

## Standing stocks and activity of Archaea and Bacteria in the western Arctic Ocean

David L. Kirchman,<sup>1</sup> Hila Elifantz,<sup>2</sup> Ana I. Dittel, Rex R. Malmstrom,<sup>3</sup> and Matthew T. Cottrell  
College of Marine and Earth Studies, University of Delaware, Lewes, Delaware 19958

### Abstract

This study examined the abundance, cell size, and activity of Bacteria and Archaea in the Chukchi Sea and the Canada Basin of the western Arctic Ocean in the spring (May–June) and summer (July–August) of 2002 and 2004. Data from fluorescence in situ hybridization (FISH) analyses indicate that bacterial abundance as a percent of total prokaryotes decreased with depth, whereas in contrast, Crenarchaeota increased from about 10% of prokaryotes in surface waters to as much as 40% in samples from 100 to 200 m. Euryarchaeota were detectable in only a few samples. Relative abundance of Crenarchaeota, expressed as a percent of total prokaryotes, correlated with ammonium concentrations, but relative bacterial abundance did not. Crenarchaeota cells were significantly larger than Bacteria by 1.5- to 2-fold in the upper 200 m. Data collected from a combination of FISH and microautoradiography indicate that often the fraction of both Bacteria and Crenarchaeota assimilating organic compounds was high (up to 55%), and both microbial groups were more active in assimilating amino acids than other compounds. However, Crenarchaeota were usually less active than Bacteria in assimilating amino acids and glucose, but were nearly as active as Bacteria in assimilating protein and diatom extracellular polymers. The fraction of Bacteria and Crenarchaeota assimilating CO<sub>2</sub> in surface waters was higher than expected by anaplerotic fixation alone, suggesting that many of these microbes are chemoautotrophic. These data add to a growing body of evidence indicating how the roles of Archaea and Bacteria differ in biogeochemical cycles of the oceans.

The abundances of Archaea and Bacteria vary differently with depth in the oceans examined to date, and these differences provide one of the first clues that the two prokaryotic domains are regulated by different factors in marine environments. Data from fluorescence in situ hybridization (FISH) studies indicate that Archaea make up a larger fraction of total prokaryote abundance in the mesopelagic and bathypelagic zones than in surface waters of the North Pacific Ocean (Karner et al. 2001). In fact, Crenarchaeota are nearly as abundant as Bacteria at about 1,000-m depth in the North Pacific but are near detection limits in surface waters where Bacteria dominate (Karner et al. 2001). There is some evidence of a similar depth distribution for Archaea and Bacteria in the North Atlantic Ocean (Herndl et al. 2005; Teira et al. 2006).

Unlike temperate oceans, Archaea may be abundant even in the surface layer of the polar oceans (DeLong et al. 1994). Probing of ribonucleic acid (RNA) blots has suggested that Archaea make up, depending on the season and location, 1–17% of the picoplankton in surface waters around Antarctica (Massana et al. 1998; Murray et al.

1999). FISH studies with polyribonucleotide probes have confirmed the high abundance of Archaea, mainly Crenarchaeota, especially in winter surface waters near the Antarctic Peninsula (Church et al. 2003). Total archaeal abundance also appears to be high (1–25%) in the Amundsen Gulf and Beaufort Shelf of the coastal Canadian Arctic (Wells and Deming 2003; Garneau et al. 2006; Wells et al. 2006), although the FISH probe (Arch915) used by these previous studies may have overestimated archaeal abundance because of nonspecific binding (Pernthaler et al. 2002). Wells et al. (2006) hypothesized that Archaea may have originated from the Mackenzie River, which dominates the region of the Canadian Arctic they examined. The abundance of Archaea in other Arctic regions less affected by rivers is unclear. In addition to abundance data, size estimates are also needed to determine biomass (standing stocks) of Archaea in the Arctic and in other oceanic regimes.

Little is known about the metabolic capacity and biogeochemical roles of Archaea in pelagic marine environments. Recent data suggest that some marine Archaea are chemoautotrophic and capable of CO<sub>2</sub> fixation (Herndl et al. 2005) using ammonium (Francis et al. 2005; Konneke et al. 2005) or perhaps other unknown reduced compounds as electron donors and energy sources. Ammonium oxidizers in the North Sea were dominated by Archaea (Crenarchaeota), more so than Bacteria, according to data on ammonium monooxygenase gene abundance (Wuchter et al. 2006). However, some oceanic Archaea are heterotrophic or mixotrophic, since a large fraction of Archaea has been shown to assimilate amino acids in the North Atlantic (Herndl et al. 2005; Teira et al. 2006) and in coastal waters of southern California (Ouverney and Fuhrman 2000). The role of marine Archaea in assimilating other organic compounds is not clear.

The goal of this study was to estimate standing stocks of Archaea and Bacteria in the western Arctic Ocean from

<sup>1</sup> Corresponding author (kirchman@udel.edu).

<sup>2</sup> Present address: Department of Microbiology, University of Massachusetts, Amherst, Massachusetts 01003.

<sup>3</sup> Present address: Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.

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data on abundance and cell size and to examine the assimilation of various components of the dissolved organic material (DOM) pool and CO<sub>2</sub> using a combination of FISH with oligonucleotide probes and microautoradiography (Micro-FISH) (Lee et al. 1999; Ouverney and Fuhrman 1999; Cottrell and Kirchman 2000). The DOM components we examined included leucine, an amino acid mixture, protein, glucose, and extracellular polymers (EPS) extracted from a diatom (Elifantz et al. 2005). These compounds were examined because leucine incorporation is used to estimate total biomass production, and the other organic compounds often support a large fraction of heterotrophic bacterial growth in the oceans (Kirchman 2004), including the Arctic (Rich et al. 1997). We found that the abundance, distribution, and activity of Archaea and Bacteria differed in the western Arctic, and this finding has implications for understanding the biogeochemical roles of these microbes in the oceans.

## Materials and methods

This study was part of the Shelf Basin Interaction (SBI) project, which examined the flux of materials from the continental shelf of the Chukchi Sea to the basin of the western Arctic Ocean (Grebmeier and Harvey 2005). Samples were taken on expeditions from 05 May to 15 June (spring) and 16 July to 26 August (summer) in 2002 and 15 May to 23 June and 16 July to 26 August in 2004. These expeditions, which were on the US Coast Guard Cutter *Healy*, were designated HLY 02-01, HLY 02-03, HLY 04-02, and HLY 04-03, respectively. The data from these expeditions are available at <http://www.eol.ucar.edu/projects/sbil/>, and the methods for the nutrient data used in this paper are described by Codispoti et al. (2005).

**Fluorescence in situ hybridization**—The abundance of Bacteria and Archaea was determined by fluorescence in situ hybridization (FISH) with oligonucleotide probes. Water for this analysis was preserved in fresh paraformaldehyde (2%, final concentration) overnight and then filtered through 0.2- $\mu$ m polycarbonate filters. The abundances of Bacteria, Crenarchaeota, and Euryarchaeota were determined by probing, respectively, with Eub338, Cren537, and Eury806 (Teira et al. 2004) labeled with CY3 (MWG Biotech). A negative control probe for nonspecific binding was also used at the same concentration (Cottrell and Kirchman 2003). Bacteria were also detected with a mixture of oligonucleotide probes (Eubmix) (Morris et al. 2002) with a negative control probe (Eub338F) at the same concentration as the Eubmix probe. For some Micro-FISH experiments, bacteria in the SAR11 clade were also identified by FISH using a previously described mixture of probes (Morris et al. 2002). Hybridization and washing were carried out as described previously (Morris et al. 2002; Teira et al. 2004), except that the hybridization was about 17 h. The filters were mounted with a mixture of 4:1 Citifluor (Ted Pella) and Vectashield (Vector Labs) containing 0.5 ng/ $\mu$ L 4'-6'-diamidino-2-phenylindole (DAPI) stain and covered with coverslips. Slides were stored at  $-20^{\circ}\text{C}$  until microscopic analysis with a semi-

automated image analysis system coupled to an Olympus epifluorescence microscope (Cottrell and Kirchman 2003).

