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Archaea in particle-rich waters of the Beaufort Shelf and Franklin Bay, Canadian Arctic: Clues to an allochthonous origin?

Llyd E. Wells,[1] *Michael Cordray*,[2] *and Sarah Bowerman*[3]
School of Oceanography, University of Washington, Box 357940, Seattle, Washington 98195-7940

Lisa A. Miller
Institute of Ocean Sciences, Fisheries and Oceans Canada, Sidney, British Columbia V8L 4B2, Canada

Warwick F. Vincent
Department of Biology, Laval University, Quebec City, Quebec G1K 7P4, Canada

Jody W. Deming
School of Oceanography, University of Washington, Box 357940, Seattle, Washington 98195-7940

Abstract

We used 4′,6-diamidino-2-phenylindole (DAPI) staining and fluorescent in situ hybridization to examine total bacterioplankton and archaeal distributions in surface waters and in deeper nepheloid layers and particle-poor waters across the Beaufort Shelf of the Canadian Arctic, including the Mackenzie River and Kugmallit Bay, as well as more distant Franklin Bay. Although the regional distribution of bacterioplankton was best explained by salinity ($r_s = -0.89$, $n = 28$, $p < 0.001$) and indicators of primary production (chlorophyll *a* [Chl *a*], total organic carbon, and the ratio of Chl *a* to particulate organic carbon [Chl *a*:POC]), that of Archaea instead reflected measures of particulate matter, specifically microscopically determined particle concentration ($r_s = 0.85$, $n = 30$, $p < 0.001$), suspended particulate matter, POC, particulate organic nitrogen (PON), and the beam attenuation coefficient. Moreover, when compared with similarly deep particle-poor waters, nepheloid layers were significantly enriched in Archaea (median concentration of 6.00×10^4 mL^{-1} [15.5% of bacterioplankton] vs. 1.79×10^4 mL^{-1} [3.6%]; $p < 0.05$), but not total bacterioplankton. The relationship between Archaea and particles, the dominance of the Mackenzie River as the regional particle source, the detection of highest archaeal concentrations (11.5–14.4 $\times$ 10^4 mL^{-1}) in the river, and the highly significant correlation ($r_s = 0.97$, $n = 12$, $p < 0.001$) between Archaea in particle-rich waters and PON (the river providing the upper end member) suggest that many of these Archaea derive from the river.

Archaea have now been recognized as ubiquitous and sometimes abundant members of the marine bacterioplankton across the oceans, in temperate and high-latitude environments, along coasts, in estuaries, and in the deep sea (e.g., Murray et al. 1998; Crump and Baross 2000; Karner et al. 2001). Despite reports of limited genetic diversity in the free-living assemblage of disparate oceanic environments (Massana et al. 2000), considerable functional and biogeochemical diversity within the planktonic marine Archaea is implied by field data and experimental evidence that some are chemoautotrophic (Hoefs et al. 1997; Pearson et al. 2001; Wuchter et al. 2003), while others appear capable of heterotrophy (Ouverney and Fuhrman 2000; Teira et al. 2004). Moreover, in interfacial environments like estuaries, archaeal diversity might be greater than in the open ocean because of allochthonous inputs, as noted in the Columbia River estuary (Crump and Baross 2000).

We became interested in the possible allochthony of Arctic Archaea on the basis of our earlier study with fluorescent in situ hybridization (FISH) of archaeal distribution in

[1] Corresponding author (chimera1@ocean.washington.edu).
[2] Present address: Department of Bioengineering, Rice University, Houston, Texas 77005.
[3] Present address: Department of Biology, University of Washington, Seattle, Washington 98195.

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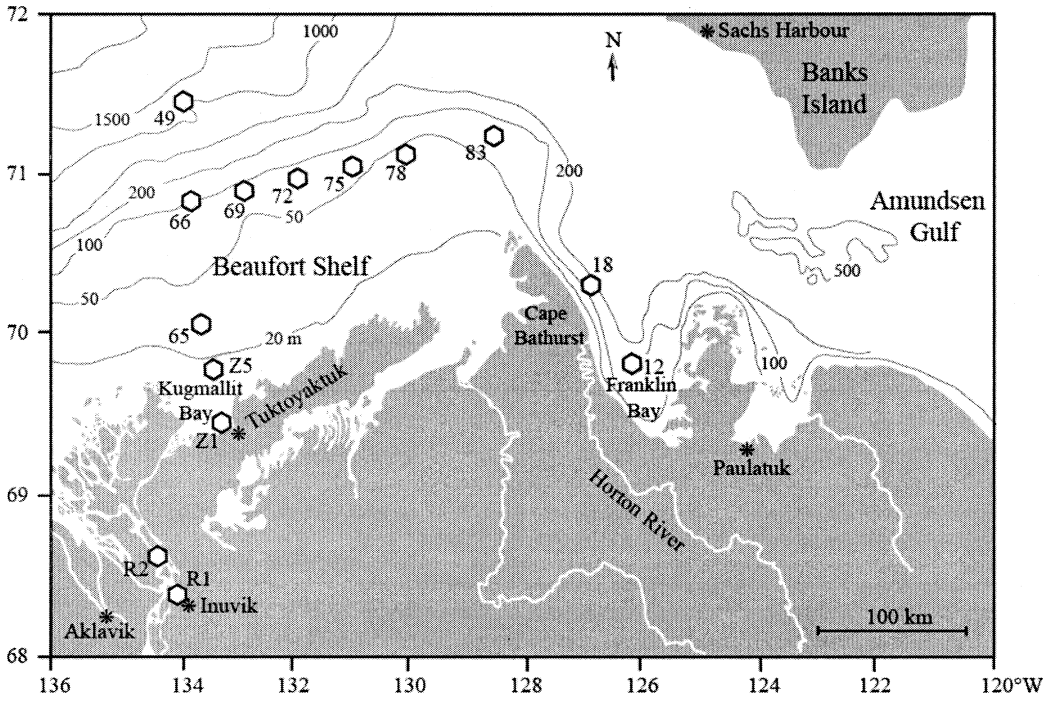


Fig. 1. Field site and stations.

Amundsen Gulf (the westernmost region of the Northwest Passage in the Canadian Archipelago) in September 2000 (Wells and Deming 2003). Archaeal abundances measured in surface (7–42 m) and deeper (150–500 m) waters were low ($\sim$1–2 $\times$ 10^3 cells mL^{-1}) and corresponded to a small fraction of the bacterioplankton ($\sim$0.5%), similar to the fractions found in Antarctic surface waters during the Austral summer (albeit with the use of different methods; Murray et al. 1998; Church et al. 2003). In contrast, distinct particle-rich regions in the water column called nepheloid layers, usually associated with the bottom, supported both higher archaeal concentrations ($\sim$8 $\times$ 10^3 cells mL^{-1}) and fractions of the bacterioplankton (typically $\sim$5% but as high as 13%). The only measured physical or chemical variable distinguishing these layers from adjacent deep waters was the abundance of particles, with which archaeal concentration correlated positively ($p < 0.001$). We hypothesized that these nepheloid-layer Archaea could be allochthonous, either resuspended with underlying shelf sediments or otherwise reflecting the predominant particle sources in the region. The opportunistic nature of our sampling in 2000 did not permit us to explore this possibility in detail.

