~900 km in a north–south direction parallel to the Antarctic Peninsula and more than 200 km offshore. The region includes the shallow, nearshore waters on the Antarctic shelf and the deep, open ocean waters off the shelf west of the Antarctic Peninsula. Sampling was conducted on cruises aboard the ASRV *Laurence M. Gould* (LMG 99-1) during the summer (January 1999) and aboard the RVIB *Nathaniel B. Palmer* (NBP 99-6) during the austral winter (June–July 1999). Water column samples were collected at ~20-km intervals on east–west transects in both the northern (600 transect line) and southern (200 transect line) portions of the LTER sampling grid (Fig. 1). Water samples were collected using a CTD rosette equipped with 24, 12-liter Niskin bottles. The instrumentation included a Sea Bird SBE 9 CTD, a Biospherical Instruments QSP-200L PAR sensor, and a Sea-Tech MK III Aquatrack fluorometer. Chlorophyll *a* (Chl *a*) concentrations were determined fluorometrically from phytoplankton pigments concentrated on glass fiber filters and subsequently extracted in acetone.

Picoplankton sampling during LMG 99-1 was most intensive in the upper water column (0–100 m); however, samples

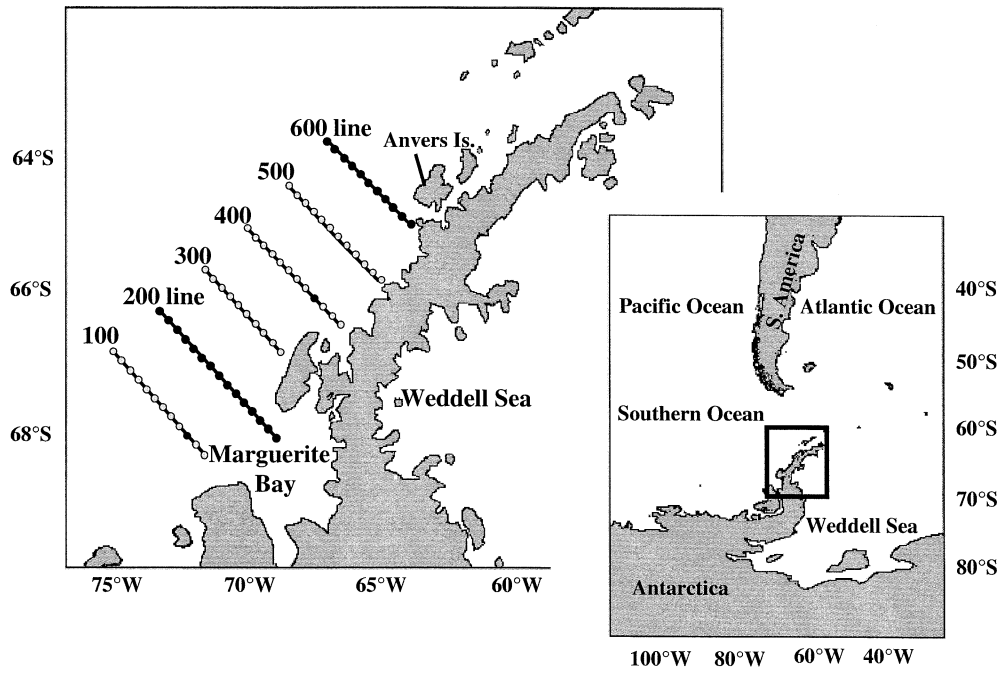

Fig. 1. Map of the sampling area, including the west Antarctic Peninsula (right), with detail of sampled transects (100 through 600 lines) and sampled stations (black dots) (left). Map constructed using Ocean Data View (Schlitzer 2002).

were collected throughout the entire water column (0–3,500 m) at several stations along the cruise transect. Samples from NBP 99-6 were collected from selected stations along the 600, 400, 200, and 100 transect lines. Samples from NBP 99-6 were collected from various depths evenly spaced throughout the water column, ranging from 2 to 2,300 m, enabling detailed vertical profiles of the abundances of *Archaea* and *Bacteria*. Samples from LMG 99-1 were collected from Niskin bottles into 50-ml polyethylene centrifuge tubes. Samples were collected, preserved, and processed as described by DeLong et al. (1999).

Samples from the NBP 99-6 cruise were collected from the Niskin bottles into 500-ml amber polypropylene bottles. Samples were fixed with formalin (2% final concentration), immediately frozen (−20°C), and transported to the University of Hawaii for subsequent analyses. Approximately a year after the sample collection date, frozen seawater samples were thawed at room temperature, gently shaken, subsampled into 50-ml polyethylene centrifuge tubes, and filtered onto 25-mm, 0.2-μm polycarbonate filters and processed as described in DeLong et al. (1999).

Polyribonucleotide FISH—Development of the polyribonucleotide probes, as well as details on the synthesis, hybridization, and wash conditions are described in DeLong et al. (1999). Fluorescein-labeled polyribonucleotide rRNA probes were used for all analyses. Each probe consisted of hydrolyzed, labeled RNA, targeting *Archaea* (GI or GII) or *Bacteria*, or a negative control probe. The control probe consisted of a nonbinding complementary strand of GI probe. Control probe counts were not subtracted from the individual determinations of GI, GII, and *Bacteria* cell abundance because it was not possible to attribute the control-positive cells to any of the three picoplankton groups examined; however, control probe counts were subtracted from estimates of total probe-positive cell abundances. Microscope counts were conducted on either a Zeiss Axiophot or a Nikon EM600 Infinity Optics epifluorescence microscope. DAPI-stained cells were enumerated on each microscope field followed by determination of probe–positive cells. Cell abundance was estimated on 15 different microscope fields per sample. The percentages of probe-positive cells were calculated as the total number of probe-positive cells relative to the DAPI-stained cells in each microscope field. In some cases, the total probe-positive cells exceeded the DAPI-stained cells, resulting in >100% of the total cells enumerated by the polyFISH probes.

Results

Environmental characteristics—The Palmer LTER study site is characterized by large temporal and spatial habitat variability. In particular, the annual advance and retreat of the pack ice, coupled with the seasonal oscillations in solar irradiance, are hypothesized to be the fundamental determinants on the magnitude and duration of biological productivity in this region (Smith et al. 1995; Karl et al. 1996).