Cell sizes were estimated from the DAPI image of cells identified as being Bacteria or Archaea by FISH as described previously (Cottrell and Kirchman 2004). The data reported here focus on the average cell size for a given sample in which >1,000 prokaryotes were examined.

**Microautoradiography combined with fluorescence in situ hybridization**—Surface layer samples were collected for Micro-FISH experiments from four locations in the western Arctic Ocean in summer 2004. The depths of these samples were 7 m (shelf; station 15), 13 m (slope; station 32), 41 m (basin; station 37), and 26 m (shelf break; station 44). These depths were at 15% of surface light, except the shelf sample, which was from the 30% light depth. Water samples (20 mL from the shelf, 60 mL from the other locations) were incubated for 2 h with <sup>3</sup>H-leucine (20 nmol L<sup>-1</sup>, final concentration, 5.55 TBq mmol<sup>-1</sup>, PerkinElmer), a <sup>3</sup>H-amino acid mixture (0.5 nmol L<sup>-1</sup>, TRK440, Amersham), or D-[6-<sup>3</sup>H(N)]-glucose (0.5 nmol L<sup>-1</sup>, 1.22 TBq mmol<sup>-1</sup>, Amersham). Other samples were incubated with <sup>3</sup>H-EPS (1.2  $\mu$ mol L<sup>-1</sup> in glucose equivalents) or <sup>3</sup>H-protein (20 ng mL<sup>-1</sup>) for 6–9 h. <sup>3</sup>H-protein and <sup>3</sup>H-EPS were prepared as described previously (Cottrell and Kirchman 2000; Elifantz et al. 2005). Dark assimilation of <sup>14</sup>CO<sub>2</sub> (3.7 MBq per incubation) was also examined in similar experiments, but the incubation was for 24 h. Controls killed with paraformaldehyde were also run simultaneously with all live incubations. Negligible numbers of cells were labeled with silver grains in the killed controls. All samples were incubated at the in situ temperature in the dark. These temperatures were 0, 5, 1.2,  $-1^{\circ}\text{C}$  for the shelf, shelf-break, slope, and basin experiments, respectively. At the end of the incubation, samples were fixed with paraformaldehyde (2% final concentration) and stored at  $4^{\circ}\text{C}$  for 24 h. Samples were then filtered onto 0.2- $\mu$ m-pore-size polycarbonate filters, which were kept at  $-20^{\circ}\text{C}$  until analysis.

The filters were analyzed by FISH and microautoradiography as described previously (Cottrell and Kirchman 2003). The microautoradiographic exposure times were 24 h for leucine and 6 d for the other compounds. These exposure times gave the highest percent of cells scoring positive for uptake (cells with silver grains) while minimizing background (Elifantz et al. unpubl. data). At the end of the exposure time, the slides were developed as described previously (Cottrell and Kirchman 2003). The <sup>3</sup>H-DOM and <sup>14</sup>CO<sub>2</sub> experiments used NTB (Kodak) and LM-1 emulsion (Amersham Biosciences), respectively.

To examine CO<sub>2</sub> fixation by the anaplerotic pathway, which is present in all heterotrophs, we measured <sup>14</sup>CO<sub>2</sub> fixation by SAR11 bacteria and compared these results with those for Bacteria and Crenarchaeota. Since a cultured representative from the SAR11 clade, *Pelagibacter ubique*, is not a chemoautotroph (Giovannoni et al. 2005), we assumed that any <sup>14</sup>CO<sub>2</sub> assimilation by these bacteria was due to anaplerotic fixation. The ribosomal gene in *P. ubique* is similar to that of SAR11 bacteria in the Arctic surface layer (unpubl. data; see also Brown and Fuhrman 2005).

Table 1. Summary of relative bacterial and archaeal abundance in depth profiles from all stations. The negative control, which was not subtracted from these data, was  $1.7 \pm 2.7\%$  of total prokaryotes (DAPI-positive cells).

Date	Depth (m)	% prokaryotes						% total detected‡	SD	n§
		Bact*	SD†	Cren	SD	Eury	SD			
Spring 2002	0–100	76.3	13.9	13.3	11.6	2.2	2.9	89.8	18.7	18
	100–499	60.7	11.4	26.1	9.0	0.9	0.8	87.7	18.1	8
	>500	55.7	10.9	6.7	3.6	1.2	1.3	67.5	3.4	3
Summer 2002	0–100	75.7	14.9	8.9	8.0	1.6	5.1	85.6	16.6	38
	100–499	55.4	8.7	23.0	14.4	0.6	0.9	79.1	17.7	9
	>500	52.3	13.0	12.6	10.5	0.7	0.4	65.7	21.3	6
Spring 2004	0–100	74.2	17.6	8.3	9.5	9.3	9.2	93.9	15.9	13
	100–499	60.1	17.0	21.1	13.8	8.6	5.8	86.9	27.9	6
	>500	58.2	7.2	9.8	14.6	9.8	9.3	77.8	18.0	5
Summer 2004	0–100	77.5	8.0	12.9	10.6	4.3	5.6	94.6	12.5	24
	100–499	54.7	9.0	14.2	6.8	1.4	0.9	70.3	9.9	9
	>500	53.6	0.6	19.9	23.1	1.9	0.9	62.3	1.4	2

\* The following abbreviations were used: Bact=Bacteria, Cren=Crenarchaeota, and Eury=Euryarchaeota.

† Standard deviation (SD) was calculated for  $n$  samples taken from each date and depth interval.

‡ % total detected is the sum of the percent of total prokaryotes detected as Bacteria, Crenarchaeota, and Euryarchaeota calculated for each sample, then averaged for the indicated date and depth interval.

§ Number of samples for the indicated date and depth interval.

We calculated biomass production and growth rates for Crenarchaeota and Bacteria from the FISH and Micro-FISH data and bulk leucine incorporation rates. Biomass was calculated assuming the applicability of either  $148 \text{ fg C } \mu\text{m}^{-3}$  (Gundersen et al. 2002) or  $12.4 \text{ fg C cell}^{-1}$  (Fukuda et al. 1998) to both Bacteria and Archaea identified by FISH. Biomass production was estimated from total leucine incorporation rates measured by the standard methods (Kirchman 2001). Triplicate samples with  $^3\text{H}$ -leucine ( $20 \text{ nmol L}^{-1}$ ) and a killed control were incubated for 2 h at the in situ temperature. The incorporated  $^3\text{H}$ -leucine was collected by microcentrifugation after precipitation by trichloroacetic acid and rinsed with trichloroacetic acid and ethanol before radioassaying. To convert to biomass production, we assumed no isotope dilution, which gives a conversion factor of  $1.5 \text{ kg C per mole of incorporated leucine}$ . This theoretical factor agrees with estimates of the empirical conversion factor for the Arctic (Kirchman unpubl. data). The Micro-FISH data were then used to determine the fraction of production contributed by Bacteria and Crenarchaeota. Growth rates were estimated by dividing the appropriate production rate by the two estimates of biomass.

**Statistical analysis**—The FISH data expressed as a percent of prokaryotes and cell size data were arcsine and log-transformed, respectively, before being analyzed statistically. Lowess lines were calculated in Sigmaplot and used in plots of relative archaeal and bacterial abundance versus depth to illustrate trends. A lowess line is similar to a regression line calculated locally for a limited number of points.

## Results

**Abundance of Bacteria and Archaea**—We used FISH and direct counts of total prokaryotes to examine standing

stocks of Bacteria and Archaea in the water column of the western Arctic Ocean during four expeditions in 2002 and 2004. To simplify the analyses and to focus on large-scale variation, sampling stations were grouped by water column depth: the shelf (waters <100-m deep), shelf break (100–200 m), slope (200–2,000 m), and the basin (>2,000 m). About 90% of total prokaryotes could be detected by FISH for samples from depths above 500 m (Table 1), and there was no significant difference between 0–99 m and 100–500 m in detection ( $p > 0.05$ ), except for the summer of 2004 (Table 1). Below 500 m, the total percentage of cells detected by FISH decreased to <70%. Consequently, we did not analyze data from >500 m in further detail. Although the mixture of oligonucleotide probes (Eubmix) often gave higher values than the single oligonucleotide probe (Eub338) for Bacteria when detection was low (<50%) in deep waters, results from Eubmix and Eub338 were not significantly different at the depths focused on here (paired Student's  $t$ -test;  $p > 0.05$ ).