In the work described here, we returned to Amundsen Gulf and especially the Beaufort Shelf further west to examine the distribution of Archaea specifically in relation to particles. The eastern Beaufort Shelf (also known as the Mackenzie Shelf) is an attractive system for such a study because of the large annual sediment load of the Mackenzie River ($\sim$128 million tons of mostly silt and clay, which is twice the combined loads of the larger Arctic rivers, Ob, Yenisey, and Lena; Carson et al. 1998) and is thought to account for $\sim$95% of the shelf sediment supply (Macdonald et al. 1998). At least during open-water season, benthic nepheloid layers and a particle-rich surface plume have regularly been observed in the vicinity of the river mouth (e.g., Iseki et al. 1987; Carmack et al. 1989); farther offshore, bottom currents and storms can mobilize sediments into additional nepheloid layers (Hill et al. 1991). These particle-rich waters might be important not only in terms of sediment transport but as sites of microbially mediated organic transformations. The latter possibility is consistent with observations of increased microbial activity in nepheloid layers (e.g., Townsend et al. 1992; Boetius et al. 2000) and sinking particles (e.g., Huston and Deming 2002) in other marine environments, as well as the often disproportionate contribution of particle-associated bacteria to bacterial secondary production in estuaries (Griffith et al. 1994; Crump et al. 1998). The likely biogeochemical significance of particle-rich waters is an argument for understanding the composition of their microbial inhabitants (the subject of parallel work in surface waters of the region; Garneau et al. unpubl. data), often presumed by default to belong to the Bacterial domain.

In this study, we examined the distribution of Archaea over a broader region and more systematically than was possible in 2000, comparing archaeal concentrations in particle-rich and particle-poor waters from the Mackenzie River, across shelf to the western reaches of Amundsen Gulf. The northernmost station allowed us to examine archaeal abundance to a depth of 968 m.

Materials and methods

Field site and sampling—Sampling was conducted at 14 stations along the Beaufort Shelf (Fig. 1) from the Canadian

Coast Guard icebreaker NGCC *Pierre Radisson* during 23 September 2002–12 October 2002 as part of the international Canadian Arctic Shelf Exchange Study. Bottom water (~5 m above the seafloor) was sampled along a roughly west–east transect along the outer shelf from north of the Mackenzie Delta to the entrance of Amundsen Gulf. Another transect extended northward from the mouth of the east channel of the Mackenzie River to the Beaufort Sea shelf break. Over this transect, both bottom and surface waters (≤6 m) were collected, except at the shallow (3–12 m total depth) Stas. Z1 and Z5 in Kugmallit Bay and at two additional stations (Stas. R1 and R2) in the Mackenzie River, where only surface waters were taken. Additionally, two stations (Stas. 12 and 18) in Franklin Bay, influenced by the Horton River, were also sampled. An SBE Carousel 24-bottle sampler equipped with 12-liter polyvinyl chloride sample bottles, a conductivity-temperature-depth (CTD) probe (SBE-911plus), and a transmissometer (WETlabs cs-25-660 [red] C-STAR) was used to profile the water column and collect water. Bottles were fired during the up-cast following identification of nepheloid layers from the transmissivity signal. At two stations (Stas. 12 and 49) more complete depth profiles were taken. Because of their shallowness, nearshore Stas. Z1 and Z5 were sampled with a 5-liter GO-FLO bottle and SBE 19 CTD (i.e., no transmissometer) deployed from a hovering helicopter or small boat, respectively. The two Mackenzie River samples were collected with a clean bucket and transported to the ship by helicopter. These river and nearshore samples were stored cold and processed as quickly as possible, within 6 h of collection. (All other samples were processed immediately.)

On recovery shipboard, water was subsampled for macronutrients (nitrite + nitrate, hereafter called ''nitrate''; dissolved reactive phosphorus, hereafter called ''phosphate''; and silicate; J.-E. Tremblay, Laval University), dissolved oxygen, chlorophyll *a* (Chl *a*), particulate organic carbon (POC), and particulate organic nitrogen (PON; K. Lacoste and S. Demers, University of Quebec at Rimouski), total organic carbon (TOC), and suspended particulate matter (SPM), as well as for microbial analyses (following). Dissolved oxygen samples were analyzed according to Carpenter (1965) with an automated colorimetric titration system with a Metrohm Dosimat 655 and a Brinkman PC910 probe colorimeter. Analyses were calibrated with a standard potassium iodate solution (estimated accuracy within 0.3% and precision of 1.2 μmol L^{-1} from multiple samples tapped from the same Niskin bottle). For the determination of Chl *a*, 0.2–1 liters of seawater was passed through a Whatman GF/F filter and extracted for 24 h in 90% acetone at 5°C without grinding (Parsons et al. 1984). Fluorescence was read on a Turner fluorometer (model 10-AU). POC and PON concentrations were measured by filtering at least 250 mL of seawater through precombusted (5 h at 500°C) Whatman GF/F filters. These were stored in precombusted paper foil at −80°C until laboratory analysis on a Perkin Elmer 2400 CHN analyzer.

TOC samples were collected directly from the rosette into 40-mL EPA vials with teflon-lined silicone septa caps (vials had been cleaned in 10% HCl and combusted at 500°C for 1 h; caps were acid washed but not combusted). Samples were immediately frozen (−20°C) and stored until analysis (within 6 months) by high-temperature combustion (Spyres et al. 2000) on a Tekmar-Dohrmann Apollo 9000 analyzer calibrated with a potassium phthalate standard (Folio Instruments). Accuracy was confirmed by analysis of Bermuda Seawater (provided by D. Hansell and W. Chen, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Florida). Precision of the analyses was 2.8 μmol L^{-1} on the basis of the average difference between replicate samples from single rosette bottles.

For SPM determination, 0.5–2.0 liters of water were filtered through 42.5-mm precombusted, preweighed GF/C filters. The filters were then rinsed with 10 mL of 1% sodium formate to remove salt and stored frozen in separate petri dishes at −20°C. The filters were later dried at 60°C for 24 h and reweighed to determine the total dry weight concentration of SPM (Williams 1985). Finally, the beam attenuation coefficient (*c*) was calculated from the transmissivity data according to McCave (1986): $c = -4 \ln \mathrm{Tr}$, where Tr is the transmissivity as a fraction of 1.

Enumeration of total cells, Archaea, and particles by epifluorescence microscopy—Preparation of samples shipboard followed the protocol described in Wells and Deming (2003). Briefly, triplicate 100-mL water samples were filtered under low vacuum onto 0.22-μm, 47-mm polycarbonate filters and fixed overnight at 4°C in ~0.5 mL of 4% paraformaldehyde (in phosphate-buffered saline). After fixation, all filters were carefully folded, sample side in, and stored in 70% ethanol at −20°C until analysis by FISH (completed within 11 months of sampling). Wells and Deming (2003) reported better preservation of cells fixed in this way than if fixed in whole (unfiltered) seawater, although both methods yielded counts highly correlated with one another.