The prominent chemical, physical, and biological properties of the region west of the Antarctic Peninsula have been described previously (Holm-Hansen and Mitchell 1991; Karl et al. 1991; Hofmann et al. 1996). The majority of samples collected for this study were from stations located on the continental shelf, where bottom depths ranged between 200

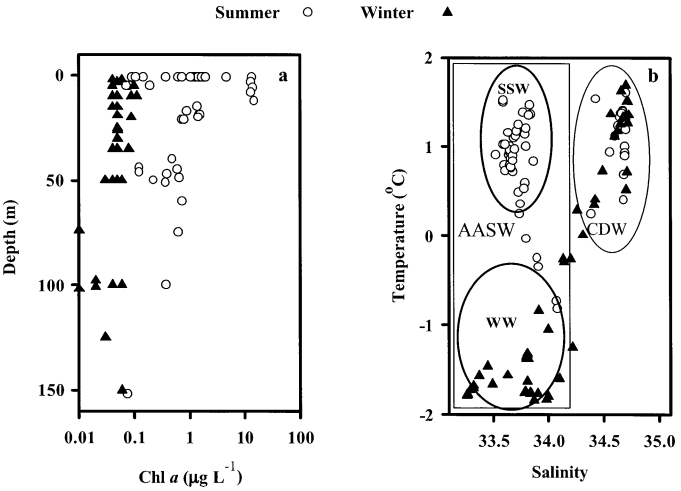

Fig. 2. (a) Chlorophyll *a* concentrations (μg L^{-1}) in the upper ocean measured during the summer and winter cruises. (b) Temperature–salinity (T-S) characteristics of stations sampled, indicating approximate T-S envelopes for water masses during the summer and winter in the waters west of the Antarctic Peninsula. Descriptions of water masses are provided in the text. Abbreviations are Antarctic surface water (AASW), in box that includes both summer surface water (SSW) and winter water (WW), and circumpolar deep water (CDW).

and 500 m (Fig. 1). The continental shelf in this region is depressed because of isostatic adjustment of the ice mass covering the Antarctic continent. Four stations sampled outside the shelf break had bottom depths exceeding 3,000 m. All stations occupied during LMG 99-1 were free of pack ice, whereas surface water at several of the stations sampled in NBP 99-6 were covered with sea ice. Seasonal ice retreat typically opens water along the northern portion of the LTER grid in the beginning of the austral spring (November), followed soon after by clearance of the pack ice to the south (Stammerjohn and Smith 1996).

Chl *a* concentrations demonstrated remarkable spatial heterogeneity in the summer. Concentrations on the January cruise ranged more than two orders of magnitude (0.1–14.9 μg Chl *a* L^{-1}) (Fig. 2a), with peak concentrations found at the nearshore stations. Chl *a* concentrations in the surface waters of stations located in the eastern end of the 200 sampling line (inside Marguerite Bay) exceeded 14 μg Chl *a* L^{-1}, whereas Chl *a* concentrations at stations offshore of the continental shelf break ranged between 0.1 and 2.0 μg Chl *a* L^{-1}. During the winter, concentrations of Chl *a* were uniformly low, averaging 0.05 μg Chl *a* L^{-1} (range 0.0–0.1 μg Chl *a* L^{-1}) (Table 1).

Hydrography—Vertical profiles of temperature and salinity (T-S) revealed the presence of distinct water masses in the waters west of the Antarctic Peninsula (Fig. 2b). The hydrography of this region has been well characterized, and the water masses sampled in this study included the Antarctic surface water (AASW) and circumpolar deep water (CDW) (Niller et al. 1991; Hofmann and Klinck 1998; Smith et al. 1999). In addition, winter water (WW) was sampled during both cruises: WW is a subcomponent of the AASW and is the remnant winter mixed-layer water isolated below the seasonal pycnocline that is produced locally by summer heating (Hofmann and Klinck 1998). All of these water masses were sampled on both the winter and summer cruises; however, the most substantial seasonal modifications in T-S signatures were observed in the surface waters (Fig. 2b).

Surface water temperatures during the austral summer ranged from 0.5 to 1.5°C, and salinities ranged between 33.5 and 33.8 (Table 1; Fig. 2b). For purposes of comparison, we define this summer surface water (SSW) component of the AASW (Table 1; Fig. 2b). Immediately below the SSW, water column temperatures reached a middepth minimum, declining to between −0.8 and 0.8°C, accompanied by an increase in salinity (~34.0) characteristic of WW. Below the WW, temperatures progressively increased to 0.2–2°C, and salinity increased to 34.7, characteristic of the oceanic CDW water mass. CDW consists of well-defined upper and lower water masses (Hofmann and Klink 1998), but for clarity in this study, we opted to group the picoplankton abundances in the CDW into a single water mass. The relatively warm, salty CDW occupied the lower water column between ~150 and 3,500 m.

T-S profiles obtained during the austral winter revealed a seasonally modified AASW mass that was both fresher and cooler than the SSW. Only two distinct water masses were observed during the winter cruise: the WW and the CDW. The surface water mass was the winter end-member of the WW (Table 1; Fig. 2b). Salinities in the WW ranged from 33.3 to 34.2, and temperatures were −1.8 to −0.3°C (Table 1; Fig. 2b). WW formed a deep mixed layer in the upper ocean, extending from the surface waters all the way to the top of the CDW at ~200 m. CDW was the densest water

Table 1. Characteristics of the principal water masses in the west Antarctic Peninsula.

Season (cruise)	Water mass*	Depth (m)†	Temperature (°C)	Salinity	Chl *a* (μg L^{-1})
Summer (LMG 99-01)	SSW	1–40	0.5–1.5	33.5–33.8	0.1–14.9
	WW	44–100	−0.8–0.8	33.7–34.1	0.1–0.7
	CDW	150–3,500	0.2–2.0	34.4–34.7	ns‡
Winter (NBP 99-06)	WW	2–222	−1.8 to −0.3	33.3–34.3	0.0–0.1
	CDW	74–2,325	0–1.7	34.3–34.7	ns

* See text for hydrographic description of water masses.
† Range of depths in each water mass where samples were collected for this study.
‡ ns, not sampled.

mass, consistently occupying the base of the water column and undergoing little seasonal modification (Fig. 2b).