Bacteria dominated the prokaryotic community throughout the water column ( $2\text{--}10 \times 10^8 \text{ cell L}^{-1}$ ), especially in surface waters (<100 m) (Table 2). Bacterial abundance significantly decreased along transects from the shelf and the Chukchi Sea to basin waters during most of the cruises (Table 2) (ANOVA;  $p < 0.05$ ), but these changes were usually less than those observed in depth profiles. Abundance was high in the upper 100 m and then decreased by as much as 10-fold below 100 m, which marked the start of the upper halocline in these waters (Fig. 1). Bacteria were significantly more abundant in summer than in spring in most areas we sampled, although often the difference was not large (Fig. 1; Table 2) (ANOVA;  $p < 0.05$ ). Of the two archaeal groups we examined, only Crenarchaeota were abundant ( $0.04\text{--}2.0 \times 10^8 \text{ cells L}^{-1}$ ), and the abundance of Euryarchaeota was usually much less than for Crenarchaeota (Table 2). Euryarchaeota were more abundant in some samples in

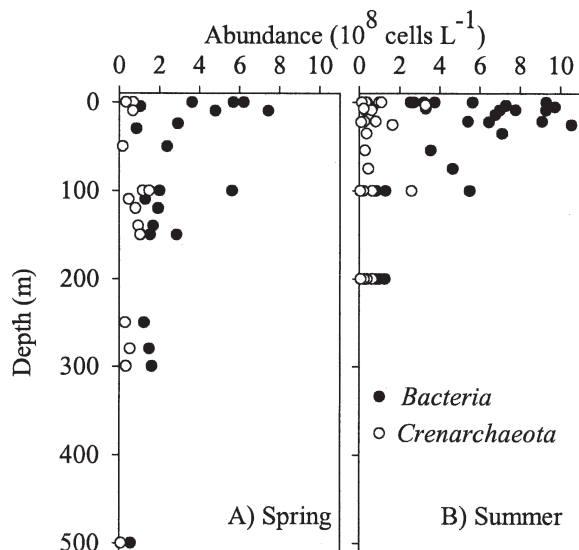


Fig. 1. Abundance of Bacteria and Crenarchaeota in slope waters of the western Arctic in the (A) spring and (B) summer of 2002 and 2004.

2004, but, again, this group was usually outnumbered by Crenarchaeota. For this reason, we focus here on Crenarchaeota.

In contrast to Bacteria, the abundance of Crenarchaeota in surface waters (<100 m) did not decrease significantly along transects from the shelf to the basin (Table 2) (ANOVA;  $p > 0.05$ ), although crenarchaeal abundance was lower in the basin than on the shelf by about 3-fold in 2004 (Table 2). Abundance of this archaeal group was the same in the spring and summer of 2002 in surface waters but was generally higher in the summer of 2004 than in spring (Table 2). The abundance of Crenarchaeota decreased with depth, but not nearly as much as did the

Bacteria (Fig. 1). Overall, bacterial abundance decreased about 8-fold more with depth than did Crenarchaeota, based on regression analyses of all data.

*Bacteria and Crenarchaeota as a fraction of total prokaryotes*—To explore in greater detail the contribution of Crenarchaeota versus Bacteria to total prokaryotic abundance, we examined the variation in the percentage of prokaryotes that could be identified as either Bacteria or Crenarchaeota. The spring and summer data were analyzed separately, but the two years were combined. This approach, along with dividing the stations into four locations, simplified the analysis and increased the number of samples per analysis.

As seen in the absolute numbers, relative bacterial abundance, expressed as a percentage of all prokaryotes, decreased with depth at most stations. Although there was no consistent pattern in the shelf and shelf-break samples in spring (Fig. 2A,B), bacterial abundance significantly decreased from over 80% of total prokaryotes to about 50% by 100 m in the slope and basin samples (Fig. 2C,D) (linear regression analysis;  $p < 0.05$ ). Relative bacterial abundance decreased with depth at all four regions in the summer (Fig. 3) (linear regression analysis;  $p < 0.05$ ). On the shelf, Bacteria made up nearly 90% of total prokaryotes but dropped to about 50% at 50 m (Fig. 3A). Relative bacterial abundance also decreased with depth in the other regions, but the decrease started at about 100 m at the shelf break and slope (Fig. 3B,C) and 200 m in the basin (Fig. 3D).

In contrast to the Bacteria, relative crenarchaeal abundance remained constant with depth on the shelf in spring and summer (Figs. 2A, 3A) or increased with depth in the other regions. Crenarchaeal abundance was as high as 40% of all prokaryotes in slope and basin waters in spring and summer (Figs. 2C,D, 3C,D). Overall, Crenarchaeota, as a percent of total prokaryotes, were significantly

Table 2. The abundance of Bacteria, Crenarchaeota, and Euryarchaeota in surface waters (<100 m) of the western Arctic Ocean.

Date	Location	Bacteria	SD*	Crenarchaeota	SD	Euryarchaeota	SD	n
		(10 <sup>8</sup> cells L <sup>-1</sup> )						
Spring 2002	Shelf	3.68	1.77	0.693	0.857	0.063	0.062	12
Spring 2002	Shelf break	2.59	1.07	0.348	0.150	0.019	0.018	4
Spring 2002	Slope	4.66	1.45	0.693		0.043		1
Spring 2002	Basin	3.71	1.79	0.928	0.703	0.334	0.329	8
Summer 2002	Shelf	7.40	2.10	0.634	0.564	0.083	0.058	12
Summer 2002	Shelf break	2.81	0.79	0.296	0.298	0.034	0.022	4
Summer 2002	Slope	6.11	3.46	0.743	0.869	0.192	0.650	20
Summer 2002	Basin	5.76	3.58	0.584	0.646	0.086	0.097	12
Spring 2004	Shelf	6.79	0.21	0.554	0.271	0.158		2
Spring 2004	Shelf break	3.99	2.50	0.039		0.059		1
Spring 2004	Slope	3.70	2.38	0.773	0.560	0.317	0.203	9
Spring 2004	Basin	2.38	1.30	0.157	0.223	0.391	0.278	6
Summer 2004	Shelf	10.25	8.70	1.907	1.900	0.358	0.233	4
Summer 2004	Shelf break	6.87	5.41	0.918		0.707		1
Summer 2004	Slope	5.89	3.02	0.528	0.441	0.102	0.084	6
Summer 2004	Basin	3.44	1.87	0.598	0.527	0.143	0.127	18

\* Standard deviation (SD) was calculated for  $n$  samples taken from each region and time.

