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Abundance and single-cell activity of bacterial groups in Antarctic coastal waters

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Abstract

We estimated the abundance and single-cell activity of bacterial groups in waters off the West Antarctic Peninsula (WAP) using a combination of microautoradiography and fluorescent in situ hybridization (FISH). The abundance of the Ant4D3 subgroup, detected by a new FISH probe, was 10% of the total community and half of the gammaproteobacterial population. The Ant4D3, *Polaribacter*, and SAR11 subgroups accounted for the majority of the *Gammaproteobacteria*, *Sphingobacteria–Flavobacteria*, and *Alphaproteobacteria*, respectively. Approximately 40% of the total microbial community actively incorporated leucine (added at 20 nmol L^{-1}), while a smaller fraction (12–22%) used protein and an amino acid mixture (added at tracer concentrations). The fractions of SAR11, *Polaribacter*, and Ant4D3 that were active differed from each other and varied among substrates. SAR11 had the largest fraction of active cells incorporating leucine, while *Polaribacter* dominated the community using protein. The fraction of Ant4D3 using different compounds did not vary, but this group dominated the incorporation of amino acids, and was an abundant and active component of the bacterial community. Bacteria in the WAP region were as active as bacteria in the Mid-Atlantic Bight, even though total bacterial production was lower in the WAP. Though persistently cold (0–1°C) and dominated by different bacterial taxa, the single-cell activity of this summertime Antarctic bacterial community was comparable to that of temperate communities.

Polar regions are important but understudied biomes with unique ecological characteristics (Kirchman et al. 2009*b*). Both Arctic and Antarctic marine waters are characterized by relatively constant low temperatures and high seasonal variability in light flux. One major difference between the two is that the Arctic Ocean receives terrestrial inputs of organic material, while the Southern Ocean and coastal waters of Antarctica do not. However, primary production is still the largest source of labile organic material to both polar oceans (Ducklow et al. 2006). Rates of primary production are often quite high in both polar systems, but bulk bacterial production (^{3}H-leucine incorporation rates) in the Ross Sea (Antarctica) and in various Arctic waters is low compared to rates in temperate regions (Ducklow et al. 2001; Kirchman et al. 2009*b*). Consequently, the ratio of bacterial production to primary production is lower in both the Arctic Ocean and Ross Sea than in temperate oceans (Kirchman et al. 2009*b*).

The low rates of bacterial production in polar regions could be due to bacteria being inactive in these perennially cold waters (Ducklow et al. 2001; Smith and del Giorgio 2003). Early work suggested that bacterial growth is limited in cold temperatures, potentially requiring higher concentrations of substrate to achieve rates similar to bacterial growth in warmer, temperate waters (Pomeroy and Wiebe 2001). Yet single-cell studies have shown that in spite of low bulk rates, the fraction of individual cells that are active in the Arctic Ocean is high (Elifantz et al. 2007; Malmstrom et al. 2007; Vila-Costa et al. 2008). Single-cell activity has been examined much less extensively in Antarctic waters. The single relevant study also found that similar fractions of cells use leucine in the Weddell Sea as in temperate waters (Grossman 1994).

Many of the broad taxa of bacteria found in lower latitudes are also present in the Arctic Ocean and in Antarctic waters as well (Bano and Hollibaugh 2002; Elifantz et al. 2007; Murray and Grzymski 2007). Culture-independent studies suggest that although gammaproteobacterial abundance is often low in temperate waters, this group may be abundant in polar systems (Glöckner et al. 1999; Pommier et al. 2007; Schattenhofer et al. 2009). The *Gammaproteobacteria* are the third-most abundant group in the western Arctic, following the *Sphingobacteria–Flavobacteria* (SF group) and *Alphaproteobacteria* (Elifantz et al. 2007; Alonso-Saez et al. 2008*a*). Antarctic bacterial communities have not been characterized as extensively as those in Arctic waters, and it is not clear if the two polar systems are dominated by the same broad taxa of bacteria.

The alphaproteobacterial clade SAR11 is abundant in many marine environments and may play a role in polar systems as well (Malmstrom et al. 2007; Schattenhofer et al. 2009). SAR11 is the most abundant group in Arctic surface waters, comprising ~20% of the bacterial community, with 15–40% of the group actively incorporating leucine (Malmstrom et al. 2007; Kirchman et al. 2010). This fraction of active SAR11 cells is similar to what has been observed in temperate waters (Malmstrom et al. 2005). SAR11 is present in Antarctic waters, but quantitative estimates of abundance are still needed (Murray and Grzymski 2007). Though lower in abundance, high fractions of *Roseobacter* and the *Polaribacter* clade of the SF group were active in Franklin Bay and the Chukchi Sea of the western Arctic Ocean (Malmstrom et al. 2007; Alonso-Saez et al. 2008*a*). The abundance and single-cell

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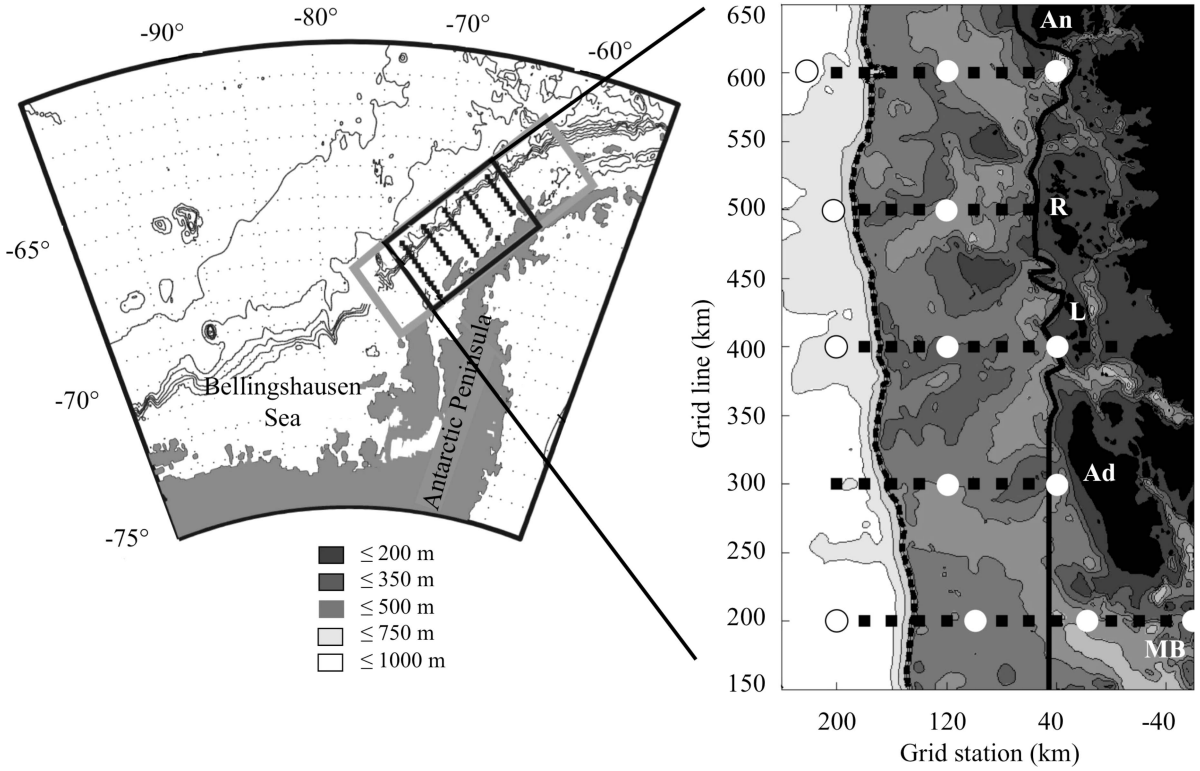