Picoplankton abundance and distributions—The abundances and distributions of both *Archaea* and *Bacteria* varied significantly between the summer and winter cruises and among the various water masses. Overall, total picoplankton (DAPI-stainable cells of <2.0 μm diameter) abundance in the summer ranged from 1.5×10^4 to 1.1×10^6 cells ml^{-1}, whereas the winter picoplankton abundance ranged from approximately 10^4 to 1.6×10^5 cells ml^{-1} (Fig. 3). In the upper ocean, picoplankton abundance declined between the summer and winter. Cell abundance in the deep, aphotic waters also varied temporally; average picoplankton abundance in the CDW declined 69% between the summer and winter (Table 2).

Overall, probe-positive cells had bright, clear fluorescence, making them easily distinguished from the background. On average, we were able to quantify >89% (range 70–108%) of the picoplankton assemblage at the domain level during the austral summer and 85% (range 50–116%) during the winter (Table 2; Fig. 4), implying the polyFISH probes were robust for identification of picoplankton assemblages in both the summer and winter, even at water depths exceeding 3,000 m. Nonspecifically stained or autofluorescent cells were generally low in all samples: negative control probe-positive cell counts averaged 1% ($n = 40$) of the total prokaryotic picoplankton.

Picoplankton abundance in the austral summer—The spatial heterogeneity of the LTER sampling area was reflected in the distributions of picoplankton cells during the summer (DAPI-stainable cells) (Fig. 3). Total picoplankton abundance in the SSW was nearly twice as great in the northern sampling transect as cell concentrations in the southern transect (two-sample *t*-test, $p < 0.01$, data not shown). There were also strong vertical gradients in cell abundance with picoplankton abundance decreasing nearly fourfold between the SSW and CDW (Table 2).

Upon hybridization with the GI probe, GI probe–positive cells were readily distinguished by a characteristic two-lobed morphology previously described by Preston et al. (1996) and DeLong et al. (1999). GI abundance was very low in the SSW (4.7×10^3 cells ml^{-1}), increasing substantially in the mid- and lower water column (Table 2; Fig. 5a). GI probe–positive cell abundances in the SSW were (on average) not significantly different from the control probe counts (two-sample *t*-test, $p > 0.05$), indicating that the average surface water GI cell concentrations were at or below the detection limits of this method. Although the relative abundance of GI cells in the SSW was low, the characteristic morphology of these cells enabled positive identification of the GI-hybridized cells.

GI abundance in the SSW accounted for ~1% of the total picoplankton assemblage (Table 2; Fig. 6a), statistically indistinguishable from the negative probe count. Abundance of GI cells had a subsurface peak, with maximal cell abundances in the WW at ~100 m (Fig. 5a). GI probe–positive cells in the middepth WW averaged 2.0×10^4 cells ml^{-1}, more than a fourfold increase relative to the SSW (Table 2).

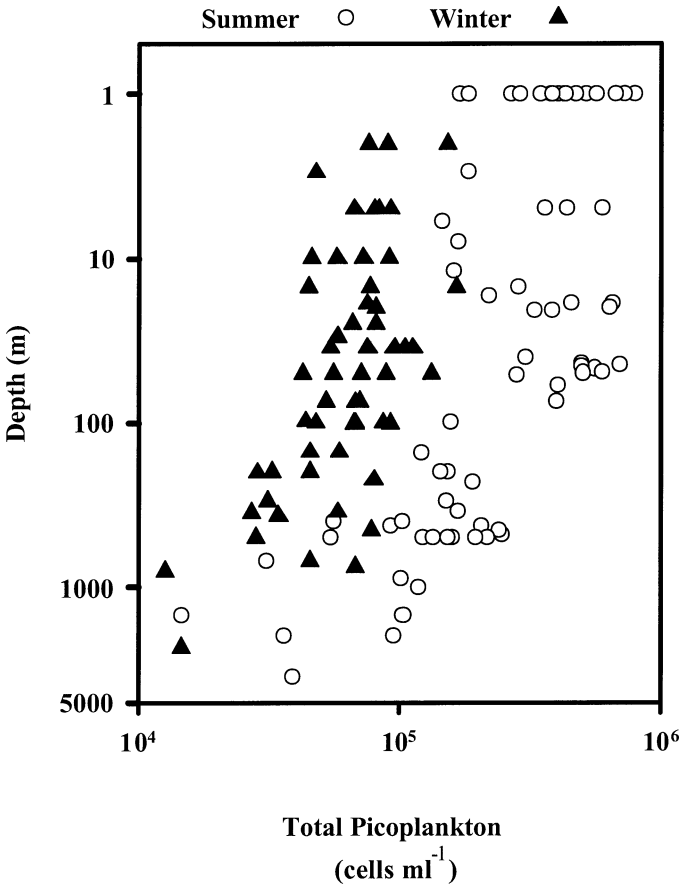

Fig. 3. Total picoplankton abundances for all stations sampled on both the summer and winter cruises. Picoplankton abundance determined by epifluorescent microscope counts of DAPI-stained cells.

Below the middepth WW, GI cell concentrations remained roughly constant into the deep CDW (mean, 2.1×10^4 cells ml^{-1}; Table 2; Fig. 5a). The increase in GI cells between the SSW and the deep CDW was notable given that total picoplankton abundance declined nearly 70% between the SSW and CDW (Table 2). The average proportion of GI cells increased with depth, averaging 5% of the total picoplankton in the WW, and increasing to 17% in the CDW (Table 2).

GII abundance was low or undetectable throughout the water column (Fig. 5b). The proportion of GII cells ranged from <1% to 6% of the total picoplankton assemblage (Fig. 6b), and there were no significant differences in the average proportion of GII cells among any of the water masses examined (one-way ANOVA, $p > 0.05$). The average abundance of GII cells in the upper ocean was not significantly different from GI cell abundance (two-sample *t*-test, $p > 0.05$); however, GII cells were an order of magnitude less abundant than the GI cells in the CDW (Table 2; Fig. 5b).

Bacteria dominated picoplankton abundances throughout the entire water column in the waters west of the Antarctic Peninsula. Bacterial abundance accounted for between 70 and 99% of the picoplankton assemblages in the upper ocean and between 55 and 84% in the CDW (Fig. 6c). Bacterial abundance was relatively constant in the upper water col-

Table 2. Abundances and relative proportions* of *Archaea* and *Bacteria* in water masses west of the Antarctic Peninsula.