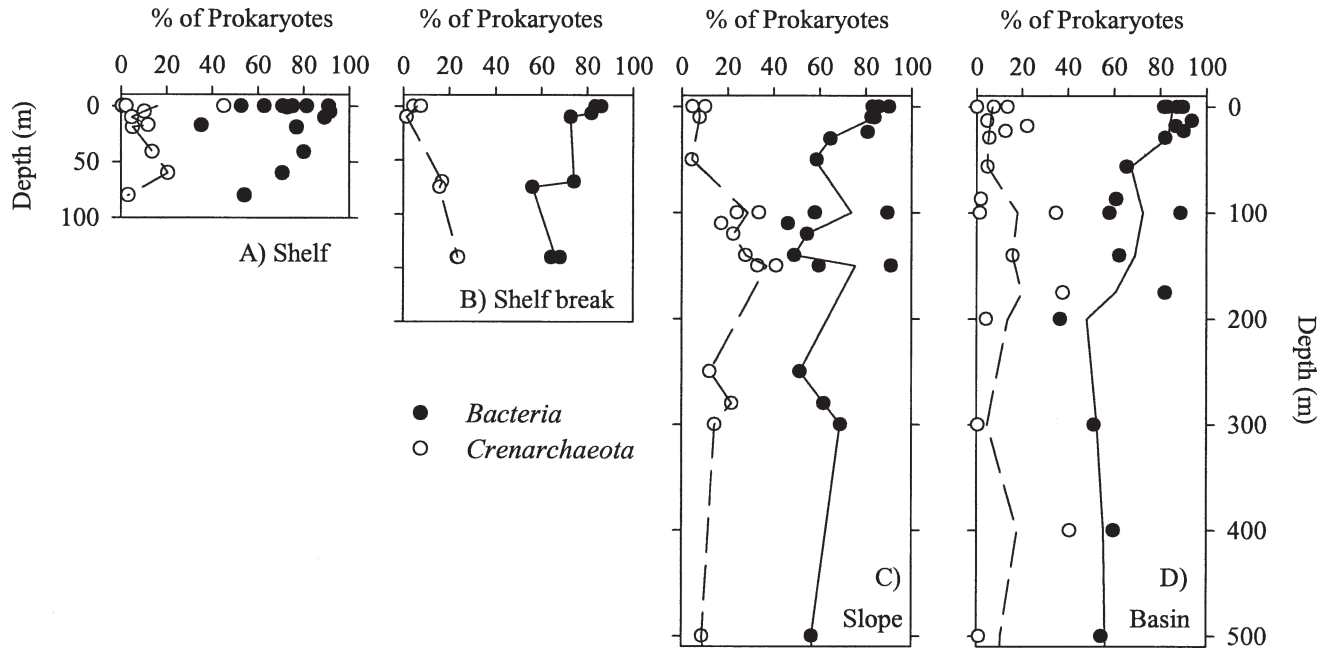


Fig. 2. Abundance of Bacteria and Crenarchaeota, as a percentage of total prokaryotes, in spring of 2002 and 2004 in the (A) shelf, (B) shelf break, (C) slope, and (D) basin of the western Arctic Ocean.

more abundant in the 100–499-m layer than in the surface layer (0–99 m) (Student’s *t*-test;  $p < 0.02$ ), except in summer 2004, when the two layers did not differ (Table 1). Except for summer 2004, Crenarchaeota were about twice as abundant (relative to the total) in the deeper layer as in the surface. In contrast, relative bacterial abundance was significantly lower in the 100–499-m layer than in the surface during all expeditions (Student’s *t*-test;  $p < 0.01$ ).

*Correlations with ammonium and other environmental factors*—Correlation analyses indicate that Bacteria and Crenarchaeota, expressed as a percentage of total prokaryotes, vary differently with select environmental factors (Table 3). Relative crenarchaeal abundance was positively correlated with nitrate and phosphate concentrations, whereas there were significant negative correlations between bacterial abundance and concentrations of these

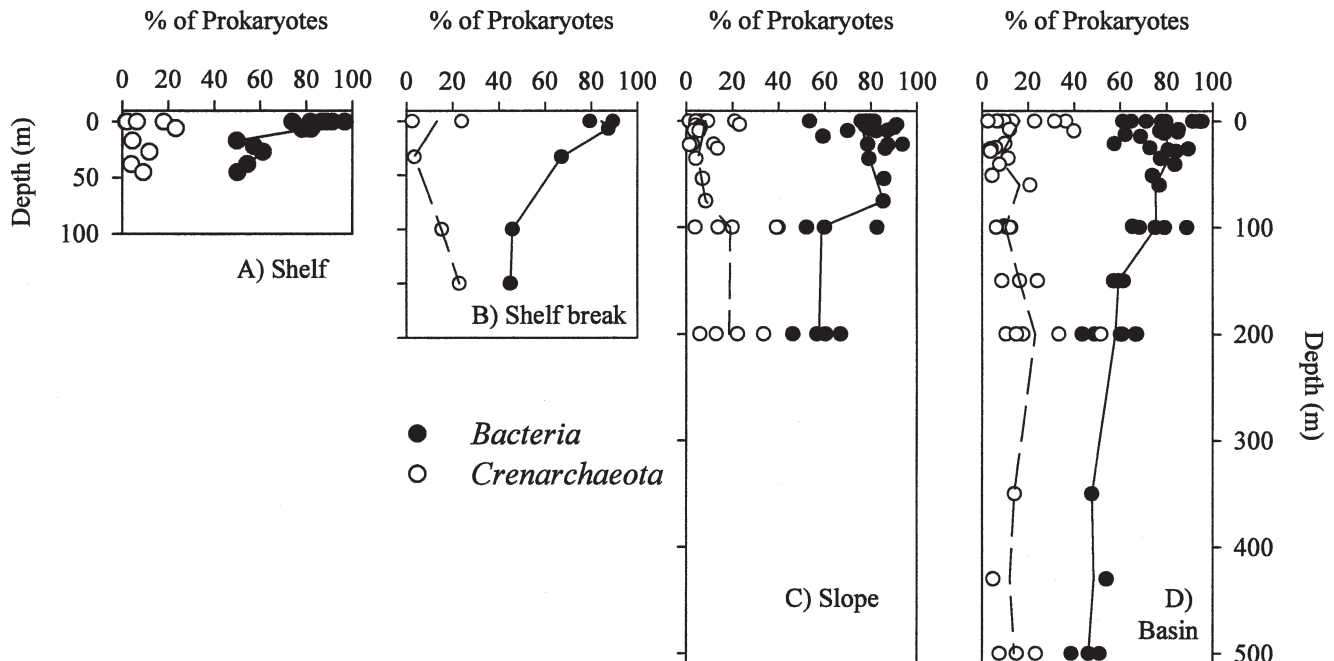


Fig. 3. Abundance of Bacteria and Crenarchaeota, as a percentage of total prokaryotes, in the summer of 2002 and 2004 in the (A) shelf, (B) shelf break, (C) slope, and (D) basin of the western Arctic Ocean.

Table 3. Correlations between select parameters and percent of prokaryotes present as Bacteria or Crenarchaeota. Particulate organic carbon (POC) data from only the 2002 expeditions were available for this analysis (Bates et al. 2005). Data from all regions and dates were analyzed (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ).

Parameter	Bacteria	Crenarchaeota	<i>n</i>
Chlorophyll ( $\mu\text{g L}^{-1}$ )	0.186*	-0.062	141
POC ( $\mu\text{mol C L}^{-1}$ )	0.500*	-0.132	46
$\text{NH}_4^+$ ( $\mu\text{mol L}^{-1}$ )	-0.066	0.226**	143
Nitrate ( $\mu\text{mol L}^{-1}$ )	-0.615***	0.332***	143
Phosphate ( $\mu\text{mol L}^{-1}$ )	-0.353***	0.356***	143

nutrients (Table 3). Relative bacterial abundance was positively correlated with chlorophyll and particulate organic carbon, whereas Crenarchaeota abundance was not. In contrast, the relative abundance of Crenarchaeota was significantly correlated with ammonium concentrations, whereas Bacteria were not (Table 3).

Ammonium concentrations from 2002 have been discussed in detail previously (Codispoti et al. 2005), and here we present concentrations only at stations with FISH data, using the same format as that for relative bacterial and crenarchaeal abundance (Figs. 2, 3). These figures illustrate why ammonium and relative crenarchaeal abundance are significantly, but weakly correlated. Except for the shelf stations (Figs. 4A, 5A), ammonium concentrations were low in surface waters and then increased to  $1.0 \mu\text{mol L}^{-1}$  or more below about 100 m (Figs. 4B,C, 5B,C,D), similar to the increase in relative crenarchaeal abundance (Figs. 2, 3). Unlike Crenarchaeota, ammonium concentrations were very low in spring in the basin (Fig. 5D). However, for the

other cases, ammonium and relative crenarchaeal abundance appeared to vary with depth similarly.

*Cell size of Bacteria and Archaea*—The cell size of Bacteria and Archaea identified by FISH did not appear to vary consistently or significantly with region (shelf to the basin) (data not shown). The cell volume of Bacteria increased significantly between spring 2002 and summer 2004 (ANOVA;  $p < 0.05$ ), but the increase was small compared to the variation with depth (see below). Consequently, we compiled all cell-size data into composite depth profiles for Bacteria and Archaea (Fig. 6). The cell sizes of Euryarchaeota were not analyzed because of their low abundance. The sizes of Bacteria identified with Eub338 and Eubmix FISH probes were not significantly different (Student's paired *t*-test;  $p > 0.05$ ).