Our FISH procedure was modified from Fuhrman and Ouverney (1998) and tested against a suite of pure cultures (representing α-Proteobacteria, γ-Proteobacteria, Cytophaga–Flavobacteria, and Euryarchaea) to optimize probe signal intensity and specificity. Under optimized conditions, no cross-hybridization with nontarget organisms was observed. Filters from each sample were sliced aseptically into sections (typically ⅛ of a 47-mm filter). The 4′,6-diamidino-2-phenylindole (DAPI) control section was immediately stained with DAPI-Vectashield (Vector Laboratories) and mounted on a slide for enumeration of total cells. Two sections (per sample) reserved for FISH were dehydrated sequentially in 2-min 50%, 80%, and 96% ethanol washes, placed on cover slips, and completely covered in hybridization buffer (5× SET = 750 mmol L^{-1} NaCl, 100 mmol L^{-1} Tris-Cl pH 7.8, 5 mmol L^{-1} EDTA [disodium salt]; 10% dextran sulfate [molecular mass 500,000]; 0.2% acetylated bovine serum albumin; 0.01% polyadenylic acid; and 0.1% sodium dodecyl sulfate). To one of the two filter sections, no probes were added to serve as an autofluorescent control; to the other, after 15 min of prehybridization at 43°C, a cocktail of four probes specific to Group I (S-O-Cenar-0554-a-A-20; Massana et al. 1997), Group II (S-O-ArGII-0554-a-A-20; Massana et al. 1997), or all Archaea (S-D-Arch-0915-a-A-20, Stahl and Amann 1991; S-D-Arch-0338-a-A-24, Giovannoni

et al. 1990) was added (final concentration of each probe was 1 ng μL^{-1}). Each probe was 5′, Cy-3–conjugated and purchased from Qiagen-Operon. For a subset of samples, an additional hybridization was done in which probe S-D-Arch-0915-a-A-20 (hereafter called Arch915) was omitted from the probe cocktail to compare archaeal percentages with three instead of four probes. Following hybridization for 3–6 h at 43°C, the filter sections were immersed in three consecutive, 10-min washes of 0.25× SET at 43°C. Filters were then air-dried, mounted on slides, counterstained with DAPI-Vectashield, and stored at −20°C. Each hybridization was accompanied by positive and negative controls of pure cultures.

Total cells (DAPI), Cy-3–labeled cells, and autofluorescent cells were enumerated on a Zeiss Universal microscope at ×1,563 magnification in a minimum of 20 randomly selected fields with the use of appropriate filter sets for DAPI (365/395 ex, 420 em) and Cy-3 (545/565 ex, 610 em). Counts were completed within 1 week of slide preparation. A cell was considered labeled with Cy-3 only if it corresponded to a DAPI-stained cell. Autofluorescent cells, rarely detected and usually ≪1% of the cells, were subtracted from probe counts, although this was a negligible correction. The archaeal percentage was determined independently for each of the triplicate samples. Particles, distinguished on the filters by their color (yellow or brown), angular shape, and frequently large size compared with cells, were also counted, as in Wells and Deming (2003). In this paper, we refer to ''bacteria,'' ''bacterioplankton,'' and ''total cells'' when no distinction is intended between the domains Bacteria and Archaea; the term ''domain Bacteria'' is used when a distinction is intended.

Statistical methods—For statistical analyses, data were grouped into four categories: surface (≤6 m), nepheloid layers (defined as $c \geq 1\ m^{-1}$ and at depths between 20 and 150 m), nonnepheloid waters ($c < 1\ m^{-1}$ and at depths between 20 and 150 m), and all data. Spearman rank correlation coefficients (r_s) were calculated for pairwise comparisons between microbial (total cells, Archaea, and percent Archaea) and environmental variables (depth, temperature, salinity, nitrate, phosphate, silicate, oxygen, TOC, Chl *a*, POC, PON, Chl *a*:POC, C:N, attenuation coefficient, particles, and SPM). Additional comparisons were made between particle numbers or SPM weight and the remaining environmental variables over the entire data set. The acceptable probability of committing a Type I error was set at 0.05 (two-tailed), with $p < 0.001$ considered highly significant. Unreported correlations were not significant. Because multiple correlations were considered in our analyses, the probability of committing a Type I error for the entire data set is underestimated by that probability for any individual correlation. For some variables, river and marine samples differed dramatically in magnitude if not also trend. To avoid spurious correlations driven by the river samples, we examined correlations both in their presence and in their absence.

Results

Field conditions—During the sampling period, winds were variable, but most frequently from the northwest, and freeze-up was beginning. Surface waters were usually brackish (Table 1; Fig. 2), reflecting large freshwater inputs, especially from the Mackenzie River (and, in Franklin Bay, the smaller Horton River). Salinities along the north–south transect did not increase linearly with distance from the river mouth, however: for example, Sta. Z5 (30.1) and Sta. 65 (27.2) had higher surface salinities than Sta. 66 (20.8). Nitrate concentrations in surface waters were low (Table 1); the molar nitrate to phosphate ratio was <1 at all stations except Sta. 18 (=1.8). Surface-water phosphate increased with salinity, whereas silicate decreased, as expected in this river-influenced region (Carmack et al. 2004). Chl *a* concentrations were modest (median 0.37 $\mu g\ L^{-1}$; Table 1), although comparatively high in the Mackenzie River (1.7–2.0 $\mu g\ L^{-1}$). Nearshore Stas. Z1 and Z5 and Sta. 12 near the mouth of the Horton River in Franklin Bay had particle and SPM concentrations typically an order of magnitude higher than in the surface waters of other marine stations (3.0–17 $\times\ 10^4$ vs. 0.28–1.4 $\times\ 10^4$ particles mL^{-1}; 9.31–60.4 vs. 0.96–1.2 mg SPM L^{-1}), with elevated POC (189–228 vs. 72.9–182 $\mu g\ L^{-1}$) and PON (31.8–42.0 vs. 15.9–31.2 $\mu g\ L^{-1}$) concentrations as well (no POC or PON measurements were made at Sta. Z1; Table 2).

An extensive network of bottom nepheloid layers, typically 10–20 m in vertical extent, was observed throughout the region (Fig. 2; Table 2 beam attenuation coefficients). The regression of the beam attenuation coefficient, *c*, against SPM concentration was highly significant across the entire data set ($c = 0.36 \times [SPM] + 0.13$; $p \ll 0.0001$). Along the north–south transect, the shallow nearshore Stas. Z1 and Z5 were extremely turbid (7.66–17.0 $\times\ 10^4$ particles mL^{-1}, 12.7–60.4 mg SPM L^{-1}). Thereafter, the pronounced benthic nepheloid layer ($c = 3.15\ m^{-1}$) seen at Sta. 65 gave way to a minor feature at Sta. 66 ($c = 0.68\ m^{-1}$) and disappeared beyond the shelf break at Sta. 49. Extending eastward from Sta. 66, nepheloid layer attenuation coefficients progressively increased (and transmissivity decreased) to Sta. 75 ($c = 6.96\ m^{-1}$; Fig. 2A) and thereafter declined at Sta. 78 ($c = 2.45\ m^{-1}$) and Sta. 83 ($c = 1.01\ m^{-1}$). At Sta. 12 in Franklin Bay, nepheloid layers were observed throughout the water column, including an ~45-m turbid (~50% transmittance, $c > 2\ m^{-1}$) surface layer that extended through the halocline and lay atop a significant (~0.7°C) temperature inversion (Fig. 2B).

Relative to surface waters, deeper samples (nepheloid and nonnepheloid) had higher salinities and macronutrient concentrations (as expected) and lower concentrations of oxygen and TOC (Table 1). The only variables (other than microbiological ones) that distinguished nepheloid and nonnepheloid waters were higher median concentrations of particles (2.02 $\times\ 10^4$ vs. 0.67 $\times\ 10^4$ mL^{-1}), POC (158 vs. 81.2 $\mu g\ L^{-1}$), and PON (32.2 vs. 20.0 $\mu g\ L^{-1}$) in nepheloid layers (Mann–Whitney *U*-test, $p < 0.05$ for each comparison). The highly significant regression of beam attenuation coefficient against SPM concentration implies that nepheloid layers also had significantly greater SPM concentrations than nonnepheloid water (which was sampled only once for SPM; Table 1).