		Abundance (10^4 cells ml^{-1})				Proportion (%)			
			Archaea			*Archaea*			
Season	Water mass	Total†	GI	GII	*Bacteria*	GI	GII	*Bacteria*	Total‡
Summer	SSW	41.9	0.5	0.3	38.5	1.2	0.8	88.5	90.5
	(n=36)§	(20.8)	(0.7)	(0.4)	(19.2)	(1.8)	(1.0)	(7.00)	(6.89)
	WW	46.2	2.0	0.3	38.5	5.0	0.9	88.0	94.5
	(n=9)	(15.7)	(2.9)	(0.4)	(13.8)	(7.2)	(1.2)	(7.33)	(8.34)
	CDW	12.9	2.1	0.3	8.9	17.1	2.0	70.0	89.0
	(n=28)	(6.5)	(1.2)	(0.4)	(4.7)	(6.46)	(1.9)	(8.96)	(9.78)
Winter	WW	8.5	0.9	0.3	6.0	10.4	3.3	69.0	82.7
	(n=45)	(3.3)	(0.5)	(0.2)	(2.8)	(4.24)	(2.46)	(12.5)	(15.1)
	CDW	4.0	0.4	0.2	2.9	12.6	4.1	72.3	88.8
	(n=20)	(2.0)	(0.2)	(0.2)	(1.5)	(5.71)	(2.6)	(8.96)	(11.9)

* Top numbers are average abundances or relative proportions of specific picoplankton groups; numbers in parentheses are standard deviations of the means.
† Total picoplankton abundance determined by DAPI-stained cells. GI + GII + *Bacteria* not necessarily equal to total picoplankton because of <100% total cell identification.
‡ Mean percent polyFISH probe GI + GII + *Bacteria*-positive cells as a percentage of total DAPI counts.
§ *n*, total number of samples from each water mass.

umn, declining with depth; the average bacterial abundance in the SSW was 3.9×10^5 cells ml^{-1}, decreasing to 8.9×10^4 cells ml^{-1} in the CDW (Table 2).

Picoplankton abundance in the austral winter—Total picoplankton abundances (DAPI-stained cells) were significantly lower during the austral winter than in the summer (Fig. 3). Average picoplankton abundances were 80% lower in the WW and 69% lower in the CDW in the winter (Table 2). Cell abundance generally decreased with depth, but vertical gradients in total picoplankton abundance were not as pronounced as during the summer (Table 2).

GI cells displayed relatively homogeneous distributions during the winter. GI abundances throughout the surface waters averaged 9.0×10^3 cells ml^{-1}, increasing by 44% relative to abundances measured in the SSW during the summer (Table 2). In general, upper ocean GI cell proportions were a greater fraction of total picoplankton abundance during the winter, averaging 10% of the picoplankton assemblage (Table 2; Fig. 6a). In the CDW, GI abundance was nearly fivefold lower in the winter than in the summer, but GI cells remained a large fraction of the total picoplankton in the CDW during the winter (averaging 13%) (Table 2; Fig. 6a).

GII cell densities were a low proportion of total picoplankton abundance during the winter (Fig. 6b). The proportion of archaeal GII cells ranged from <1% to 10% of the identifiable picoplankton cells (Fig. 6b). There were no significant differences in GII abundance among the water masses sampled during the winter cruise, nor were there significant differences in GII abundances between the summer and winter (two-sample *t*-test, $p > 0.05$).

Bacterial abundances during the winter cruise were significantly lower than cell abundances during the summer. The average bacterial abundance during the winter in the WW was 6.0×10^4 cells ml^{-1}, an 84% decline in abundance relative to the SSW. CDW bacterial abundance was also significantly lower than during the summer, averaging 2.9×10^4 cells ml^{-1}, equating to a 67% decline relative to the summer. Vertical gradients in cell abundance were somewhat less pronounced during the winter: bacterial abundance decreased ~50% between the WW and the CDW during the winter, compared to a ~75% decrease between the SSW and CDW during the summer (Table 2).

Discussion

The primary objectives of this study were to quantify the abundances and distributions of archaeal Group I Crenarchaeota, archaeal Group II Euryarchaeota, and *Bacteria* cells in the waters west of the Antarctic Peninsula. By examining how the abundance and distributions of these populations changed between the summer and winter, we hoped to gain insight into the temporal dynamics of microbial food webs in these poorly understood remote habitats. By using the recently developed polyFISH method, we successfully discriminated the major components of the picoplankton assemblage in the waters west of the Antarctic Peninsula. The two most important observations obtained from this study were that picoplankton abundance and phylogenetic composition varied among the water masses found off the Antarctic Peninsula and that the abundances of both *Archaea* and *Bacteria* varied significantly between the summer and winter cruises within each water mass. These results indicate that, on seasonal time scales, there were imbalances in net population growth of both *Archaea* and *Bacteria*, suggesting selective losses of the individual prokaryote subpopulations.

FISH detection of Archaea *in the Southern Ocean*—A few other studies have utilized FISH to quantify the abundance of *Archaea* in the Southern Ocean. Murray et al. (1998) used a suite of oligonucleotide rRNA FISH probes to enumerate planktonic *Bacteria* and *Archaea* from coastal seawater off the west Antarctic Peninsula in the early austral spring. They estimated that GI abundance in the upper ocean (0–50 m) ranged from 9.0×10^3 to 2.7×10^4 cells ml^{-1}, and *Bacteria* were 6.0×10^4–1.4×10^5 cells ml^{-1} (Murray et al. 1998). During the austral summer in the upper ocean of offshore