Crenarchaeota were usually larger than Bacteria, although the difference and absolute sizes varied greatly with depth (Fig. 6). The average cell volume of Crenarchaeota was  $0.09\text{--}0.14 \mu\text{m}^3$ , whereas Bacteria average cell volume was  $0.05\text{--}0.09 \mu\text{m}^3$  (the range of averages for the four expeditions). The difference in cell volume, compared per sample, was statistically significant (Student's paired *t*-test;  $p < 0.001$ ). However, most of the difference was in the upper 200 m where cell volumes of Crenarchaeota were  $>1.6$ -fold greater on average than those of Bacteria (Fig. 6). Below 500 m, there was no significant difference in cell size (Student's paired *t*-test;  $p > 0.05$ ).

*Assimilation of  $^3\text{H}$ -DOM by Bacteria and Crenarchaeota*—We used the Micro-FISH approach to examine how many Bacteria and Crenarchaeota assimilated leucine and

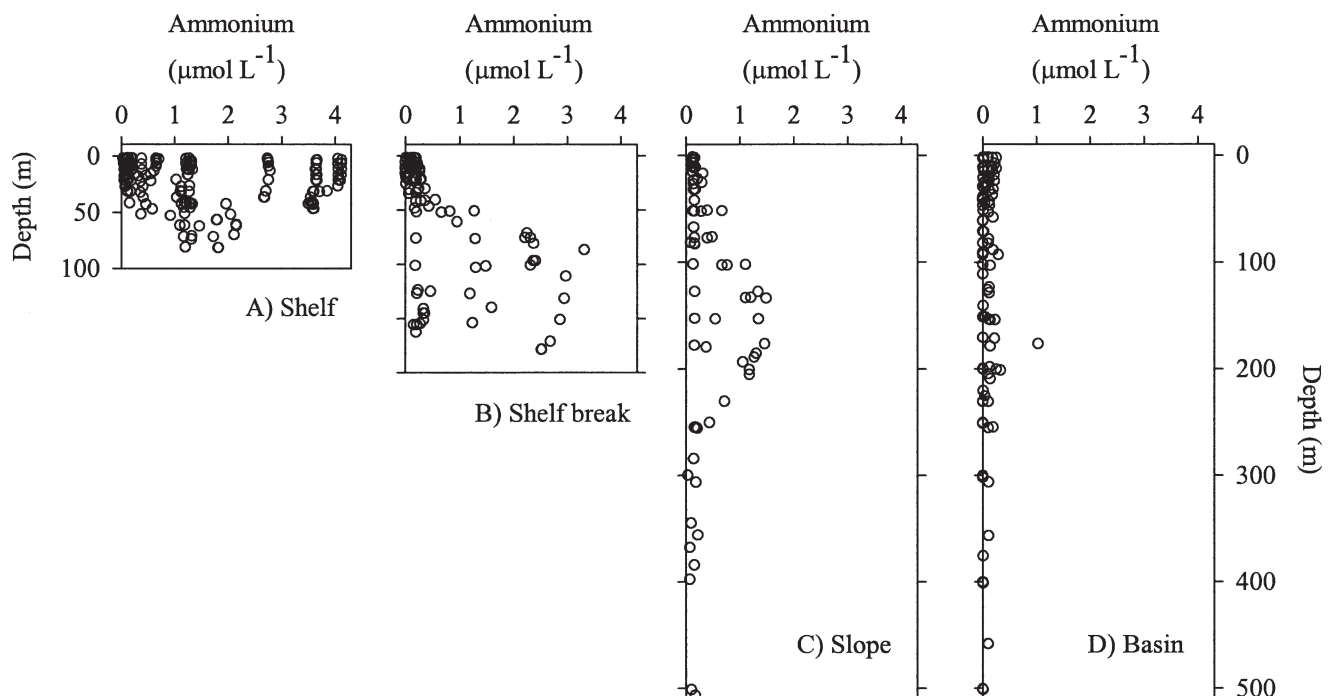


Fig. 4. Ammonium concentrations in spring of 2002 and 2004 in the (A) shelf, (B) shelf break, (C) slope, and (D) basin of the western Arctic Ocean. Data are presented from only those stations with FISH data (see Fig. 2).

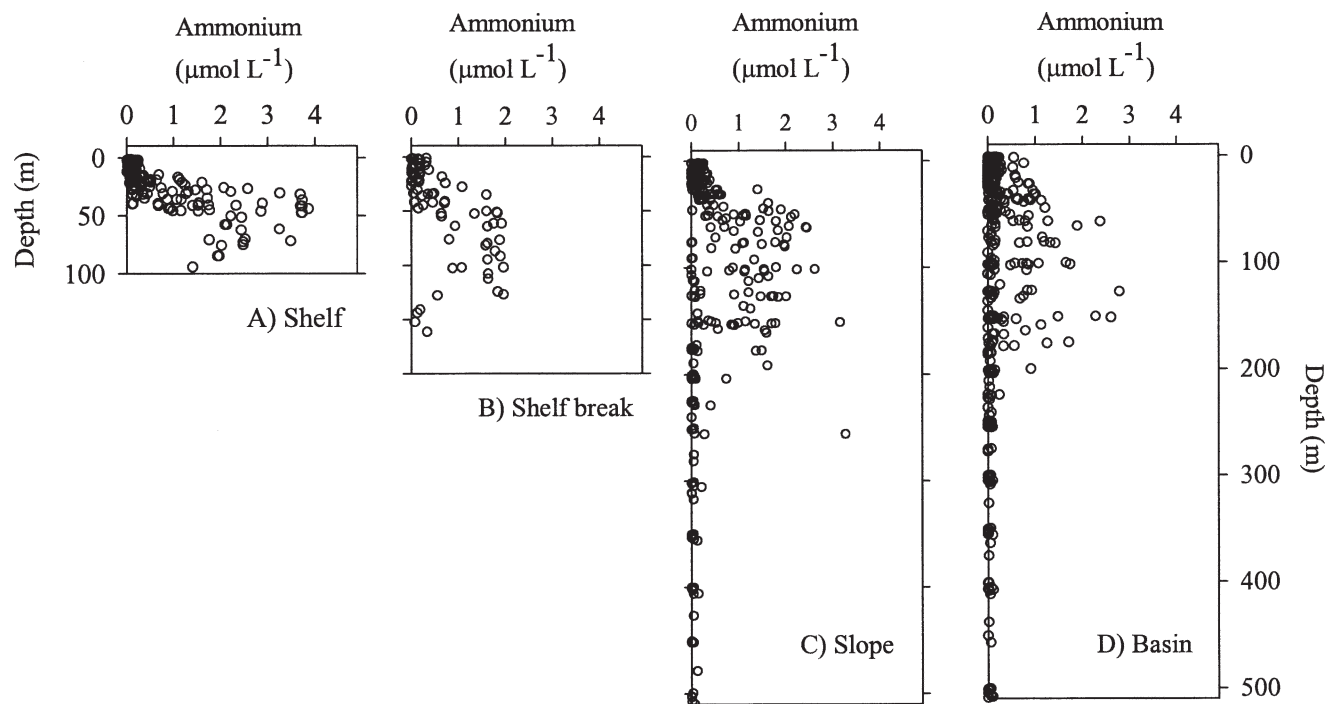


Fig. 5. Ammonium concentrations in summer of 2002 and 2004 in the (A) shelf, (B) shelf break, (C) slope, and (D) basin of the western Arctic Ocean. Data are presented from only those stations with FISH data (see Fig. 3).

four DOM components in western Arctic surface waters. Overall, a remarkably high fraction of both Bacteria and Archaea assimilated these  $^3\text{H}$ -organic compounds, although the percentages varied greatly (5–60%) depending on the region and the compound (Fig. 7). The percentage of Bacteria that assimilated leucine and the amino acid mixture was significantly higher than that of Crenarchaeota in all experiments (Fig. 7). The fraction of Bacteria assimilating these compounds varied from 30% to >60%, whereas the fractions for Crenarchaeota were always lower (20% to 45%), depending on the region (Fig. 7). Relative assimilation of glucose by both microbial groups was lower than leucine and amino acid assimilation, but again the fraction of Bacteria assimilating glucose was higher than that of Crenarchaeota in three of the four locations (Fig. 7). The difference was significant at two locations and insignificant at the other two (Fig. 7).