The ratio of Chl *a*:POC, a proxy for organic carbon freshness, was highest, as expected, in overlying surface waters

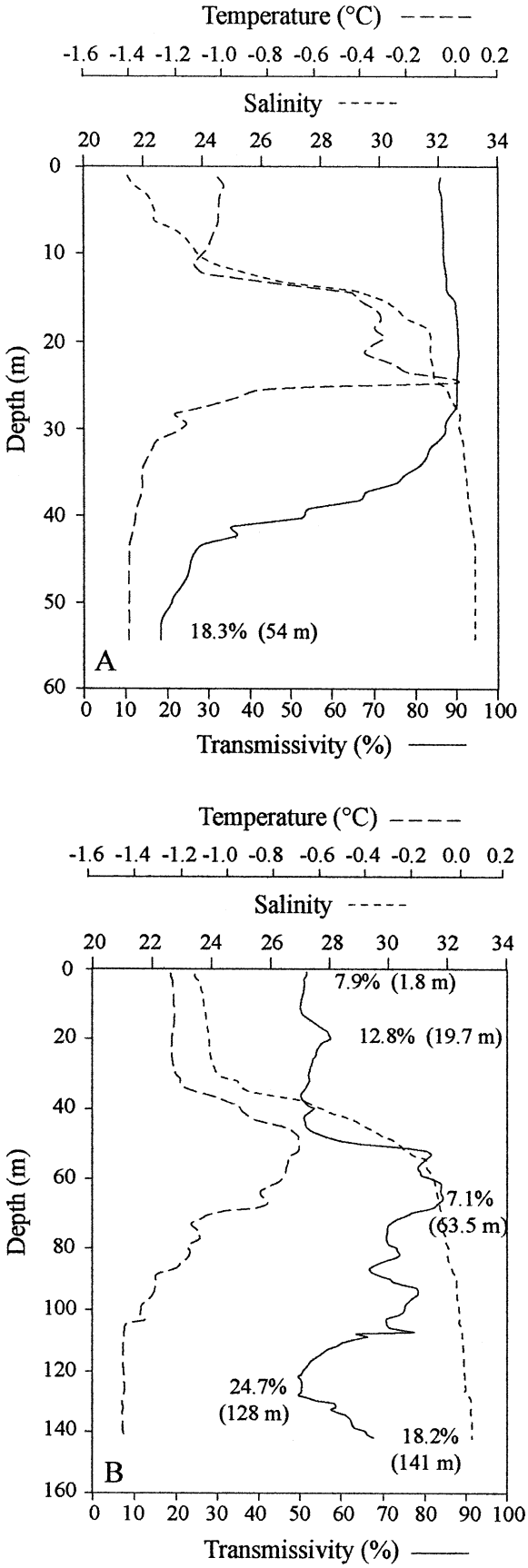
Table 1. Summary of environmental variables. For each, the median, range, and *n* (in parentheses) are reported. ND, not determined.

Variable	River (n=2)	Surface (<6 m)	Waters below surface (20–150 m)	
			Nepheloid	Nonnepheloid
Depth (m)	~0	2.5 0.5–5.5(7)	54.0 19.7–141.4(9)	60.5 19.7–90.1(7)
Temperature (°C)	2.9–3.9	−0.42 −1.26–0.42(7)	−1.36 −1.47–−1.07(9)	−0.76 −1.41–−0.064(7)
Salinity	0.2	24.46 20.85–30.09(7)	32.29 23.95–32.83(9)	31.85 28.40–32.51(7)
Nitrate (μmol L^{-1})	ND	0.34 0.12–0.64(5)	9.08 0.51–11.5(9)	3.78 0.05–11.7(6)
Phosphate (μmol L^{-1})	ND	0.38 0.30–0.56(5)	1.68 0.75–2.10(8)	1.16 0.53–1.95(6)
Silicate (μmol L^{-1})	ND	3.81 0.89–11.1(5)	25.2 3.50–28.5(9)	9.06 0.22–26.9(6)
Oxygen (μmol kg^{-1})	ND	388 386–390(4)	312 304–380(8)	353 302–397(7)
Chl *a* (μg L^{-1})	1.7–2.0	0.37 0.09–0.79(6)	0.20 0.05–0.57(8)	0.20 0.06–0.24(5)
TOC (μmol L^{-1})	430–490	100 69–120(7)	69 62–95(5)	66 63–70(7)
POC (μg L^{-1})	846–870	169 72.9–228(6)	158 88.6–322(8)	81.2 58.4–106(5)
PON (μg L^{-1})	60–78	27.1 15.9–42.0(6)	32.2 20.1–59.6(8)	20.0 11.7–23.0(5)
SPM (mg L^{-1})	27.8–42.5	5.3 0.96–60.4(6)	5.4 2.2–18.4(7)	1.34 (1)
Particles ($\times 10^4$ mL^{-1})	6.41–7.29	1.37 0.28–17(7)	2.02 1.36–4.68(9)	0.67 0.10–2.08(7)

(Table 2), with two exceptions on the north–south transect: at shallow (30.2 m) Sta. 65 the Chl *a*:POC ratio (and Chl *a*) was three to four times higher at the bottom than the surface, and at deep Sta. 49, the Chl *a* maximum was subsurface (60 m; Fig. 3). The low-salinity surface waters of Franklin Bay and Sta. 66 had the highest ratios (31.1–34.5 $\times 10^{-4}$; Table 2)—greater even than the two river stations (20.4–23.4 $\times 10^{-4}$). At deep Sta. 49, relatively high ratios of 16.0–21.1 $\times 10^{-4}$ in the upper 20 m gave way to values one and two orders of magnitude lower at 200 and 800 m, respectively, with a slight increase at the bottom. At Sta. 12, the ratio declined roughly linearly with depth (Table 2). Along the west–east bottom transect, the ratio was 20.9–22.2 $\times 10^{-4}$ at Stas. 66 and 69 (and 26.7 $\times 10^{-4}$ at Sta. 65), then declined progressively to a minimum of 5.9 $\times 10^{-4}$ at Sta. 83 (Table 2). The C:N ratio was useful only in recognizing river sample material (ratios by weight of 11.2 and 14.1) as more refractory than that in marine samples (median 5.0, range 3.0–12.8, $n = 24$; the highest value came from a depth of 800 m at Sta. 49).

Particles and SPM—On the north–south transect, surface particle concentrations increased northward from river Sta. R1 (6.41 $\times 10^4$ mL^{-1}) to a maximum near the delta at Z5 (1.70 $\times 10^5$ mL^{-1}), then declined to the observed minimum of 2.75 $\times 10^3$ mL^{-1} at off-shelf Sta. 49 (Table 2). SPM concentrations showed similar trends, with highest concentrations at river and coastal stations (maximum of 60.4 mg L^{-1} at Z1) and progressively lower values northward (minimum of 0.96 mg L^{-1} at Sta. 49). The featureless transmissivity profile at Sta. 49 ($c \leq 0.42$ m^{-1} at all sampled depths) corresponded to low particle and SPM concentrations, with maxima of each at the bottom (4.07 $\times 10^3$ particles mL^{-1} and 1.33 mg SPM L^{-1}; Table 2). Along the west–east bottom transect, particle concentration, low at Sta. 66, remained relatively constant from Stas. 69 to 75 (~2 $\times 10^4$ mL^{-1}), peaked at Sta. 78 (3.13 $\times 10^4$ mL^{-1}), and then decreased by half at Sta. 83 (Table 2). SPM concentrations along the bottom transect peaked at Sta. 75 (18.4 mg L^{-1}) and declined progressively both east and west of there (Table 2). In Franklin Bay, surface concentrations of particles and SPM were both higher at Sta. 12 than at Sta. 18 (3.00 $\times 10^4$ vs. 8.92 $\times 10^3$ particles mL^{-1} and 9.31 vs. 1.20 mg SPM L^{-1}, respectively). At Sta. 18, particle and SPM maxima coincided at 60-m depth, but at Sta. 12, the particle maximum was at the bottom (4.68 $\times 10^4$ mL^{-1}) despite a surface SPM concentration almost twice that at the bottom (Table 2). Such discrepancies notwithstanding, particle and SPM concentrations correlated not only with one another but with the same environmental variables (Table 3) across the marine data set. For both, the strongest physical or chemical correlate (after beam attenuation coefficient) was the PON concentration (Fig. 4).