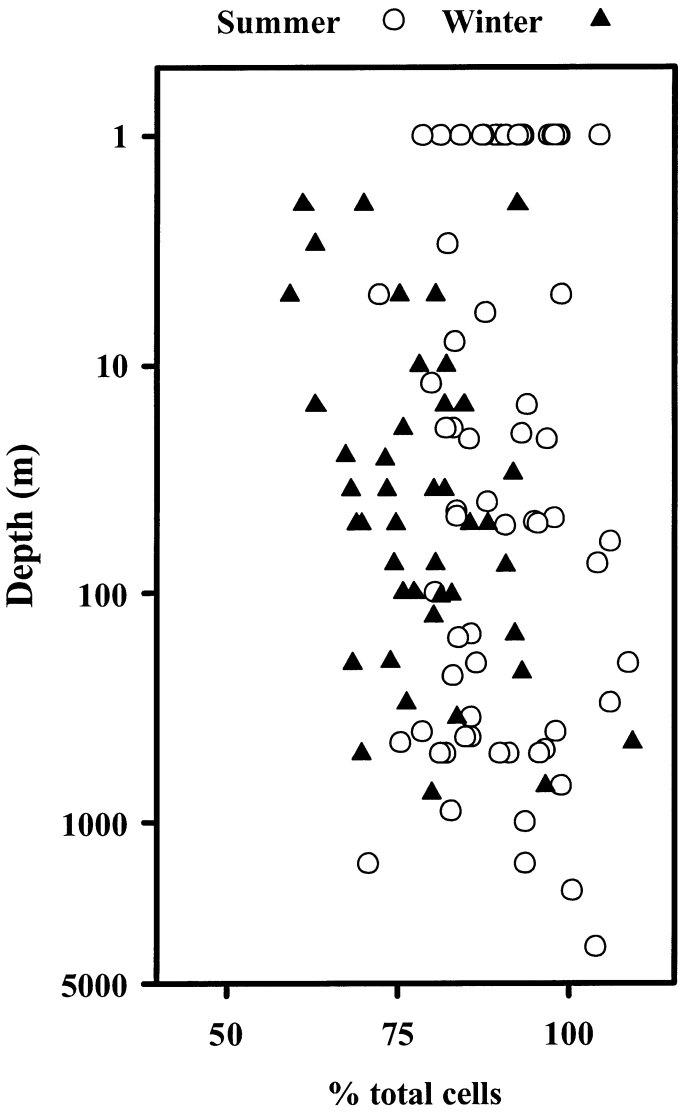

Fig. 4. Depth profile of probe-positive picoplankton normalized to total picoplankton abundance (DAPI-stained cells) for both summer and winter cruises.

Southern Ocean waters, Glöckner et al. (1999) did not detect *Archaea* by oligoFISH; however, only 50–60% of the total picoplankton assemblage was identified in their study. Similarly, using oligoFISH, Simon et al. (1999) failed to identify *Archaea* in the surface waters of the Southern Ocean during the austral summer despite characterizing >80% of the total picoplankton assemblage. OligoFISH methods appear less sensitive than the polyFISH method for enumerating marine picoplankton in coastal and open ocean habitats (Pernthaler et al. 2002), and these methodological differences might explain the apparent discrepancies between the present study and past studies using oligoFISH.

Vertical segregation of Archaea *and* Bacteria—Our observations on the vertical abundance of planktonic *Archaea* and *Bacteria* corroborate past investigations describing the relative distributions of planktonic archaeal rRNA in Antarctic marine picoplankton communities. In a study conducted in the Gerlache Strait, Antarctica, Massana et al. (1998) estimated that *Archaea* accounted for <1–15% of the surface water microbial rRNA, increasing to 25% at 500 m depth. Murray et al. (1998) also described a subsurface maximum in archaeal rRNA in the waters of the Palmer Basin, Antarctica, during the early austral spring, with archaeal rRNA accounting for up to 17% of the total picoplankton rRNA. Subsequent evaluation of the relative proportion of archaeal rRNA to total bulk picoplankton rRNA revealed that *Archaea* constitute a significant but variable fraction of the picoplankton community in subsurface Antarctic waters (Murray et al. 1999*b*).

The summertime distributions and abundances of GI cells appeared consistent with GI cell distributions in temperate ecosystems. DeLong et al. (1999) found greatest GI abundance off the coast of California at water depths of 60–150 m (total water depths ranged from ~250 to 4,400 m). Consistent with these results, GI appeared consistently more abundant near the base of the photic zone (~150–200 m) in the oligotrophic subtropical North Pacific (Karner et al. 2001). Surface water GI abundances ($\sim 5 \times 10^3$ cells ml^{-1}) in the western Antarctic Peninsula were approximately an order of magnitude lower than abundances measured in the North Pacific ($\sim 5 \times 10^4$ cells ml^{-1}; Karner et al. 2001), but roughly equivalent to those measured off California (5×10^3–1×10^4 cells ml^{-1}; DeLong et al. 1999). In the mesopelagic waters (150–1,000 m) off the Antarctic Peninsula, GI cell concentrations were approximately equal to those measured in the North Pacific midwaters (~ 2–5×10^4 cells ml^{-1}), but roughly sevenfold lower than GI abundance in California coastal waters (DeLong et al. 1999). In the deep, bathypelagic waters (1,000–3,500 m), GI abundances in the Antarctic were approximately equivalent to cell concentrations measured in both the North Pacific and off the California coast ($\sim 2 \times 10^4$ cells ml^{-1}).

The metabolic capabilities of planktonic *Archaea* remain unclear (Ouverney and Fuhrman 2001; Pearson et al. 2001; Wuchter et al. 2003), but the vertical distributions of *Archaea* and *Bacteria* observed in our study might reflect utilization of different resources by these picoplankton groups. The relative success of bacterial populations during the productive summer suggests that bacterial growth is tied to the seasonally phased production of labile organic matter; conversely, the high abundance of GI populations in the mid- and deep water column suggests these organisms were successful in the region of the water column where fluxes of labile organic matter are restricted, light fluxes are low or nonexistent, inorganic nutrient concentrations are high, and both remineralization and oxidation are the dominant picoplankton processes.