In contrast to the low molecular weight monomers, the percentage of Crenarchaeota assimilating protein and EPS was similar to that of Bacteria in most experiments (Student's *t*-test;  $p > 0.05$ ). The exceptions were EPS assimilation at the shelf station (Fig. 7A) and protein assimilation in the basin (Fig. 7D), but there was no significant difference in EPS and protein assimilation between Bacteria and Crenarchaeota for the other six experiments. When data from all stations were considered together, significantly more Bacteria than Crenarchaeota assimilated the amino acids and glucose, whereas there was no significant difference in the fractions of both groups assimilating the polymeric DOM components (two-way ANOVA;  $p > 0.05$ ).

To examine relative activity in more detail, we estimated production and growth rates for Bacteria and Crenarchaeota

using bulk leucine incorporation rates, FISH data on size and abundance of Bacteria and Crenarchaeota, and Micro-FISH data on the fraction of leucine assimilation attributable to either Bacteria or Crenarchaeota. Most of biomass production was by Bacteria rather than Crenarchaeota (Table 4), mainly because of the low abundance of Crenarchaeota in these samples. However, leucine incorporation rates per cell, i.e., growth rates, were also lower for this archaeal group. Growth rates of Bacteria were usually 1.5- to 3-fold faster than those of Crenarchaeota, depending on the region and the assumptions (Table 4). The rates varied from 0.011 to 0.185  $\text{d}^{-1}$ , corresponding to generation times of a couple days to over a month.

*Assimilation of  $^{14}\text{CO}_2$  by Bacteria and Crenarchaeota*—To explore possible chemoautotrophy in the western Arctic, we examined assimilation of  $^{14}\text{CO}_2$  by microbes using the Micro-FISH approach. FISH probes for SAR11 bacteria were used to estimate the level of anaplerotic fixation as detected by the Micro-FISH approach (see Methods and materials).

The fraction of Bacteria and Crenarchaeota assimilating  $^{14}\text{CO}_2$  was quite high, ranging from nearly 20% in the shelf (Fig. 7A) to about 5% in the basin (Fig. 7D). There was no difference between Bacteria and Crenarchaeota at any location. In contrast, the relative number of SAR11 bacteria assimilating  $^{14}\text{CO}_2$  was significantly lower than for Bacteria or Crenarchaeota in three of the four locations (Student's *t*-test;  $p < 0.05$ ); only in the basin was there no significant difference (Fig. 7D). The three samples with  $^{14}\text{CO}_2$  assimilation apparently exceeding anaplerotic fixation had detectable ammonium (0.1–0.3  $\mu\text{mol L}^{-1}$ ), but the

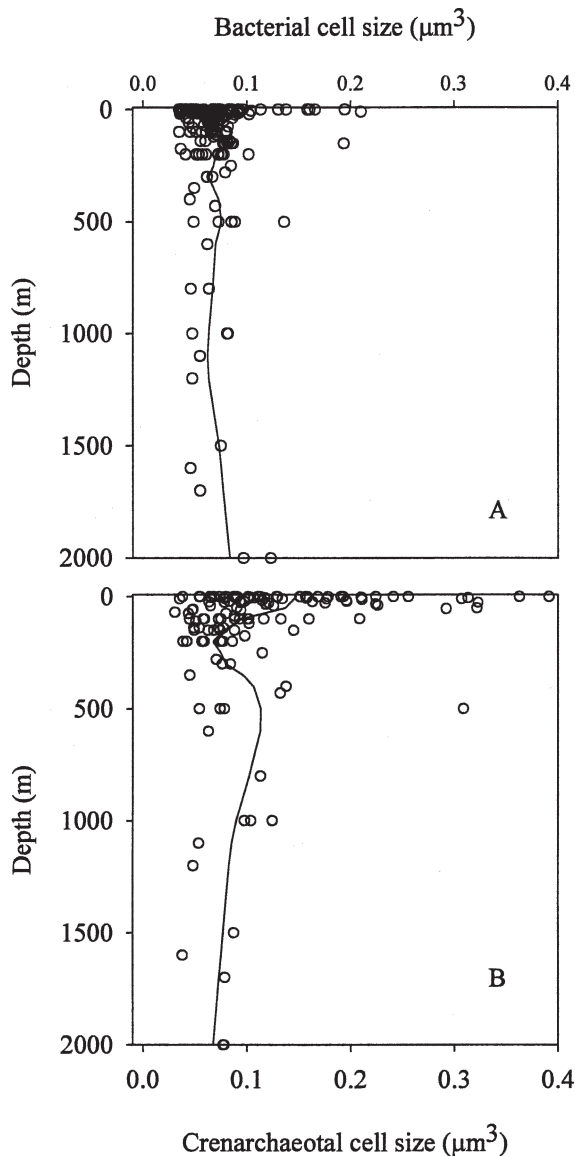


Fig. 6. Cell volume of (A) Bacteria and (B) Crenarchaeota in 2002 and 2004. Each point represents the average size for the entire sample. Over 1,000 cells were measured for each sample.

basin sample did not. Although the percentage of SAR11 bacteria assimilating  $^{14}\text{CO}_2$  was low, this group was active in assimilating amino acids in these samples—the percentages of these bacteria taking up amino acids were  $53.5 \pm 4.6\%$ ,  $66.3 \pm 3.2\%$ ,  $67.5 \pm 1.8\%$ , and  $69.8 \pm 2.5\%$  for the shelf, shelf break, slope, and basin, respectively. Even if  $\text{CO}_2$  assimilation by SAR11 is subtracted out, as many as 10% of all Bacteria and Archaea appear to be chemotrophic in these western Arctic waters.

## Discussion

The standing stock data give some clues about the regulation of Bacteria and Archaea in the western Arctic Ocean and how these microbial groups in the Arctic differ

from oceanic regimes examined to date. In the Arctic Ocean, Archaea vary greatly over time, among shelf, slope, and the basin stations, and with depth. Crenarchaeota were often abundant even in surface waters of the western Arctic Ocean during our study, and their abundance as percent of total prokaryotes usually increased with depth while Bacteria declined. These and other patterns were observed even though the analyses combined data from different years, and the geographical regions defined here agglomerated diverse oceanographic regimes (Codispoti et al. 2005). The abundance data alone point to differences in the biogeochemical roles of Bacteria and Archaea in the western Arctic Ocean.

The archaeal community was usually dominated by Crenarchaeota in the Arctic waters we sampled, and often Euryarchaeota were not even detectable. This dominance by Crenarchaeota has been found in deep waters of other oceanic regimes using FISH approaches (Karner et al. 2001), most notably in the Southern Ocean off of the west Antarctic Peninsula (Church et al. 2003). Based on data from other approaches, Euryarchaeota have been found to be more abundant than Crenarchaeota in surface waters (Massana et al. 1998, 2000; DeLong et al. 2006), but this archaeal group may be abundant even in some deep-water masses. Metagenomic data indicate that Euryarchaeota (Group 2) and Crenarchaeota are equally common in the North Pacific at 500 m (DeLong et al. 2006). Although Crenarchaeota appear to be more abundant in the North Atlantic, Euryarchaeota numbers can be substantial in the North Atlantic, reaching 25% of total prokaryotic abundance in some deep waters (Teira et al. 2006). In contrast to our results, Crenarchaeota accounted for about 60% of the archaeal clones in nine 16S rDNA (rRNA genes) libraries from the Arctic Ocean, but the remaining 40% were from Euryarchaeota (Bano et al. 2004), a higher percentage than any of our estimates, and Euryarchaeota dominated 16S rDNA libraries in the Beaufort Sea (Galand et al. 2006). In addition to the possibility that the difference is due to methodology, our FISH study and the clone library studies sampled quite different regions of the Arctic Ocean at different times. Variation in archaeal communities over time and space is to be expected.