Abundance and distribution of bacterioplankton—To assess whether cells were lost during the FISH procedure, we compared cell counts between the DAPI control and corresponding filter sections that underwent FISH. The counts were highly correlated ($r_s = 0.88$, $n = 117$, $p \ll 0.001$) with a slope of 1.0. Accordingly, we averaged DAPI counts from



the autofluorescence, probed, and DAPI control slides to generate the final cell count reported per sample.

Total cell concentrations in the river were about twice those found in the surface marine environment (14.6–16.0 $\times 10^5$ mL^{-1} vs. a median of 6.73 $\times 10^5$ mL^{-1}; Table 2). Along the north–south transect from marine Stas. Z1 to 49, cell concentrations tended to decrease with distance offshore (from 6.73 $\times 10^5$ to 4.63 $\times 10^5$ mL^{-1}; Table 2) except at Sta. 66, which had the highest surface concentration (8.89 $\times 10^5$ mL^{-1}) along this transect and the highest Chl *a* content (0.58 μg L^{-1}, two to five times higher) and Chl *a*:POC ratio (32.0 $\times 10^{-4}$ vs. 8.0–16.0 $\times 10^{-4}$). Its surface salinity of 20.8 was the lowest of all marine samples. Surface samples in distant Franklin Bay had comparably high total cell concentrations (7.51 $\times 10^5$ and 1.07 $\times 10^6$ mL^{-1}), also corresponding to high Chl *a* concentrations (0.49 and 0.79 μg L^{-1}), Chl *a*:POC ratios (31.1 $\times 10^{-4}$ and 34.5 $\times 10^{-4}$), and low salinities (23.5 and 24.2). Although no significant correlations (only trends) were observed between total cell concentrations and other measured variables in marine surface samples, if the river stations were included, significant correlations were observed with temperature ($r_s = 0.68$, $p = 0.05$), POC ($r_s = 0.76$, $p < 0.04$), TOC ($r_s = 0.85$, $p < 0.006$), Chl *a* ($r_s = 0.86$, $p < 0.02$), salinity ($r_s = -0.87$, $p < 0.005$), and the C:N ratio ($r_s = 0.90$, $p < 0.005$).

Nepheloid and nonnepheloid waters supported similar densities of bacterioplankton (median 4.51 $\times 10^5$ and 5.51 $\times 10^5$ mL^{-1}, respectively; Mann–Whitney *U*-test, $p > 0.20$) that were typically lower than those found in the overlying surface waters (Table 2). Cell numbers tended to decline along the west–east bottom transect (Table 2), except at Sta. 78, where cell concentrations were 4.93 $\times 10^5$ mL^{-1}; the only noticeably different features were higher concentrations of PON (59.6 vs. 20.1–33.4 μg L^{-1} at the other stations) and particles (3.13 $\times 10^4$ vs. 0.76–2.02 $\times 10^4$ mL^{-1}). In nepheloid layers, total cell concentration correlated significantly with temperature ($r_s = 0.70$, $p < 0.05$), salinity ($r_s = 0.78$, $p < 0.02$), Chl *a* ($r_s = 0.79$, $p < 0.03$), and the Chl *a*:POC ratio ($r_s = 0.90$, $p < 0.01$). Total cell concentrations of nonnepheloid waters correlated significantly only with Archaea ($r_s = 0.86$, $p < 0.03$). In terms of depth profiles, bacterioplankton decreased approximately linearly with depth at Sta. 12 (Table 2), but deeper Sta. 49 showed a subsurface maximum coinciding with the Chl *a* maximum, followed by a precipitous decline to concentrations of $<1 \times 10^5$ mL^{-1} at and below 600 m (Fig. 3). Over the entire marine data set, the strongest correlates of total cell concentration were salinity, Chl *a*, the Chl *a*:POC ratio, TOC, depth, and nitrate (Table 3).

←

Fig. 2. Water column profiles showing temperature, salinity, and transmissivity at (A) Sta. 75 and (B) Sta. 12. Percentages recorded on the graph indicate the archaeal fraction of the bacterioplankton at the corresponding depth. The profile shown for Sta. 12 is a composite of two casts: one from the surface to ~60 m and the other from 60 m to the bottom. Note that the figures are not drawn to the same depth scale.

Table 2. Particulate and microbial variables. The error indicated is the standard error of the mean of triplicate samples or the range when only two replicates were available (*). Microbial data for Sta. 49 between surface and bottom are shown in Fig. 3. *c*, beam attenuation coefficient; ND, not determined. Samples were classified as surface (S), nepheloid (N), nonnepheloid (NN), or deep (D).

Sta.	Depth (m)	c (m^{-1})	SPM (mg L^{-1})	Particles ($\times 10^4$ mL^{-1})	POC (μg L^{-1})	PON (μg L^{-1})	Chl *a*: POC ($\times 10^{-4}$)	Total cells ($\times 10^5$ mL^{-1})	Archaea ($\times 10^4$ mL^{-1})	% Archaea	Sample type
River											
R1	~0	ND	42.5	6.41	870	78.0	23.4	14.6±1.29	14.4±0.73	9.1±0.4	S
R2	~0	ND	27.8	7.29	846	60.0	20.4	16.0±0.63*	11.5±0.68*	9.0±0.6*	S
North–south surface and bottom transect											
Z1	0.5	ND	60.4	7.66	ND	ND	ND	6.73±2.03	4.05±0.92	7.6±0.2	S
Z5	0.5	ND	12.7	17.0	189	42.0	12.8	6.23±0.71*	9.52±2.48*	15.1±2.6*	S
65	3.3	0.92	1.20	1.37	112	22.9	8.0	6.08±0.93	2.90±0.45	4.6±0.2	S
	30.2	3.15	6.72	1.36	148	34.6	26.7	4.96±0.22	7.00±0.45	15.5±0.9	N
66	3.4	0.84	ND	0.67	182	31.2	32.0	8.98±0.63	1.35±0.09	1.7±0.1	S
	65.5	0.68	ND	0.76	102	20.5	20.9	5.51±0.68	1.79±0.13	3.3±0.2	NN
49	2.5	0.42	0.96	0.27	72.9	15.9	16.0	4.63±0.19	2.36±0.47	4.8±0.4	S
	968	0.40	1.33	0.41	38.6	13.0	0.5	0.51±0.04	1.14±0.24	22.2±3.1†	D
West–east bottom transect (starting with Sta. 66 above)											
69	55.9	1.05	2.20	2.02	100	20.1	22.2	5.01±0.52	3.23±0.69	5.9±1.1†	N
72	55.4	1.31	2.74	1.89	127	25.9	12.1	4.51±0.29	5.01±0.75	14.5±3.9†	N
75	54.0	6.96	18.4	1.86	322	33.4	9.6	3.39±0.47	6.00±1.99	18.3±4.4	N
78	34.1	2.45	5.36	3.13	229	59.6	7.4	4.93±0.18	9.08±0.21	17.3±0.9	N
83	43.2	1.01	2.53	1.36	88.6	29.2	5.9	3.21±0.52	3.96±0.46	10.8±0.6	N
Franklin Bay											
12	1.8	2.61	9.31	3.00	228	31.8	34.5	7.51±0.26	5.76±1.36	7.9±2.2	S
	19.7	2.15	ND	3.94	196	48.8	29.2	6.28±0.14*	7.73±0.14*	12.8±1.1*	N
	63.5	0.67	ND	2.08	106	20.0	22.8	5.73±0.10	4.20±0.08	7.1±0.6†	NN
	128	2.79	ND	2.77	ND	ND	ND	3.47±0.10	7.70±1.26	24.7±1.7†	N
	141	1.61	5.41	4.68	168	31.0	4.4	2.85±0.08	5.82±1.02	18.2±3.3	N
18	5.5	0.64	1.20	0.89	157	21.9	31.1	10.7	5.02	4.4	S
	19.7	0.52	ND	0.67	58.4	11.7	ND	7.25±0.10*	2.90±0.89*	3.9±1.3*	NN
	59.4	0.57	1.34	1.45	81.2	23.0	ND	4.64±0.65*	1.52±0.27*	3.3±0.1*	NN
	226	0.54	1.28	0.61	129	20.1	1.1	1.70	ND	ND	D