Fig. 1. Sampling locations (white dots) on transects off the West Antarctic Peninsula. An: Anvers Island; R: Renaud Island; L: Lavoisier Island; Ad: Adelaide Island; MB: Marguerite Bay. Solid line denotes coastal domain; dashed line denotes continental shelf break; area between the lines is the continental shelf domain (shelf) and area beyond shelf break is continental slope and deep ocean domain (offshore).

activity of these groups have not been examined in Antarctic waters.

The goal of this study was to characterize the abundance and activity of bacterial groups in continental shelf waters off the West Antarctic Peninsula (WAP). Surface waters were sampled on transects up to 200 km offshore and 500 km along the Peninsula, including the water column on the shelf and deeper open oceanic waters. We used fluorescent in situ hybridization (FISH) and microautoradiography to examine the abundance of numerically important bacterial groups and their uptake of selected substrates. We found that the use of leucine, a mixture of amino acids, and protein differed within the total community and among dominant subgroups SAR11, *Polaribacter*, and the Ant4D3 clade of *Gammaproteobacteria*.

Methods

Surface waters were sampled in the austral summer of January 2007 along transects off the west coast of Antarctica aboard the icebreaker *LM Gould*. The sampling stations were in the Palmer Long Term Ecological Research sampling grid along the WAP (<http://pal.lternet.edu>; Fig. 1). The oceanographic setting and microbial ecology of this region are described in Ducklow et al. (2006). Abundance of all prokaryotes was measured using flow cytometry with SYBR green staining (Applied Biosystems). We use 'bacteria' throughout this paper because archaeal abundance in surface waters is low at this time of the year (Murray et al. 1998). Concentrations of chlorophyll *a* (Chl *a*) were measured by standard fluorescence methods. Total bacterial production was estimated using ^{3}H-leucine incorporation (20 nmol L^{-1} additions) with the microcentrifuge method, using 3–4-h incubations in the dark (Kirchman 2001). Bulk growth rates were calculated by dividing bacterial production (mg C m^{-3} d^{-1}) by biomass (mg C m^{-3}). Bacterial production was estimated from leucine incorporation, assuming a conversion factor of 1.5 kg C mol^{-1} of leucine, while biomass was estimated from bacterial abundance assuming 20 fg C $cell^{-1}$. SYPRO Ruby (Molecular Probes) protein staining to estimate cell volumes was carried out as described by Straza et al. (2009).

Quantification of bacterial groups—The abundance of bacterial groups was estimated using FISH. Water samples were fixed overnight at 4°C with paraformaldehyde (PFA, 2% final concentration). The water was filtered through 0.22-μm polycarbonate filters (Millipore) with a 0.45-μm nitrocellulose support filter (Millipore), rinsed with 0.22-μm–filtered deionized water, and stored at −20°C until further processing. Probes for *Alphaproteobacteria* (Alf968), *Polaribacter* (mixture of three probes), SAR11 clade (mixture of four probes), *Gammaproteobacteria* (Gam42a), *Sphingobacteria–Flavobacteria* group (CF319a),

all bacteria (EUB338), and a negative control were used (Malmstrom et al. 2007; Amann and Fuchs 2008). Two probe sets were used separately for *Roseobacter*: RSB67 with helper probes RSB67h3 and RBS67h5 (Zubkov et al. 2002) and a probe mixture designed for Arctic *Roseobacter* cells (Malmstrom et al. 2007).

We designed probe Ant4D3a (5′-CAA GCC AGG GCG TCG CCT-3′) for a subgroup of *Gammaproteobacteria* based on the Ant4D3 fosmid clone (Grzymski et al. 2006). The probe was tested in silico using ARB software with SILVA database release 98 (Mar 2009); it matched the 43 sequences in the Ant4D3 clade with 98 ± 2.6% similarity (Pruesse et al. 2007). A cultivated representative of the Ant4D3 clade was not available to serve as a positive control, but we tested probe specificity by varying formamide concentrations from 10% to 35%, on cultures of *Alteromonas*, *Vibrio alginolyticus*, and a *Cytophaga* strain DB362, as well as a sample from the mouth of the Delaware Bay. Probe binding was below 2% in all these tests and was not different from the negative control. Filter pieces were hybridized with cyanine-3 (Cy3)–labeled oligonucleotide probes in 30% formamide, or 35% formamide for probe CF319a. Following a final washing step, the filter pieces were either taken on to microautoradiography or transferred onto slides and stained with 4′-6-diamidino-2-phenylindole (DAPI; 0.5 ng L^{-1}) in a 4 : 1 mixture of Citifluor (Ted Pella) and Vectashield (Vector Labs) anti-fading mountants.

Incorporation of substrates by specific bacterial groups—The uptake of selected compounds by specific bacterial groups was examined using microautoradiography combined with FISH (Micro-FISH). Water samples for Micro-FISH were incubated with 20 nmol L^{-1} ^{3}H-leucine (Perkin Elmer), ^{3}H-glucose (0.5 nmol L^{-1}; Perkin Elmer), a mixture of tritiated amino acids including leucine and 14 other protein amino acids (total concentration of 0.5 nmol L^{-1}; American Radiolabeled Chemicals), or 0.4 g mL^{-1} ^{3}H-protein. Tritiated protein was made using a *Vibrio alginolyticus* culture (Cottrell and Kirchman 2000). Incubations with leucine, glucose, and the amino acid mixture lasted 4 h, while protein incubations were 8 h. All incubations were at in situ temperatures in the dark. After incubation, samples were fixed with PFA (2% final concentration) overnight at 4°C and filtered as for FISH. PFA was added to killed controls prior to the addition of labeled compounds.