The relative abundance of Crenarchaeota increases with depth in both low-latitude oceans (Karner et al. 2001; Herndl et al. 2005) and polar seas (this study, Church et al. 2003), but this archaeal group appears to become abundant at a shallower depth in the western Arctic than observed elsewhere to date. Microbes detected by the Arch915 FISH probe, which are probably mostly Archaea, also were abundant at relatively shallow depths in a study of the Beaufort Shelf (Wells et al. 2006). The difference is probably due to unique features of the Arctic Ocean. Much more so than other oceans, the Arctic Ocean is affected by terrestrial inputs and exchange with the continental shelf (Shiklomanov et al. 2000). One example of this impact is seen in the ammonium data. Codispoti et al. (2005) pointed out how ammonium and other material seem to stream out from sediments of the continental shelf into what we refer to here as shelf-break and slope waters. The actual distribution of ammonium varies among the SBI

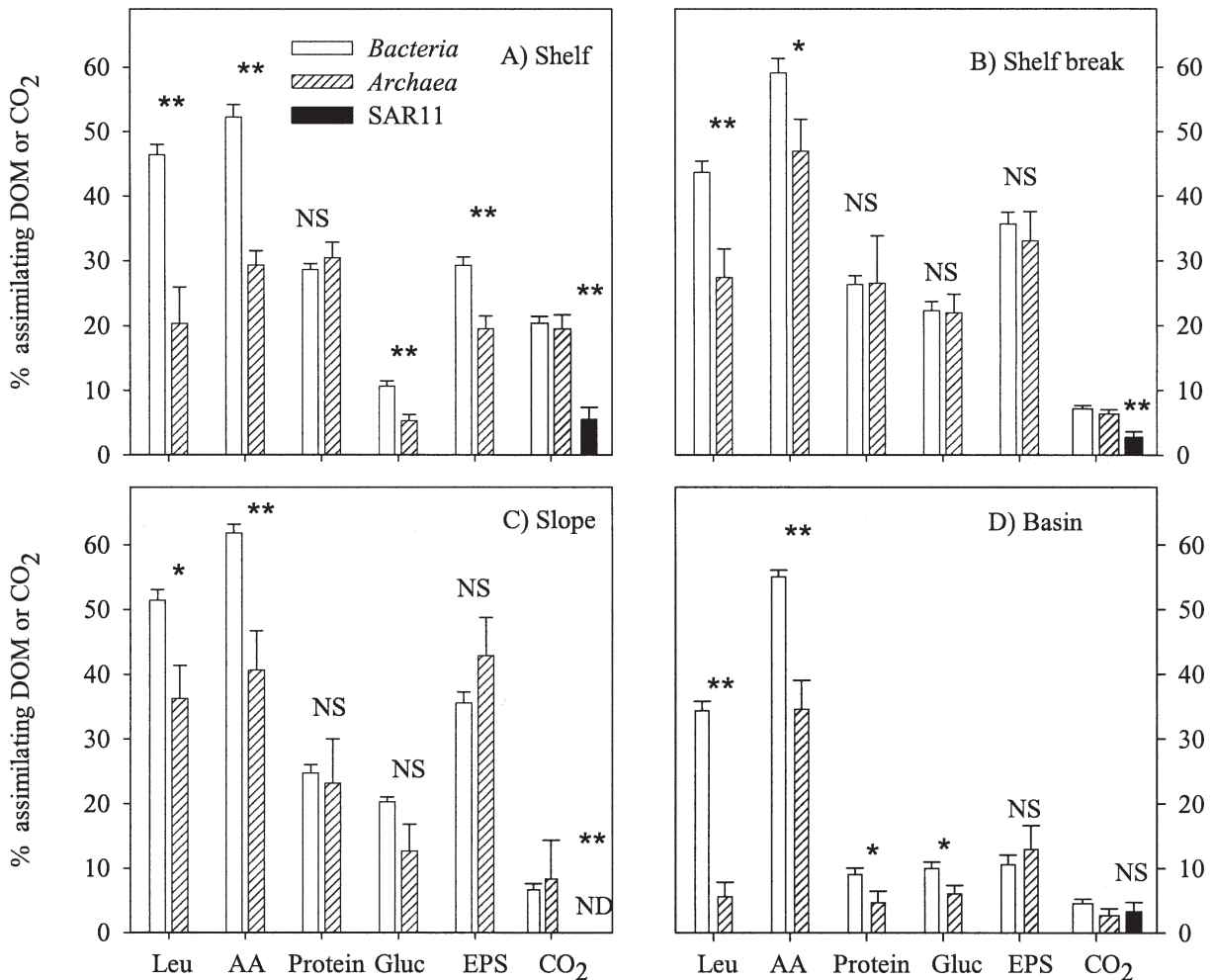


Fig. 7. Percentage of Bacteria, Crenarchaeota, and SAR11 bacteria that assimilated the indicated compounds in samples from the (A) shelf, (B) shelf break, (C) slope, and (D) basin. The organic compounds included leucine (Leu) at 20 nmol L<sup>-1</sup> and amino acids (AA), protein, glucose (Gluc), and extracellular polysaccharides (EPS) at tracer concentrations. Error bars are of standard errors for 20 microscope fields of view. <sup>14</sup>CO<sub>2</sub> assimilation by SAR11 bacteria was not detectable (ND) in the slope sample. Percentages of DOM assimilation for Bacteria versus Crenarchaeota were compared by a Student's *t*-test with \*\*, \*, and NS indicating  $p < 0.001$ ,  $p < 0.05$ , and no significant difference, respectively. The tests for the <sup>14</sup>CO<sub>2</sub> assimilation refer to comparisons with SAR11 bacteria. There was no significant difference in <sup>14</sup>CO<sub>2</sub> assimilation by Bacteria versus Crenarchaeota ( $p > 0.05$ ).

transects of the western Arctic (Barrow Canyon and West and East Hanna Shoals), but the general pattern is similar (Codispoti et al. 2005).

The correlation analysis suggests that ammonium or some co-varying parameter helps to explain why Crenarchaeota are abundant in relatively shallow waters (100–200 m) of the upper halocline in the western Arctic. In addition to ammonium, this depth layer has high concentrations of other plant nutrients and particulate organic carbon (Bates et al. 2005; Codispoti et al. 2005) from continental shelf sediments and degradation of material sinking from the surface layer. These organic and inorganic compounds may help to fuel the growth of heterotrophic and chemoautotrophic Crenarchaeota and allow either or both to be more competitive with Bacteria below the surface layer. This hypothesis is consistent with previous work in Antarctic waters (Massana et al. 1998; Murray et al. 1999; Church et al. 2003), which has suggested that

Archaea depend on energy sources not tightly coupled to contemporaneous primary production. Bacteria appear to be linked to phytoplankton biomass (chlorophyll), but Crenarchaeota are not in the western Arctic.

The significant, albeit weak, correlation between ammonium and relative crenarchaeal abundance is consistent with the hypothesis that several members of this archaeal group are chemolithotrophic. Other data indicate chemoautotrophy by Archaea in the Arctic and elsewhere. In our study, a higher fraction of Bacteria and Archaea than of SAR11 cells assimilated <sup>14</sup>CO<sub>2</sub> in three of four Micro-FISH experiments. Because one representative of the SAR11 clade found in the Arctic is not chemoautotrophic (Giovannoni et al. 2005), we assumed that the observed <sup>14</sup>CO<sub>2</sub> assimilation by SAR11 cells was due to anaplerotic fixation, which is carried out by all heterotrophs. If we also assume that the apparent anaplerotic fixation by SAR11 and other heterotrophic microbes is similar, then the

Table 4. Biomass, production, and growth rates of the total prokaryotic community, Bacteria, and Crenarchaeota in the western Arctic Ocean. Biomass was calculated using the FISH data to estimate the abundance of Bacteria and Crenarchaeota and assuming either 148 fg C  $\mu\text{m}^{-3}$  or 12.4 fg C cell $^{-1}$  for the first and second estimate, respectively. Production was estimated by applying the fraction of production contributed by Bacteria and Crenarchaeota to total leucine incorporation rates. Growth rates were estimated by dividing the production rate by the two estimates of biomass.