Temporal variability in archaeal abundance—In addition to vertical gradients in picoplankton abundance, we also observed pronounced temporal changes in the abundances of specific groups of picoplankton. GI generally comprised an insignificant component of surface water picoplankton assemblages during the summer, becoming more abundant during the winter. Murray et al. (1998) estimated that archaeal rRNA contributed between 14 and 29% to the wintertime

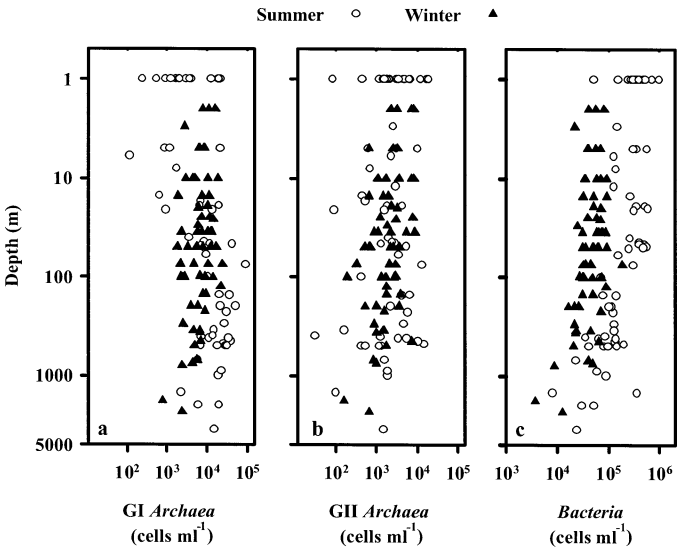

Fig. 5. Depth profiles of absolute abundance (cells ml^{-1}) of GI, GII, and *Bacteria* during summer and winter cruises.

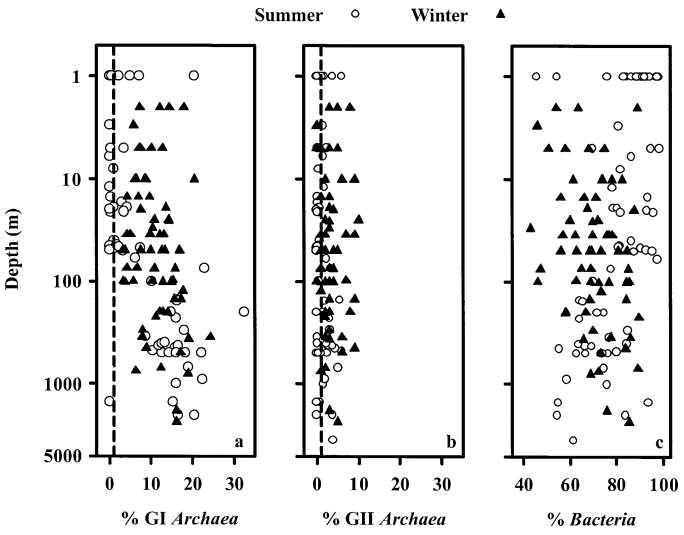

Fig. 6. Depth profiles showing the relative proportion of picoplankton cells identified using either the GI, GII, or *Bacteria* polyFISH probes. Dotted lines are mean control probe counts, indicating the detection limit of probe-positive cell enumerations.

universal probe-bound rRNA in the surface waters near Anvers Island. The same study found that archaeal rRNA fell to <1% of the universal probe during the austral summer. In support of these observations, Murray et al. (1999*b*) estimated that *Archaea* accounted for 7–36% of the surface water picoplankton rRNA in the winter, declining to 1% in the summer. Seasonality in archaeal populations does not appear restricted to polar marine environments. Murray et al. (1999*a*) observed large temporal fluctuations in populations of both GI and GII archaeal rRNA in the coastal waters of the Santa Barbara Channel, and Pernthaler et al. (2002) observed a large seasonal change in surface water GII abundances in the North Sea.

In the deeper waters west of the Antarctic Peninsula, the temporal response of GI cells was fundamentally different from that observed in the upper ocean. Assuming that physical transport of cells by seawater circulation was the same for all picoplankton, the differences between archaeal and bacterial cell accumulations in the CDW suggest that net archaeal population growth might have exceeded bacterial growth by as much as 60% in the meso- and bathypelagic waters. Over relatively long timescales (>1 month), even small imbalances in growth and removal among these two subpopulations of picoplankton could result in large changes in the abundance of these groups. Similar ''summer blooms'' of GII Archaea have been observed in coastal California waters and in the North Sea (Pernthaler et al. 2002; DeLong et al. unpubl. data). Our observations suggest that in polar waters, GI abundances in the deep water column also experience seasonal imbalances in population growth and removal.

This study confirmed several previous observations describing the low relative proportion of GII *Archaea* in Southern Ocean ecosystems (DeLong et al. 1998; Massana et al. 1998, 2000; Murray et al. 1998, 1999*b*; Béjà et al. 2002). In contrast to these reports, euryarchaeotal rRNA gene sequences were retrieved from the meso- and bathypelagic waters around Antarctica (López-Garcia et al. 2001*a*,*b*), but the quantitative significance of this study is difficult to assess since the analyses relied solely on PCR-amplified rRNA gene libraries. Through direct polyFISH identification of *Archaea*, we did not find appreciable GII cell abundances in any of our samples, irrespective of season. The average proportion of GII cells in the Antarctic was indistinguishable from the control probe counts throughout the entire water column, with no significant differences in the average GII abundance between the two cruises. To date, it is not known what ecological or biogeochemical role the *Archaea* play in marine ecosystems, but our results and prior phylogenetic picoplankton surveys suggest that GII populations are not numerically abundant members of Southern Ocean plankton communities (DeLong et al. 1998; Massana et al. 1998, 2000; Murray et al. 1999*b*).

Bacterial populations also experienced substantial seasonal modification; however, unlike the distributions of GI cells, changes in bacterial abundance were most pronounced in the upper ocean. Bacterial abundance in the SSW averaged 3.9 $\times$ 10^5 cells ml^{-1}, approximately sixfold greater than concentrations measured in the winter surface waters (Table 2). The seasonal removal of bacterial biomass in the surface waters during the winter coincided with a 40% enrichment in GI cells, suggesting that removal processes were highly selective for *Bacteria* or were not balanced as effectively by growth as that of GI *Archaea.* The large increase in bacterial abundance with the onset of the austral summer presumably reflects an enhancement of bacterial growth over removal in the upper water column. Given the large increase in phytoplankton biomass in the summer (Fig. 2a), it is likely that surface water bacterial assemblages responded to enhanced organic matter fluxes brought about by the large seasonal increase in phytoplankton biomass. The response of bacterial abundance to seasonally driven upper ocean processes suggests that *Bacteria* contribute most substantially to prokary-

ote abundance when energy fluxes through the planktonic community are at their seasonal peak. As a corollary, *Archaea* either cannot successfully compete with *Bacteria* for the seasonally accumulated phytoplankton-derived organic matter, or do not use organic matter as their primary source of carbon and energy.