Microautoradiography was carried out as described by Cottrell and Kirchman (2003). Slides were dipped in film emulsion (Amersham Hypercoat EM-1), then filter pieces were placed cell-side down onto the emulsion. A time series of exposure in the emulsion before developing was used to select the shortest time at which the number of active cells identified reached a maximum. The exposure time for leucine and amino acids samples was 2 d, and for protein samples 4 d (results not shown). At the end of the exposure time, slides were developed and fixed (Dektol developer and fixer; Kodak). After drying overnight, the filter pieces were carefully peeled away and the cells were stained with DAPI in the 4 : 1 mountant as described above.

Cells were counted using a semiautomated microscope system previously described (Cottrell and Kirchman 2003). Ten fields of view were counted from each sample, with a constant exposure time of 100 ms for silver grain images. DAPI image exposure times ranged 75–100 ms, and Cy3 image exposure times were set by the negative control and ranged 300–500 ms. Group abundances are presented as the percentage of the total number of DAPI objects that were also Cy3-labeled. The proportion of the total community that was active was calculated as the number of DAPI objects with associated silver grain clusters divided by the total number of DAPI-stained objects. Similarly, the number of probe- and DAPI-stained objects with silver grains was divided by the number of probe-positive cells to calculate the percentage of a group that was actively taking up a given compound. The contribution of each group to the total use of an organic compound was estimated from the fraction of all active cells (those with silver grains) using the compound that were probe-positive. Nonspecific binding of the negative control probe was below 5% for all samples. Percentages were arcsine-transformed prior to statistical tests.

Live and killed control filters from selected glucose experiments ($n = 5$) and amino acid experiments ($n = 3$) were radioassayed and used to calculate bulk uptake. The turnover rate constant was calculated as the radioactivity taken up per hour divided by the radioactivity added.

Bacterial activity and abundance in the mid-Atlantic Bight—To compare the activity of cells in Antarctic waters to bacterial activity in a lower latitude system, we examined leucine incorporation, single-cell activity, and other parameters in the mid-Atlantic Bight (MAB) during 2006 (Nov) and 2008 (May, Jul, and Sep). Representative samples from the Delaware Bay were chosen from a larger data set (Straza 2010) to have the same number of samples as in the WAP study ($n = 9$). The Delaware samples were from two stations at the mouth of the Delaware Bay (38°51.0′N, 75°5.9′W) and just beyond (38°50.9′N, 75°06.5′W). Incubations for total leucine incorporation and microautoradiography were similar as described above, but incubation times were shorter (0.5 h and 2 h for bulk leucine incorporation and single-cell activity, respectively) at the in situ temperature of the bay. We measured the silver grain area of cells using leucine as described by Cottrell and Kirchman (2003). Each sample was counted in duplicate from the same filter.

Results

We examined the single-cell activity of selected bacterial groups and other biogeochemical properties in transects off the West Antarctic Peninsula. The sampling region was divided into three regions (Ducklow et al. 2006): 'coast' for stations up to 40 km offshore, 'shelf' for the mid-shelf region 40–200 km from shore, and 'offshore' for deep waters beyond the shelf break (Fig. 1). Bacterial production (bulk incorporation of ^{3}H-leucine) and growth rates were highest in coastal waters (ANOVA; $p = 0.001$; Fig. 2). Chl *a* concentrations varied among regions (ANOVA; $p =$

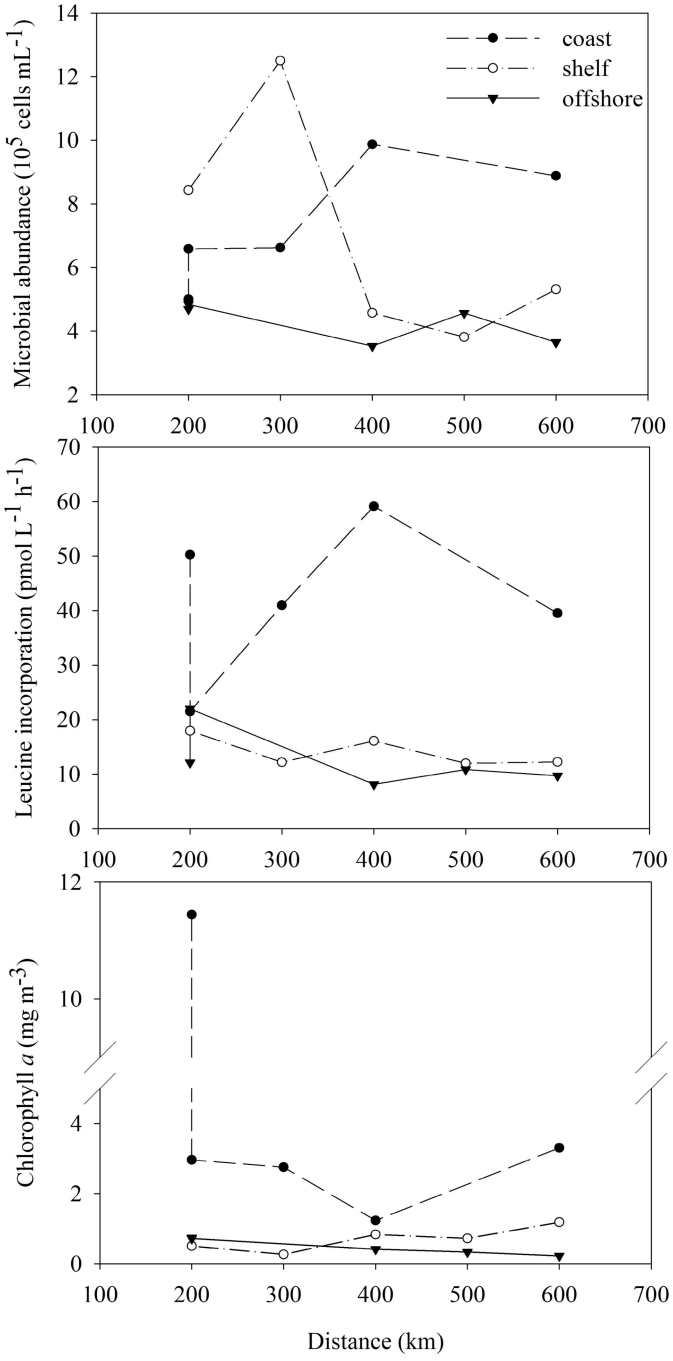

Fig. 2. Microbial abundance and bulk ^{3}H-leucine incorporation along the West Antarctic Peninsula. Horizontal axis is the distance (km) running roughly from south to north along the WAP, shown in Fig. 1.