Location		Biomass ( $\mu\text{g C L}^{-1}$ )	Production ( $\mu\text{g C L}^{-1} \text{d}^{-1}$ )	Growth rates ( $\text{d}^{-1}$ )
Shelf	Total	9.05–9.35	1.61	0.178–0.172
	Bacteria	8.10–8.37	1.50	0.185–0.179
	Crenarchaeota	0.59–0.63	0.06	0.111–0.104
Shelf break	Total	5.70–9.89	0.53	0.092–0.053
	Bacteria	4.38–7.60	0.48	0.110–0.063
	Crenarchaeota	0.33–0.41	0.03	0.093–0.075
Slope	Total	7.26–8.76	0.98	0.135–0.112
	Bacteria	6.48–7.82	0.95	0.147–0.122
	Crenarchaeota	0.48–0.54	0.06	0.125–0.110
Basin	Total	3.90–4.71	0.07	0.019–0.015
	Bacteria	2.47–2.98	0.05	0.020–0.017
	Crenarchaeota	0.16–0.18	0.002	0.012–0.011

percentage of Bacteria and Archaea above the level observed for SAR11 cells is due to chemoautotrophy. These results support previous studies that suggest that marine Crenarchaeota are chemoautotrophic (Ingalls et al. 2006) and are ammonium-oxidizers (Francis et al. 2005; Konneke et al. 2005). Using the Micro-FISH approach, Herndl et al. (2005) found that over 20% of Euryarchaeota but <10% of Bacteria and Crenarchaeota assimilate  $^{14}\text{CO}_2$  at 200-m depth in the North Atlantic, and Crenarchaeota are more active in  $^{14}\text{CO}_2$  assimilation than the other two groups only at 1,000 m. In our experiments with surface waters, the percentages of Crenarchaeota and Bacteria assimilating  $^{14}\text{CO}_2$  were similar, about 10%, excluding the basin sample. These levels of chemoautotrophy are much less than those reported by Ingalls et al. (2006), who used natural radiocarbon data to estimate that 80% of archaeal biomass was synthesized by autotrophy at 670-m depth in the North Pacific Gyre. Of course, microautoradiography is probably less sensitive than the radiocarbon approach in detecting chemoautotrophy. But the high percentage suggested by the radiocarbon data seems inconsistent with the observed assimilation of DOM components by Crenarchaeota in shallow (this study) and deep waters (Teira et al. 2004, 2006). To resolve these apparent discrepancies, more work is needed to examine whether the prevalence of chemoautotrophy among Archaea varies among oceanic regimes and whether some of these microbes are mixotrophic.

In addition to an extensive shelf region, another unique feature of the Arctic Ocean is the large input by rivers. This input and its impact vary among regions in the Arctic (Shiklomanov et al. 2000). The Beaufort Shelf and other locations in the Canadian Arctic examined by previous studies of archaeal abundance (Wells and Deming 2003; Wells et al. 2006) are heavily affected by the Mackenzie River and other, smaller rivers in the region (Macdonald et al. 1998). In these river-affected regions, Archaea were more abundant in waters enriched with suspended particular material and detritus (Wells et al. 2006). These and

other data led the authors to hypothesize that the Mackenzie River is a large source of the Archaea found in Beaufort Shelf waters. However, the archaeal community in the Mackenzie River appears to be quite distinct from the Beaufort Sea community (Galand et al. 2006). Rivers are likely to have even less of an impact on Archaea and Bacteria in the waters we sampled, because direct river flow into the Chukchi Sea and Canadian Basin is much lower than in the Beaufort Shelf (Shiklomanov et al. 2000). Also, rivers do not appear to affect the composition of the bacterial community in the western Arctic Ocean; beta-proteobacteria and Actinobacteria, which often dominate river bacterial communities (Kirchman et al. 2005), including the Mackenzie (Garneau et al. 2006), are rare in our samples (Elifantz et al. unpubl. data). If western Arctic Archaea are from rivers, then they must survive the transport from coastal regions to slope and Arctic basin waters more successfully than typical riverine bacterial groups.

Cell size and total biomass are other measures of the success of Archaea in the oceans. Assuming that the factors for converting cell size of Archaea to cell biomass are the same as those for bacteria, our data suggest that Archaea can make up an even greater fraction (by >1.6-fold) of prokaryotic biomass than suggested by their abundance alone. Because of differences in biochemical composition, the proportions of biomass distributed between Bacteria and Archaea may indirectly affect DOM composition and processes. In particular, the supply of peptidoglycan to the DOM pool will vary depending on the biomass of Bacteria versus Archaea. Peptidoglycan, which is synthesized by Bacteria but not Archaea, is potentially a major component of the organic nitrogen in DOM (McCarthy et al. 1998).

Our estimates of cell sizes and thus biomass may be affected by problems in using epifluorescence microscopic images of DAPI-stained cells. One problem is that DAPI images may reflect DNA content more than total cell size because of the specificity of DAPI for DNA. If so, our data would suggest that Crenarchaeota have larger genomes

than Bacteria in the western Arctic surface layer. The difference may be even larger than indicated by our data because DNA in Crenarchaeota may be more localized than DNA in Bacteria (Preston et al. 1996). We suspect, however, that DAPI staining is nonspecific in the procedure we used and that sizes estimated from the DAPI image reflect total cell size, not just DNA content.

Assuming that the DAPI image varies not just with genome size, the cell-size data may indicate differences in the ecology and biogeochemical roles of Bacteria and Archaea in the oceans. The larger cell size of Crenarchaeota may result in higher mortality due to cell size-dependent grazing (Sherr et al. 1992), but this effect may be offset by their lower population size, which would lead to lower grazing pressure. These competing effects have been used to explain the negative relationship between average cell size and the abundance of major bacterial groups in the Delaware Estuary (Cottrell and Kirchman 2004). The difference in cell size between Bacteria and Archaea is not because Archaea are more active than Bacteria; if anything Archaea are less active according to the Micro-FISH data presented here.

The Micro-FISH data provide some important clues to the metabolic activity and biogeochemical role of Crenarchaeota in the western Arctic Ocean. As has been observed for bacterial groups (Cottrell and Kirchman 2003; Elifantz et al. 2005), the relative abundance of Crenarchaeota is a good predictor (within 2-fold) of its contribution to DOM assimilation (data not shown). Assuming this relationship between abundance and activity holds for other times and regions, the abundance data imply that Crenarchaeota may account for <10% of DOM assimilation in surface waters and up to >40% in the 100–200-m layer in the spring. However, these percentages may not apply to all DOM components, since our Micro-FISH data suggest that Bacteria and Crenarchaeota do not assimilate all organic compounds equally. Teira et al. (2006) also found differences in D- and L-amino acid use by bacterial and archaeal groups in the mesopelagic zone of the North Atlantic Ocean.

Regardless, the fraction of cells within each group that was active in assimilating DOM was remarkably high for both Crenarchaeota and Bacteria, comparable to values we have observed for bacterial groups in the Delaware Estuary and North Atlantic Ocean (Cottrell and Kirchman 2004; Elifantz et al. 2005; Malmstrom et al. 2005) and higher than what has been usually observed elsewhere for total prokaryotic assemblages (Smith and del Giorgio 2003). The fractions of both Bacteria and Archaea assimilating amino acids in surface waters of the Mediterranean Sea and Monterrey Bay have also been found to be high (Ouverney and Fuhrman 2000), but have been reported to be much lower (<35%) for aspartic acid assimilation in deep waters ( $\geq 200$  m) of the North Atlantic Ocean (Teira et al. 2004, 2006). Our microautoradiography results are consistent with the FISH results; a high fraction of cells (about 90%) was detectable in these Arctic samples by FISH using oligonucleotides without enzymatic amplification (Teira et al. 2004). Bacteria and Crenarchaeota are apparently quite active in the western Arctic in spite of low water

temperatures ( $-1.7$  to  $5^{\circ}\text{C}$ ) and low primary production, especially in the Arctic basin (Hill and Cota 2005).

Our data point to differences in the roles of Bacteria and Archaea in the carbon cycle of the western Arctic Ocean. These differences are evident in how Bacteria and Archaea vary over time, among geographical regions, and with depth. The cell-size data also indicate differences between Bacteria and Archaea, even if some of the variation is due to DNA content and genome size. Finally, the Micro-FISH data suggest that while Crenarchaeota are quite active and contribute to DOM uptake, this archaeal group appears to assimilate different DOM components than do Bacteria. What is less clear is the prevalence of chemoautotrophy among Crenarchaeota and Bacteria, since both groups appeared to be equally active in assimilating  $\text{CO}_2$ . More data are needed to resolve this important question.

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