† If Arch915 was not included in the probe mix, archaeal percentages were as follows: 6.5±0.9 (Sta. 49, 800 m), 11.8±0.0 (Sta. 49, 968 m), 5.1±0.9 (Sta. 69), 3.9±0.6 (Sta. 72), 4.9±0.4 (Sta. 12, 63 m), and 5.6±1.0 (Sta. 12, 128 m).

Abundance and distribution of Archaea—Because of reports of apparent cross-hybridization of Arch915 with members of the domain Bacteria (Pernthaler et al. 2002; Brinkmeyer et al. 2004), we compared archaeal percentages on 16 samples (counting replicates) from four stations (Sta. 12 at 63 and 128 m, Sta. 49 at 800 and 968 m, Sta. 69 at 56 m, and Sta. 72 at 55 m) in the presence or absence of this probe. Percentages determined by the two methods correlated with one another ($r_s = 0.61$, $n = 16$, $p < 0.02$) but were an average of 2.5 times greater in the presence of Arch915 (Table 2). Hereafter, results refer to archaeal counts from all four probes.

Like the total bacterioplankton, Archaea were roughly twice as abundant at the river stations as along the shelf (Table 2). They represented ~9% of the river bacterioplankton at both R1 and R2. Along the north–south surface transect, Archaea varied from 1.35×10^4 mL^{-1} and 1.7% of the total cells (at Sta. 66) to 9.52×10^4 mL^{-1} and 15.1% of the total cells (at Sta. Z5). No discernible pattern was seen between archaeal distribution in surface waters (either in absolute abundance or as a percentage of the cells) and distance from shore or salinity. Over the limited range of 0–6 m, Archaea and percent Archaea correlated negatively with depth; if river samples were excluded, this correlation was only significant for percent Archaea. The only other variables that correlated with surface Archaea were particle related: concentrations of particles ($r_s = 0.68$, $p = 0.05$), POC ($r_s = 0.83$, $p < 0.02$), and PON ($r_s = 0.83$, $p < 0.02$). Excluding river samples, the Archaea correlated significantly only with particles ($r_s = 0.79$, $p < 0.05$). The percent Archaea correlated with particles only if the river samples were included ($r_s = 0.78$, $p < 0.02$).

Archaea were three to four times more prevalent in nepheloid than in nonnepheloid waters (Mann–Whitney *U*-test, $p < 0.05$), both in terms of abundance (medians of 6.00×10^4 vs. 1.79×10^4 mL^{-1}) and as a fraction of the cells (15.5% vs. 3.6%; Table 2). In nepheloid layers, archaeal concentration correlated with silicate ($r_s = -0.77$, $p = 0.02$), POC ($r_s = 0.79$, $p < 0.03$), oxygen ($r_s = 0.81$, $p < 0.03$), and PON ($r_s = 0.98$, $p < 0.001$; Fig. 5A). The archaeal fraction of the cells correlated with POC ($r_s = 0.79$, $p < 0.03$), SPM ($r_s = 0.89$, $p < 0.02$), and the beam attenuation coefficient ($r_s = 0.75$, $p < 0.03$). In nonnepheloid waters, no significant correlation was observed between Archaea

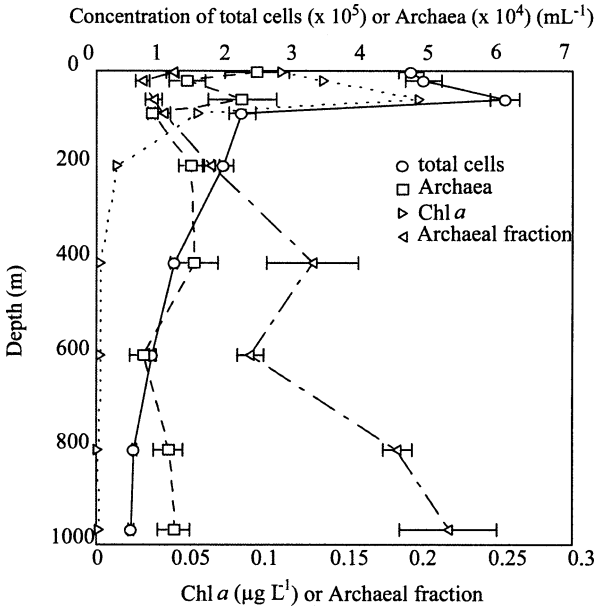


Fig. 3. Depth profile of Chl *a*, total cells, Archaea, and percent Archaea at Sta. 49. Error bars represent the standard error of triplicate samples. Archaea were determined with a cocktail of all four archaeal probes.

Table 3. Spearman rank correlation coefficients (r_s) of variables correlating significantly ($p < 0.05$) with concentrations of particles, SPM, total cells or Archaea over the entire marine data set. The inclusion of river samples marginally affected correlations (usually strengthening them) except where noted. ns, not significant. * $p < 0.005$; ** $p < 0.001$.

Variable	Particles	SPM	Total cells	Archaea
Depth	ns	ns	−0.86**	−0.47
Temperature	ns	ns	0.44†	ns
Salinity	ns	ns	−0.89**	ns
NO_3	ns	ns	−0.80**	ns
PO_4	ns	ns	−0.48	ns
TOC	0.58*	ns‡	0.87**	0.57*
Chl *a*	0.58*	ns§	0.89**	0.63
Chl *a* : POC	ns	ns	0.89**	ns
POC	0.76**	0.68*	0.51	0.84**
PON	0.80**	0.82**	0.42	0.83**
c	0.86**	0.86**	0.40	0.85**
Particles	—	0.77**	0.46	0.85**
SPM	0.77**	—	ns	0.70*
Total cells	0.46	ns	—	0.43
Archaea	0.85**	0.70*	0.43	—

† $r_s = 0.30$, $p < 0.20$ if the river was included.
‡ $r_s = 0.63$, $p < 0.01$ if the river was included.
§ $r_s = 0.60$, $p < 0.05$ if the river was included.