0.04) and were highest in coastal samples even if the very high concentration at one coastal station was excluded (Fig. 2). Total bacterial abundance and bacterial biovolume based on protein staining did not differ significantly among the three regions (Table 1).

Bacterial group abundance—The abundance of bacterial groups was examined using FISH. The EUB338 probe for all bacteria labeled ~ 80% of all cells (Table 2). The three major groups examined here accounted for 70% of all microbial cells and 90% of bacterial cells (EUB338-positive). The abundances of *Alphaproteobacteria*, *Gammaproteobacteria*, and the SF group each averaged 20–30% of all microbial cells and did not differ significantly from each other or among locations. Alphaproteobacterial abundance (coefficient of variation [CV] = 0.36) varied more than the abundance of the SF group (CV = 0.21) and *Gammaproteobacteria* (CV = 0.21; Fig. 3).

The clades within the broad phylogenetic groups varied in relative abundance among locations (Fig. 4). All subgroups were equally abundant offshore, ranging from 8% to 18% of the community (Fig. 4). In coastal samples, the gammaproteobacterial clade Ant4D3 was 11% of the community, about half as abundant as *Polaribacter*. SAR11 abundance in coastal samples was between that of the other two groups, but it was the most abundant group (27%) in shelf samples. Only cells identified by the Arctic *Roseobacter* probe mixture varied in abundance by location. These *Roseobacter* cells were ~ 10% offshore, which is three-fold higher than in coastal waters (Table 2).

The Ant4D3, *Polaribacter*, and SAR11 subgroups accounted for most of the *Gammaproteobacteria*, SF group, and *Alphaproteobacteria*, respectively (Table 2). Ant4D3 averaged 10% of the bacterial community, accounting for about half of the *Gammaproteobacteria* (Table 2). The *Polaribacter* comprised 57% of the SF group, while SAR11 made up > 80% of the *Alphaproteobacteria*. The abundance of Ant4D3 cells (CV = 0.25) varied less than that of SAR11 (CV = 0.47) or *Polaribacter* (CV = 0.54).

Single-cell activity of the total community—Uptake of ^{3}H-labeled organic compounds by the total prokaryote community as assessed by microautoradiography differed among the selected compounds. Overall a greater fraction (41 ± 3%) of cells used leucine (added at 20 nmol L^{-1}) than the fraction that incorporated either the amino acid mixture (12 ± 1%) or protein (22 ± 2%; Fig. 5) given at tracer concentrations. The fraction of cells using glucose was < 5% (data not shown), and glucose samples were not further analyzed. In coastal samples, a greater fraction of cells were active (using leucine) than incorporated the amino acid mixture (ANOVA, $p = 0.036$), but the fraction using protein did not differ from the fraction of active cells as seen from leucine uptake (Fig. 5). In offshore samples, the fraction taking up leucine was three-fold greater than the fractions using amino acids and protein ($p = 0.006$; Fig. 5). However, the relative number of cells using these compounds did not differ significantly among regions.

Single-cell use of compounds by bacterial groups—The bacterial groups differed in use of the substrates we examined (Fig. 6). About 24% of *Polaribacter* cells took up leucine, less than half of the active fractions of Ant4D3 and SAR11 (~ 55%, $p = 0.022$). In contrast, four-fold larger fractions of *Polaribacter* and Ant4D3 (32–40%) used protein than did SAR11 (9.5%, $p = 0.002$). The use of the amino acid mixture was also different among the groups, with 30% of Ant4D3 cells active vs. only 10% of SAR11 and *Polaribacter* active in taking up the amino acids (p =

Table 1. Basic biogeochemical properties in waters off the West Antarctic Peninsula. Biovolume of bacterial cells was determined by protein staining. Mean ± standard error, nd = no data. Bulk measurements were taken in triplicate, and microscopy measurements are based on 10 fields of view from each sample (n = 14–15 for each sampling region).

Property	Coast	Shelf	Offshore	Mean
Total abundance (10^5 cells mL^{-1})	7.4±0.9	7.0±1.6	4.3±0.3	6.2±1.2
Chl *a* concentration (mg m^{-3})	4.2±1.8	0.7±0.16	0.5±0.1	1.9±1.3
Leucine incorporation (pmol L^{-1} h^{-1})	42±6.3	14±1.2	13±2.5	23±7.3
Bulk growth rate (d^{-1})	0.11±0.02	0.04±0.01	0.05±0.01	0.07±0.02
Leucine silver grain area (μm^2)	1.3±0.11	nd	0.99±0.03	1.2±0.63
Bacterial biovolume (μm^3)	0.13±0.03	0.13±0.04	0.13±0.06	0.13±0.01

0.046; Fig. 6). The fraction of *Polaribacter* using protein was four times higher than the fraction using amino acids (p = 0.001; Fig. 6). While a large fraction of SAR11 was actively taking up leucine added at saturating concentrations, a five-fold smaller fraction incorporated protein or amino acids added at tracer levels ($p < 0.001$; Fig. 6). In contrast to the variation seen for *Polaribacter* and SAR11, the fraction of active cells in the Ant4D3 group and in the total bacterial community (all EUB338-positive cells) did not vary among compounds (Fig. 6).

The abundance of *Roseobacter* cells was below 10% of the community in the majority of our samples, and the abundance of the active fraction was below the limit of detection by Micro-FISH. Because of this methodological limitation, the single-cell activity of this group was not measured.

Previous sections analyzed the active fraction of cells in each bacterial group. Another, separate question is the contribution of each group to the total uptake of a given compound, an independently estimated parameter that reflects both the abundance and the active fraction of a group. The contribution to the use of each compound by different groups mirrored the patterns seen within each group (Table 3). SAR11 accounted for the largest fraction of cells using leucine (22%), while Ant4D3 and *Polaribacter* contributed smaller fractions (12–15%). Around 68% of the cells that incorporated the amino acid mixture were Ant4D3. The majority of cells that incorporated protein were *Polaribacter* (Table 3).

Comparison with a low-latitude system—For comparison with the Antarctic samples, selected biogeochemical and microbial properties in the MAB were also studied (Table 4). The average temperature in the MAB during summer and autumn for the samples examined here was 18 ± 1.5°C, much higher than in WAP waters. Chl *a* concentrations averaged 8.5 ± 0.3 mg m^{-3}, only two-fold higher than in coastal WAP waters, although substantially higher than in the other WAP regions. Bacterial abundance was 10-fold greater and bulk bacterial production was six-fold greater in the MAB than in the WAP (Tables 1, 4). However, bacterial growth rates were the same in the WAP (0.07 ± 0.02 d^{-1}) and MAB (0.06 ± 0.01 d^{-1}). Around 30% of the cells were actively incorporating leucine in the MAB, about the same as observed in WAP waters.