Distributions of pelagic Archaea *and* Bacteria—Little is known about the ecophysiological niche or niches of marine *Archaea*; however, several recurring trends appear in the emerging information on archaeal distributions in the sea. First, the Crenarchaeota are widespread in a variety of aquatic environments that include both coastal and open ocean ecosystems (Massana et al. 2000). Second, the abundance and relative proportion of Crenarchaeota generally appear to increase with depth (Massana et al. 1997; DeLong et al. 1999; Karner et al. 2001). Third, Euryarchaeota cell abundance appears mostly restricted to the upper water column in temperate ecosystems (Murray et al. 1999*a*; Massana et al. 2000). Finally, temporal fluctuations in archaeal abundance suggest they are active members of marine plankton food webs (Murray et al. 1998; Murray et al. 1999*a*).

Our results provide the first evidence that the abundances of *Archaea* in the west Antarctic Peninsula undergo pronounced seasonal changes. Most remarkably, the phylogenetic composition and abundances of picoplankton populations in both the meso- and bathypelagic waters also demonstrated large seasonal changes. The observed temporal shifts in archaeal and bacterial abundance could reflect the dependence of these picoplankton populations on seasonally variable biogeochemical and trophodynamic processes. The coincident summertime reduction in *Archaea* and increased abundance of *Bacteria* suggests that these two picoplankton populations have different input and removal mechanisms and likely play different roles in ocean ecology.

References

AMANN, R. I., L. KRUMHOLZ, AND D. A. STAHL. 1990. Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. J. Bacteriol. **172:** 762–770.

BARNES, S. M., C. F. DELWICHE, J. D. PALMER, AND N. R. PACE. 1996. Perspectives on archaeal diversity, thermophily and monophyly from environmental rRNA sequences. Proc. Natl. Acad. Sci. USA **93:** 9188–9193.

BÉJÀ, O., AND OTHERS. 2002. Comparative genomic analysis of archaeal genotypic variants in a single population and in two different oceanic provinces. Appl. Environ. Microbiol. **68:** 335–345.

CONNON, S. A., AND S. J. GIOVANNONI. 2002. High-throughput methods for culturing microorganisms in very-low-nutrient media yield diverse new marine isolates. Appl. Environ. Microbiol. **68:** 3878–3885.

DELONG, E. F. 1992. Archaea in coastal marine environments. Proc. Natl. Acad. Sci. USA **89:** 5685–5689.

———, G. S. WICKHAM, AND N. R. PACE. 1989. Phylogenetic stains: Ribosomal RNA-based probes for the identification of single microbial cells. Science **243:** 1360–1363.

———, K. Y. WU, B. B. PREZELIN, AND R. V. M. JOVINE. 1994. High abundance of *Archaea* in marine picoplankton. Nature **371:** 695–687.

———, L. L. KING, R. MASSANA, H. CITTONE, A. MURRAY, C. SCHLEPER, AND S. G. WAKEHAM. 1998. Diphytanyl ether lipids in nonthermophilic crenarchaeotes. Appl. Environ. Microbiol. **64:** 1133–1138.

———, L. T. TAYLOR, T. L. MARSH, AND C. M. PRESTON. 1999. Visualization and enumeration of marine planktonic *Archaea* and *Bacteria* by using polyribonucleotide probes and fluorescent in situ hybridization. Appl. Environ. Microbiol. **65:** 5554–5563.

FUHRMAN, J. A., AND A. A. DAVIS. 1997. Widespread *Archaea* and novel *Bacteria* from the deep sea as shown by 16s rRNA gene sequences. Mar. Ecol. Prog. Ser. **150:** 275–280.

———, AND C. C. OUVERNEY. 1998. Marine microbial diversity studied via 16s rRNA sequences: Cloning results from coastal waters and counting of native *Archaea* with fluorescent singe cell probes. Aquat. Ecol. **32:** 3–15.

———, K. MCCALLUM, AND A. A. DAVIS. 1992. Novel major archaebacterial group from marine plankton. Nature **356:** 148–149.

GIOVANNONI, S. J., T. B. BRITSCHGI, C. L. MOYER, AND K. G. FIELD. 1990. Genetic diversity in Sargasso Sea bacterioplankton. Nature **345:** 60–63.

GLÖCKNER, F. O., B. M. FUCHS, AND R. I. AMANN. 1999. Bacterioplankton compositions of lakes and oceans: A first comparison based on fluorescence in situ hybridization. Appl. Environ. Microbiol. **65:** 3721–3726.

HOFMANN, E. E., AND J. M. KLINCK. 1998. Hydrography and circulation of the Antarctic continental shelf: 150°E to the Greenwich meridian, p. 997–1042. *In* A. R. Robinson and K. H. Brink [eds.], The Sea. Vol. 11. Wiley.

———, ———, C. M. LASCARA, AND D. A. SMITH. 1996. Water mass distribution and circulation west of the Antarctic Peninsula and including Bransfield Strait. *In* R. Ross, E. E. Hofmann, and L. B. Quetin [eds.], Foundations for ecological research west of the Antarctic Peninsula. Antarctic Research Series, American Geophysical Union.

HOLM-HANSEN, O., AND B. G. MITCHELL. 1991. Spatial and temporal distribution of phytoplankton and primary production in the western Bransfield Strait region. Deep-Sea Res. **38:** 961–980.

KARL, D. M., O. HOLM-HANSEN, G. T. TAYLOR, G. TIEN, AND D. F. BIRD. 1991. Microbial biomass and productivity in the western Bransfield Strait, Antarctica during the 1986–87 austral summer. Deep-Sea Res. II **38:** 1029–1055.

———, J. R. CHRISTIAN, J. E DORE, AND R. M. LETELIER. 1996. Microbiological oceanography in the region west of the Antarctic Peninsula: Microbial dynamics, nitrogen cycle and carbon flux. *In* R. Ross, E. E. Hofmann, and L. B. Quetin [eds.], Foundations for ecological research west of the Antarctic Peninsula. Antarctic Research Series, American Geophysical Union.

KARNER, M. B., E. F. DELONG, AND D. M. KARL. 2001. Archaeal dominance in the mesopelagic zone of the Pacific Ocean. Nature **409:** 507–510.