(concentration or percentage) and any environmental variable (aside from total cell concentration, as already noted).

Regarding depth profiles, archaeal abundance in Franklin Bay (Sta. 12) varied roughly by a factor of two (4.20×10^4 to 7.70×10^4 mL^{-1}) over the five depths sampled, whereas archaeal percentages ranged from 7.1% to 24.7%. Variability appeared to be as associated with the turbidity structure of the water column as with depth (Fig. 2B). In contrast, at Sta. 49, where no significant transmissivity features were observed, archaeal concentrations were highest in the upper water column ($\sim 2 \times 10^4$ mL^{-1}), then relatively constant at greater depths (Fig. 3). The Archaea made up a larger fraction of the total cells at depth, however, reaching a maximum of 22% near the seafloor (Fig. 3). Considering the whole marine data set, archaeal concentrations correlated most strongly with particulate variables (Table 3; Fig. 5) and, unlike the bacterioplankton, showed no significant correlation with the Chl *a* : POC ratio (or salinity and nitrate).

Discussion

General environmental characteristics—The Beaufort Shelf is the most estuarine of Arctic shelves, with an equivalent freshwater surface layer of >6 m, mostly from discharge from the Mackenzie River and melting sea ice (recently reviewed in Carmack et al. 2004). The irregular distribution of surface water salinities along the north–south transect likely reflects the complicated river plume structure, consistent with other observations (*see* fig. 4 in Macdonald et al. 1989) and plume wisps and eddies recorded by satellite (Kulikov et al. 1998). It might also reflect inshore upwelling (Garneau et al. unpubl. data), as suggested by the high salinity at Sta. Z5. Although our data set of surface Chl *a* concentrations is too small to evaluate links of such physical complexity, the importance of the river plume structure on the shelf is suggested by the much greater concentrations of Chl *a* (and other variables) recorded in the river itself (Tables 1, 2) and the observation of highest Chl *a* concentration (and Chl *a* : POC ratio) in the relatively fresh surface waters of marine Sta. 66 (20.8). Chl *a* concentrations in surface marine

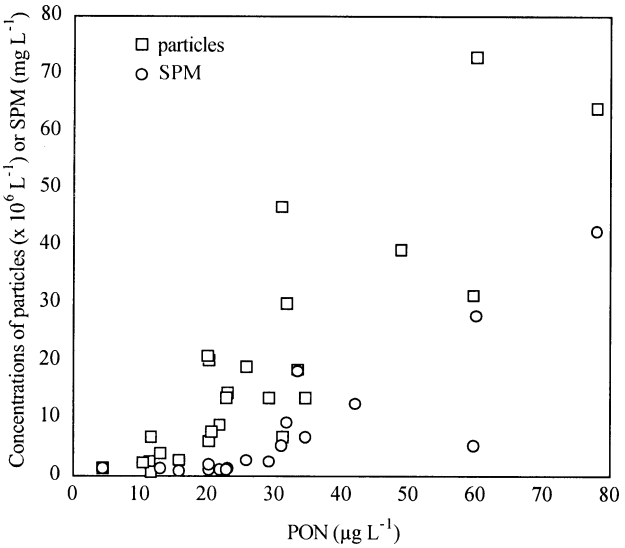


Fig. 4. Relationship between the concentrations of particles or SPM and PON. All data (including river samples) are shown, except for the particle concentration at Sta. Z5 (17×10^4 mL^{-1} corresponding to 42 μg L^{-1} PON), which is off scale. The correlation coefficients are $r_s = 0.83$, $p < 0.001$ for particles (including Sta. Z5) and $r_s = 0.87$, $p < 0.001$ for SPM; *see Table 3* for correlation coefficients excluding the river.

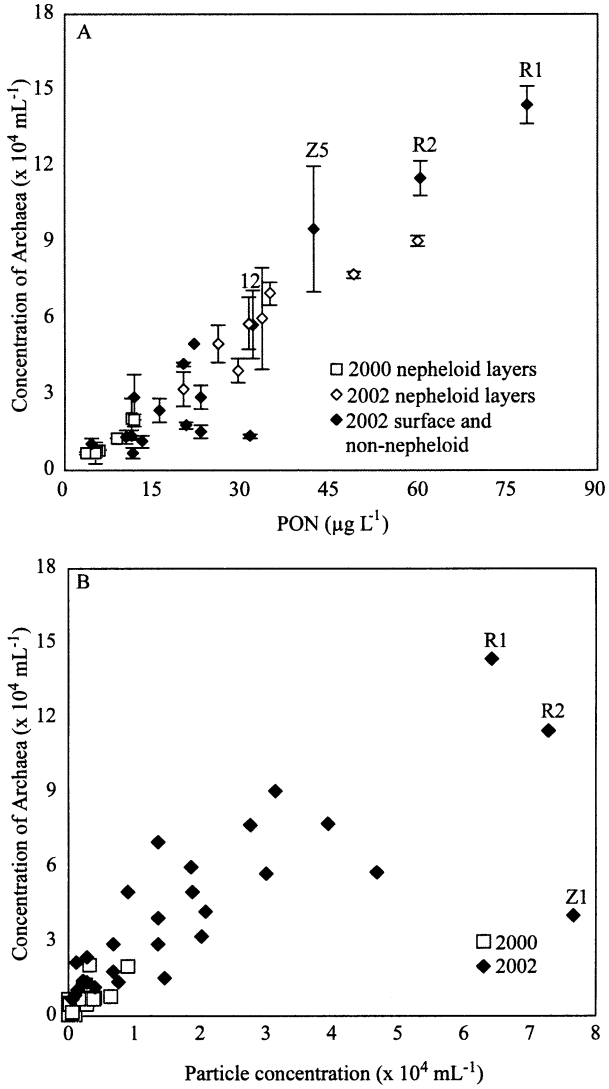


Fig. 5. (A) Relationship between the concentrations of Archaea and PON ($n = 7$ for nepheloid layer archaeal concentrations sampled in 2000; $n = 25$ for 2002 data, including $n = 8$ for nepheloid layers), with particle-rich surface waters indicated by station (Stas. 12, Z5, R1, and R2). Error bars show the standard error of the mean. (B) Relationship between the concentrations of Archaea and particles. All data are shown ($n = 26$ in 2000; $n = 29$ in 2002) except for Sta. Z5, which is off scale (17×10^4 particles mL^{-1} and 9.5×10^4 Archaea mL^{-1}). Because of the large number of 2000 data plotting near the origin, error bars (comparable to those in A) are omitted to preserve clarity. For both (A) and (B), correlations reported in the text used only 2002 data, and Archaea were determined with a cocktail of all four archaeal probes.

waters throughout our study were comparable to the lower values reported in Parsons et al. (1988, 1989) for studies done earlier in the season (July–August). The low molar nitrate-to-phosphate ratio (<1) implied nitrogen limitation over most of the region at the time of our study. Overall, the region was less oligotrophic than during our 2000 fieldwork further east, when nitrate was frequently undetectable and Chl *a* concentrations were typically about one-third those reported here (Wells and Deming 2003).