We examined the area of the silver grain clusters around bacteria in microautoradiographic preparations in order to estimate single-cell activity more quantitatively (Sintes and Herndl 2006). Mean silver grain area around cells taking up leucine in WAP samples (1.18 ± 0.18 μm^2; n = 17; Table 1) was significantly larger (t-test, t = 2.86, df = 32, $p < 0.05$) than the area around cells in MAB waters (0.65 ± 0.06 μm^2, n = 17; Table 4).

Discussion

The abundance and single-cell activity of bacterial groups have been examined in the Arctic Ocean (Malmstrom et al. 2007; Alonso-Saez et al. 2008*a*), although

Table 2. Abundance of bacterial groups in the three WAP regions. Mean ± standard error. n = 14–15 for each group. The main phylogenetic group for the four subgroups is indicated in parentheses: Gamma = *Gammaproteobacteria*, Alpha = *Alphaproteobacteria* and SF = S*phingobacteria* and *Flavobacteria*, members of the Bacteriodetes phylum.

Group	% of Total abundance			Total
	Coast	Shelf	Offshore	
Bacteria*	84.8±0.8	75.1±1.1	79.5±1.0	79.5±0.6
Sphingobacteria–Flavobacteria group	32.5±1.0	26.8±0.6	29.0±0.6	29.4±0.4
Gammaproteobacteria	19.7±0.8	17.2±0.4	22.5±0.6	19.8±0.4
Alphaproteobacteria	24.9±0.8	22.8±0.9	20.1±0.7	22.6±0.5
Polaribacter (*Sphingobacteria–Flavobacteria*)	21.9±0.8	10.2±0.4	18.5±0.4	16.9±0.3
Ant4D3 (gamma)	10.6±0.8	9.7±0.4	10.8±0.4	10.4±0.3
SAR11 (alpha)	17.3±0.8	26.5±0.9	15.4±0.8	19.7±0.5
Roseobacter† (alpha)	9.6±0.4	4.5±0.3	6.6±0.3	6.9±0.2
Arctic *Roseobacter*‡ (alpha)	3.4±0.3	5.3±0.3	9.8±0.4	6.2±0.2

* EUB338-positive cells.
† Ros67-positive cells.
‡ Cells positive after probing with Arctic *Roseobacter* probe mix.

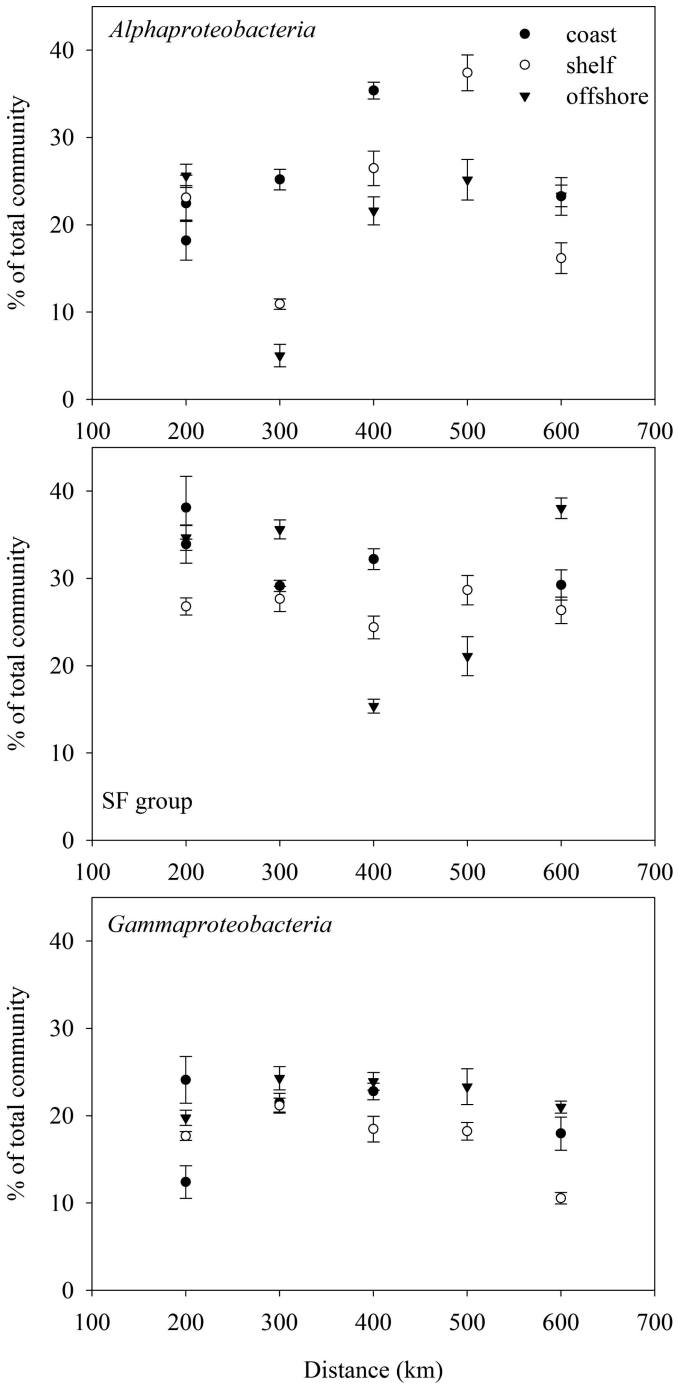

Fig. 3. Abundances of three major bacterial groups along the West Antarctic Peninsula. Error bars are one standard error. Horizontal axis is as in Fig. 2.