LÓPEZ-GARCÍA, P., A. LÓPEZ-LÓPEZ, D. MOREIRA, AND F. RODRÍGUEZ-VALERA. 2001*a*. Diversity of free-living prokaryotes from a deep-sea site at the Antarctic Polar Front. FEMS Microbiol. Ecol. **36:** 193–202.

———, D. MOREIRA, A. LÓPEZ-LÓPEZ, AND F. RODRÍGUEZ-VALERA. 2001*b*. A novel haloarchaeal-related lineage is widely distributed in deep oceanic regions. Environ. Microbiol. **3:** 72–78.

———, F. RODRÍGUES-VALERA, C. PEDRÓS-ALIÓ, AND D. MOREIRA. 2001*c*. Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. Nature **409:** 603–607.

MASSANA, R., A. E. MURRAY, C. M. PRESTON, AND E. F. DELONG.

1997. Vertical distribution and phylogenetic characterization of marine planktonic *Archaea* in the Santa Barbara Channel. Appl. Environ. Microbiol. **63:** 50–56.

———, L. T. TAYLOR, A. E. MURRAY, K. Y. WU, W. H. JEFFREY, AND E. F. DELONG. 1998. Vertical distribution and temporal variation of marine planktonic *Archaea* in the Gerlache Strait, Antarctica, during early spring. Limnol. Oceanogr. **43:** 607–617.

———, E. F. DELONG, AND C. PEDRÓS-ALIÓ. 2000. A few cosmopolitan phylotypes dominate planktonic archaeal assemblages in widely different oceanic provinces. Appl. Environ. Microbiol. **66:** 1777–1787.

MOYER, C. L., J. M. TIEDJE, F. C. DOBBS, AND D. M. KARL. 1998. Diversity of deep-sea hydrothermal vent *Archaea* from Loihi Seamount, Hawaii. Deep-Sea Res. II **45:** 303–317.

MURRAY, A. E., AND OTHERS. 1998. Seasonal and spatial variability of bacterial and archaeal assemblages in the coastal waters near Anvers Island, Antarctica. Appl. Environ. Microbiol. **64:** 2585–2595.

———, AND OTHERS. 1999*a*. A time series assessment of planktonic archaeal variability in the Santa Barbara Channel. Aquat. Microb. Ecol. **20:** 129–145.

———, K. WU, C. MOYER, D. M. KARL, AND E. F. DELONG. 1999*b*. Evidence for circumpolar distribution of planktonic *Archaea* in the Southern Ocean. Aquat. Microb. Ecol. **18:** 263–273.

NILLER, P. P., A. AMOS, AND J. H. HU. 1991. Water masses and 200 m relative geostrophic circulation in the western Bransfield Strait region. Deep-Sea Res. **38:** 943–959.

OLSEN, G. J., D. J. LANE, S. J. GIOVANNONI, N. R. PACE, AND D. A. STAHL. 1986. Microbial ecology and evolution: A ribosomal RNA approach. Annu. Rev. Microbiol. **40:** 337–365.

OUVERNEY, C. C., AND J. A. FUHRMAN. 2001. Marine planktonic *Archaea* take up amino acids. Appl. Environ. Microbiol. **66:** 4829–4833.

PEARSON, A., A. P. MCNICHOL, B. C. BENITEZ-NELSON, J. M. HAYES, AND T. I. EGLINTON. 2001. Origins of lipid biomarkers in Santa Monica Basin surface sediment: A case study using compound-specific $\Delta^{14}C$ analysis. Geochim. Cosmochim. Acta **65:** 3123–3137.

PERNTHALER, A., C. M. PRESTON, J. PERNTHALER, E. F. DELONG, AND R. AMANN. 2002. A comparison of fluorescently labeled oligonucleotide and polynucleotide probes for the detection of pelagic marine *Bacteria* and *Archaea*. Appl. Environ. Microbiol. **68:** 661–667.

PRESTON, C. M., K. Y. WU, T. F. MOLINSKI, AND E. F. DELONG. 1996. A psychrophilic crenarchaeon inhabits a marine sponge: *Cenarchaeum symbiosum* gen. nov., sp. nov. Proc. Natl. Acad. Sci. USA **93:** 6241–6246.

RAPPÉ, M. S., S. A. CONNON, K. L. VERGIN, AND S. J. GIOVANNONI. 2002. Cultivation of the ubiquitous SAR 11 marine bacterioplankton clade. Nature **418:** 630–633.

SCHLITZER, R. 2002. Ocean data view. Internet site http://www.awi-bremerhaven.de/GEO/ODV. Accessed 2002.

SIMON, M., F. O. GLÖCKNER, AND R. AMANN. 1999. Different community structure and temperature optima of heterotrophic picoplankton in various regions of the Southern Ocean. Aquat. Microb. Ecol. **18:** 275–284.

SMITH, D. A., E. E. HOFMANN, J. M. KLINCK, AND C. M. LASCARA. 1999. Hydrography and circulation of the West Antarctic Peninsula Continental Shelf. Deep-Sea Res. I **46:** 925–949.

SMITH, R. C., AND OTHERS. 1995. The Palmer LTER: A long-term ecological research program at Palmer Station, Antarctica. Oceanography **8:** 77–86.

STAMMERJOHN, S. E., AND R. C. SMITH. 1996. Spatial and temporal variability of Western Antarctic Peninsula sea ice coverage, *In* R. Ross, E. E. Hofmann, and L. B. Quetin [eds.], Foundations for ecological research west of the Antarctic Peninsula. Antarctic Research Series, American Geophysical Union.

TREBESIUS, K., R. AMANN, W. LUDWIG, K. MÜHLEGGER, AND K. H. SCHEIFER. 1994. Identification of whole fixes bacterial cells with nonradioactive 23s rRNA–targeted polynucleotide probes. Appl. Environ. Microbiol. **60:** 3228–3235.

WOESE, C. R. 1987. Bacterial evolution. Microbiol. Rev. **51:** 221–271.

WUCHTER, C., S. SCHOUTEN, H. T. S. BOSCHKER, AND J. S. S. DAMSTÉ. 2003. Bicarbonate uptake by marine Crenarchaeota. FEMS Microbiol. Lett. **219:** 203–207.

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