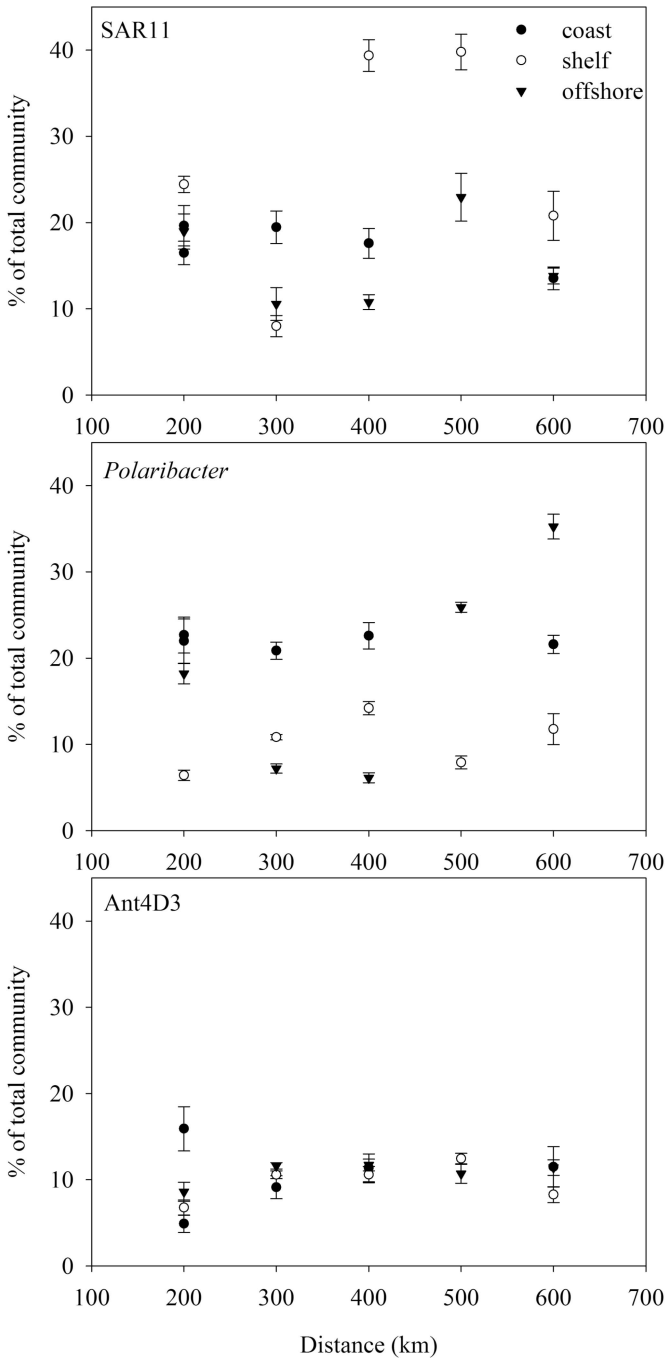

Fig. 4. Abundances of three bacterial subgroups: SAR11 (*Alphaproteobacteria*), *Polaribacter* (SF group), and Ant4D3 (*Gammaproteobacteria*). Error bars are one standard error. Horizontal axis is as in Fig. 2.

mostly at the class or phylum level, as is the case for most previous studies in other systems (del Giorgio and Gasol 2008). Because analogous data were lacking for Antarctic waters, we assessed the abundance and single-cell activity of bacterial groups in waters off the West Antarctic Peninsula during the austral summer. In this study, we examined the abundance of clades within the *Gammaproteobacteria* and SF group, and the use of leucine, a mixture of amino acids, and protein by the SAR11, *Polaribacter*, and Ant4D3 clades. We found that bacterial groups differed in abundance and substrate use between the groups and among different compounds.

A striking finding was the abundance of the Ant4D3 cluster and of the *Gammaproteobacteria* as a whole. Although the abundance of *Gammaproteobacteria* often averages $\leq 10\%$ in temperate oceanic areas (Glöckner et al.

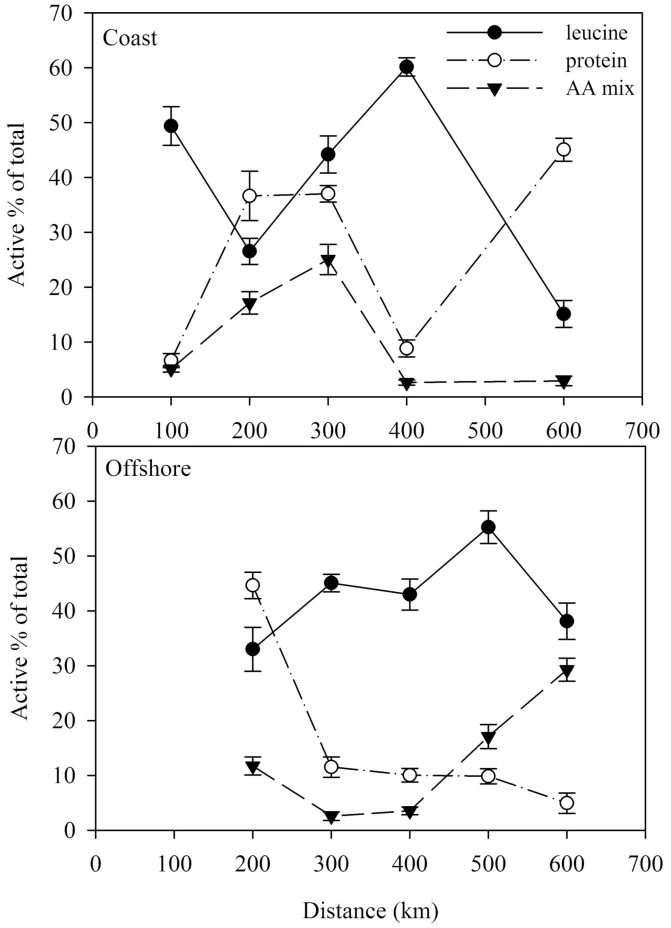

Fig. 5. Fractions of the total bacterial community actively using selected compounds in coastal and offshore waters along the West Antarctic Peninsula. Error bars are one standard error. Horizontal axis is as in Fig. 2.

1999; Pommier et al. 2007; Alonso-Saez et al. 2008*b*), we found that these bacteria were quite abundant in the WAP (25% of total abundance). The *Alphaproteobacteria*, *Gammaproteobacteria*, and SF group dominated the WAP bacterial community, as has been seen in waters off eastern Antarctica (Abell and Bowman 2005), as well as in clone libraries from a nearshore station in our study region (Murray and Grzymski 2007). These three groups are also the dominant members in Arctic waters (Elifantz et al. 2007; Vila-Costa et al. 2008; Kirchman et al. 2010). Characterization of clades within the *Gammaproteobacteria*, especially by FISH, has been limited in part due to the low abundance of the whole group in many regions (Glöckner et al. 1999; Alonso-Saez et al. 2008*a*,*b*). In the North Sea, each of nine clades within the *Gammaproteobacteria* formed 1% of the community, with the exception of the SAR86 cluster, which peaked at 10% in one sample (Eilers et al. 2000). Malmstrom et al. (2007) found three gammaproteobacterial clades that together averaged 16% of total abundance in the Chukchi Sea. In contrast, the Ant4D3 cluster comprised 50% of *Gammaproteobacteria* in Antarctic waters and 10% of the total community (Grzymski et al. 2006; this study). The Ant4D3 clade appears to be specialized to cold environments and may play an important role in polar microbial communities (Murray and Grzymski 2007). Further work is needed to describe the abundance of this group in different seasons and environmental regimes.

The Ant4D3 clade does not appear to be very diverse based on analyses with the most recent ARB database (Pruesse et al. 2007). The complete sequences of the 16S rRNA gene for this gammaproteobacterial clade are 98 ± 2.6% similar (minimum 86% similar) based on 43 sequences that were matched exactly by the probe (Murray and Grzymski 2007). Members of the Ant4D3 clade are more closely related to each other than seen for the SAR11 clade and *Polaribacter*. Bacteria targeted by the *Polaribacter* probes were 95 ± 2.8% similar on average (81% minimum), based on 98 16S rRNA gene sequences available for this group. The number of available sequences from the SAR11 clade of *Alphaproteobacteria* is greater (3001 sequences), but diversity of this clade was the same as that of *Polaribacter* (93 ± 4.3% similarity, 74% minimum).

Some of the clades within the larger groups appear to be adapted to polar regions, but there are differences between the Arctic and Antarctica. Using probes designed for Arctic *Roseobacter* representatives, Malmstrom et al. (2007) found low abundance (10%) but a large active fraction of the group in the Arctic. Using the same probe set, we found related bacteria in Antarctic waters but slightly lower abundance (6%). These *Roseobacter* representatives may be specialized to polar regions, but it remains to be seen if the Antarctic *Roseobacter* are as active as the Arctic representatives. Similarly, members of the *Polaribacter* clade of the SF group have been found to be abundant in polar oceans (Pommier et al. 2005; Malmstrom et al. 2007) where they are active and probably important in fluxes of dissolved organic material (DOM; Malmstrom et al. 2007; this study). SAR11 was about four-fold more abundant than *Polaribacter* in the Chukchi Sea (Malmstrom et al. 2007), but in WAP waters we found roughly equal abundances of SAR11 and *Polaribacter*.

We found that single-cell activity differed among the tested compounds, but in general the fraction of active bacteria was comparable to temperate regimes (Cottrell and Kirchman 2003; Elifantz et al. 2005). The fraction of the community incorporating leucine, a proxy for bacterial biomass production, was 40% in our WAP samples, similar to what we observed in the mid-Atlantic Bight and what other microautoradiographic studies found in other marine regions (Smith and del Giorgio 2003). Even though a large fraction of cells were active in biomass production, bulk rates by the total community were low, similar to what has been observed in Arctic environments (Elifantz et al. 2007; Malmstrom et al. 2007; Vila-Costa et al. 2008). Bulk bacterial production was six-fold greater in the low-latitude mid-Atlantic Bight, but this was driven by 10-fold greater cell abundance (Table 4). The bacterial abundances we observed are similar to other measurements from the Arctic (Garneau et al. 2008; Kirchman et al. 2009*a*) and Antarctic (Ducklow et al. 2001; Kirchman et al. 2009*b*). In contrast, growth rates in the WAP (0.07 d^{-1}) in this season were comparable to those of temperate bacterial communities

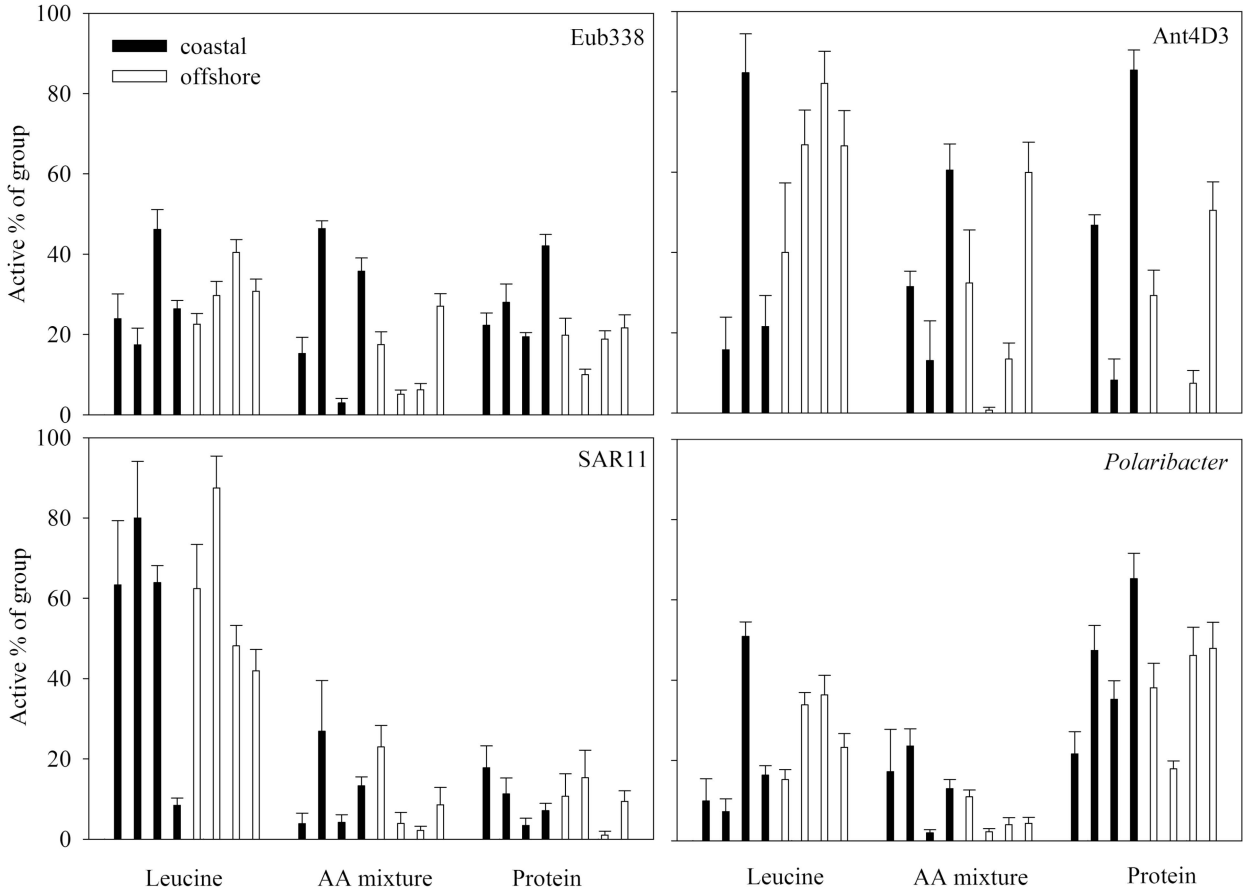

Fig. 6. Fractions of bacterial groups actively using selected compounds along the West Antarctic Peninsula. Samples from coastal stations are grouped together followed by offshore stations. Within the location groups, samples are arranged left to right from 200 km to 600 km in the WAP sampling grid. Error bars are one standard error.

(Kirchman et al. 2009*a*). Growth rates of WAP bacteria closest to the coast were 0.11 d^{-1}, the same as mean growth rates (0.12 d^{-1}) in the equatorial Pacific Ocean (Kirchman et al. 2009*a*). However, silver grain areas around cells taking up leucine in WAP samples were significantly larger than the areas around cells in the mid-Atlantic bight, suggesting greater uptake of leucine by active cells. Unequal cell abundance between the WAP and MAB waters may drive the difference observed between single-cell and bulk properties when we sampled. In turn, the causes of the difference in abundance may include grazing and viral lysis, which are largely unexplored in this polar system. Assessing the temporal variability in bacterial abundance and growth is important for our understanding of the factors controlling bacterial communities in the WAP system.

Table 3. Contribution of each group to the total fraction of cells taking up a given compound. Mean ± standard error. n = 14–15 for each group.

	Coast	Offshore	Total
Leucine			
Ant4D3	8.9±4.0	18.8±4.3	15.4±4.1
Polaribacter	13.5±2.9	10.0±1.8	12.0±2.7
SAR11	27.2±4.0	15.7±3.3	22.2±5.1
Amino acids			
Ant4D3	76.9±45.9	33.5±11.4	68.0±28.7
Polaribacter	38.9±15.1	6.3±2.2	24.9±9.5
SAR11	28.9±15.4	10.5±4.1	21.0±8.8
Protein			
Ant4D3	11.0±4.6	44.3±17.8	29.5±11.2
Polaribacter	40.3±9.4	125.3±48.2	76.7±25.3
SAR11	6.1±2.2	9.6±5.0	7.8±3.5

One apparent difference in single-cell activity between the waters of the coastal WAP and other oceanic regions is the use of organic compounds other than leucine. The fraction of cells using glucose was negligible in our samples, probably because glucose uptake was very low; the turnover rate constant for glucose was 17-fold lower than the rate constant for amino acids. In the North Sea and western Arctic a smaller fraction of bacteria used glucose than all other compounds, but the fraction using glucose still was 10–30% (Alonso and Pernthaler 2006; Elifantz et al. 2007; Alonso-Saez et al. 2008*b*). In the Delaware Bay, 15–30% of cells used glucose (Elifantz et al. 2005). Amino acids support ~ 50% of bacterial production in the oceans (Kirchman 2000), though the fraction of cells using amino acids was ~ 12%, less than half of the fraction incorporating leucine in our samples. However, the fraction of cells using protein in these

Table 4. Bacterial community data from two stations in the mid-Atlantic Bight (Delaware Bay). Mean ± standard error based on 10 fields of view for microscopy-based measurements, or three replicates for bacterial production. nd = no data.

Date	Station	Total abundance ($\times 10^5$ cells mL^{-1})	Leucine incorp. (pmol L^{-1} h^{-1})	Fraction active (%)	Growth rate (d^{-1})	Silver grain area (μm^2)
Nov 10, 2006	Coast	17.6±3.6	nd	27.3±2.8	nd	0.81±0.03
May 12, 2008	Bay	20.8±7.1	78±11.1	32.8±2.5	0.07±0.02	0.46±0.04
May 12, 2008	Coast	20.1±7.5	30±4.6	46.4±2.7	0.03±0.01	0.52±0.03
Jul 12, 2008	Coast (night)	36.6±8.2	181±14.6	16.7±1.5	0.09±0.02	0.37±0.02
Jul 12, 2008	Coast	39.8±7.4	140±9.0	42.8±3.1	0.06±0.01	0.52±0.05
Jul 12, 2008	Bay	41.7±9.1	134±15.4	34.9±1.6	0.06±0.01	0.80±0.07
Sep 12, 2008	Bay	67.1±8.8	232±18.6	15.3±2.0	0.06±0.01	0.77±0.05
Sep 12, 2008	Coast	62.7±8.0	192±14.2	21.1±1.9	0.05±0.01	0.94±0.07
Sep 12, 2008	Coast (night)	54.6±19.1	194±9.1	23.6±4.0	0.06±0.02	0.67±0.32
Mean		42.9±3.2	148±4.0	29.2±0.8	0.06±0.01	0.65±0.31

Antarctic waters (~ 22%) was comparable to that seen in the North Atlantic (Malmstrom et al. 2005), and higher than the fraction incorporating protein in estuaries such as the Delaware Bay (Cottrell and Kirchman 2000).

Phylogenetic subgroups may have different ecological niches, as seen in the varying contribution to the use of different organic compounds. Large fractions of the gammaproteobacterial clade Arctic96B-16 and *Polaribacter* actively incorporated leucine in the Arctic despite the low abundances of the clades (Malmstrom et al. 2007). Although the fractions of Ant4D3 and *Polaribacter* actively using leucine in our samples were lower than observed in the Arctic, the Ant4D3 group dominated the incorporation of the amino acid mixture, and the *Polaribacter* were very important in the use of protein. Our observations of *Polaribacter* are similar to those of other studies in which high fractions of the SF group used high molecular weight compounds (Cottrell and Kirchman 2000; Malmstrom et al. 2007). In contrast, the ubiquitous SAR11 clade has been suggested to specialize on low-molecular-weight compounds (Malmstrom et al. 2005), a hypothesis supported by our observations here. Malmstrom et al. (2005) found fewer SAR11 cells incorporating protein than low-molecular-weight compounds in the North Atlantic. Importantly, the SAR11 clade accounted for the largest fraction of cells using leucine in this study, suggesting that it was responsible for most of bacterial biomass production and accounted for a large part of DOM fluxes in WAP waters. This is not necessarily the case in all oceanic regions, even when SAR11 cells are abundant (Alonso-Saez et al. 2008*a*).

Our data indicate that the bacterial community actively contributes to DOM fluxes in Antarctic waters, but this flux is smaller than seen in low-latitude oceans, as suggested by lower ratios of bacterial production to primary production (Ducklow et al. 2006). This ratio is not low because bacterial growth rates are low in WAP waters. We observed bulk growth rates to be similar to those in temperate regions, and single-cell activity was also high in these cold Antarctic waters. Causes for the low ratio of bacterial production to primary production may include low biomass due to grazing by bacterivores (Duarte et al. 2005), but the extent of uncoupling of bacterial production and primary production remains to be explored. Insights into this uncoupling may be gained by examining data such as ours on the abundance and activity of different phylogenetic groups of bacteria. With single-cell, group-specific, and whole-community approaches available, we can now explore the seasonal and spatial variations in DOM use by this active polar community.

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