Physical characteristics of particle-rich waters—The high concentrations of particles and SPM found nearshore in the vicinity of the Mackenzie River mouth represent both the direct contribution of sediment-laden river water and resuspension of bottom sediments at the shallow stations (Stas. Z1 and Z5). The latter is implied by greatest SPM concentrations at Sta. Z1, rather than in the river (Table 2). Other studies have also reported high levels of resuspension around the 5-m isobath in Kugmallit Bay, attributable on this microtidal shelf predominantly to wind-generated wave action (Iseki et al. 1987; Hill and Nadeau 1989). If resuspended rather than newly arrived from the river, the vast majority of resuspended sediments should nonetheless be of river origin (Hill et al. 1991; Macdonald et al. 1998). Beyond this nearshore environment of high turbidity from surface to bottom, particle-rich waters during open-water season are usually limited to a surface plume and a bottom nepheloid layer (Carmack et al. 1989). Both have been documented to extend at least 50 km offshore, as we also observed, often with SPM concentrations in the bottom layer exceeding those at the surface (Hill et al. 1991). Wave-generated resuspension and particles sinking from the surface are thought to feed the bottom nepheloid layer (Hill et al. 1991), linking the two particle-rich environments.

Seaward of the 20-m isobath, wind-induced resuspension is rare. Under northwesterly winds, the surface plume propagates to the east (Carmack et al. 2004), perhaps explaining why turbid surface waters were not detected north of Sta. Z5 on the north–south transect (and consistent with the attenuation profiles in Kugmallit Bay of Parsons et al. [1988] and Carmack et al. [1989]). The near-bottom layer is also driven in this direction by an easterly bottom current (especially beyond the 50-m isobath; Hill et al. 1991). Such a northeastward advance is suggested by our observations of benthic nepheloid layers along the shelf: they declined north of Sta. 65 but strengthened to the east as far as Sta. 75 (compare the attenuation coefficients listed in Table 2). Because mean bottom current speeds of 0.08–0.11 m s^{-1} are unlikely to support more than weak resuspension (Hill et al. 1991), most of the particles in the bottom layer were probably derived from the inner shelf. This supposition is supported by the progressive decline of the bottom water Chl *a* : POC ratio with distance from the mouth (Table 2), indicative of aging organic matter and pointing to a riverine or deltaic origin. Such a scenario conforms with other work demonstrating a marked affiliation specifically between SPM composition in bottom nepheloid layers and in rivers (Sicre et al. 1994).

Compared with the shallow, relatively flat Beaufort Shelf (average bottom slope of 10^{-4}; Carmack et al. 1989), Franklin Bay has a steep bathymetry (Fig. 1) that might contribute to the explanation of the complex nepheloid layer structure observed at Sta. 12. Whereas along the west–east transect all nepheloid layers were associated with the bottom at depths between 34 and 66 m, at Sta. 12, nepheloid layers extended from the surface to the bottom (~141 m; Fig. 2B). Given the depth, the upper nepheloid layers were unlikely to have been significantly affected by bottom resuspension (and, in fact, the SPM concentration at the surface was almost twice that at the bottom; Table 2). It is unclear whether

the Mackenzie River plume can account for surface SPM concentrations of the magnitude observed (9.31 mg L^{-1}) this far east, especially given the low value at Sta. 18 farther north. An additional and perhaps dominant particle source for the surface nepheloid layer at Sta. 12, and likely an important contributor (through particle settling and resuspension) to the underlying nepheloid layers observed there, might be the plume of the nearby Horton River, about which little published information is available. The linear decrease of the Chl *a*:POC ratio with depth (Table 2) suggests that biogenic material sinking from the surface was a substantial component of the Sta. 12 nepheloid layers.

Distribution of bacterioplankton—Concentrations of total cells in marine surface waters (4.63–10.7 $\times$ 10^5 mL^{-1}) were higher than those observed farther east in September 2000 (1.23–5.11 $\times$ 10^5 mL^{-1}; Wells and Deming 2003) but well within the range reported for the summertime Mackenzie River estuary (0.1–50 $\times$ 10^5 mL^{-1}; Parsons et al. 1988, 1989). These higher autumn concentrations are consistent with the substantially less oligotrophic nature of the region in 2002. Correlations with organic resources (Chl *a*, POC, and TOC) on inclusion of the river samples pointed to a predominance of heterotrophs within the bacterial assemblage. As for Chl *a*, the river appeared to play an important role in the marine distribution of bacteria on the shelf, given the pocket of high Chl *a* and bacterial concentrations in the low-salinity water of Sta. 66, the negative correlation of bacteria with salinity, and the tendency for surface cell concentrations to decrease with distance offshore. Relatively high surface concentrations of bacterioplankton and Chl *a* were also observed in the brackish waters of Franklin Bay, where the Horton River discharge might be locally important.

The median cell concentrations in both nepheloid and nonnepheloid waters were 2–2.5 times greater than those reported in 2000, again likely reflecting the less oligotrophic nature of the environment in 2002 and possibly the greater depth of nepheloid layers sampled in 2000 (median depth in 2000, 422 m). Although other studies have reported higher bacterial concentrations in benthic boundary or nepheloid layers compared with adjacent particle-poor waters (Ritzrau et al. 1997; Boetius et al. 2000), we did not observe statistically different concentrations either in 2000 (Wells and Deming 2003) or in 2002. Instead of correlating with any of the particulate variables that distinguished nepheloid and nonnepheloid waters, total cell concentrations in these subsurface waters were best explained by organic carbon freshness (Chl *a*:POC ratio). For example, at Sta. 12 in Franklin Bay, both cell concentration and the Chl *a*:POC ratio decreased linearly with depth, resulting in a ratio between the two that varied little (factor of <1.2) in the upper 64 m (Table 2). The marine data set as a whole strengthens these conclusions: bacterioplankton distribution was better explained by organic freshness, Chl *a*, and TOC concentrations (as well as salinity, depth, and nitrate) than by any specific measure of particulate matter (Table 3).

Distribution of Archaea—In contrast, particles and related variables were the important descriptors of archaeal distribution, whether in surface waters, nepheloid layers, or considered over the entire data set (Fig. 5). The profiles of archaeal percentage at Sta. 12 and along the west–east bottom transect appeared to track the attenuation coefficient at least qualitatively (Table 2; Fig. 2B) and were consistent with concentrations of particles, POC and PON being the strongest correlates of archaeal concentration (Table 3). These observations strengthen several of those made in 2000, when we also found a highly significant positive correlation between concentrations of Archaea and particles (Fig. 5B) and significantly more Archaea in nepheloid layers than adjacent waters. Few other studies have looked at the relationship between Archaea and particles, but Archaea have been detected in association with SPM in the North Sea (van der Maarel et al. 1998) and Black Sea (King et al. 1998) and with particles in the Columbia River estuary (Crump and Baross 2000). Archaea have also been identified in shelf sediments (Vetriani et al. 1998), including those from the Arctic (Ravenschlag et al. 2001), although as a small ($\sim$5% or less) component of the benthic bacterial community.

Further emphasizing the importance of particles to archaeal distribution in this region was the significant correlation of nepheloid-layer Archaea with PON, extending our earlier finding in 2000 ($r = 0.97$, $p < 0.001$; Wells and Deming 2003), with both data sets plotting along the same line (Fig. 5A). In 2002, however, we found that this correlation was highly significant across the entire data set, not just in nepheloid layers. In fact, the overall correlation was still driven by particle-rich samples, whether from the river, the surface, or nepheloid layers and including Franklin Bay (Fig. 5A). Separate analyses of particle-rich ($c \geq 1$ m^{-1} and including Stas. Z5, R1, and R2) and particle-poor ($c < 1$ m^{-1}) waters yielded a (highly) significant correlation with PON ($r_s = 0.97$, $n = 12$, $p \ll 0.001$) in particle-rich waters, but no significant correlation in particle-poor water whether or not the deep ($\geq$200 m) samples of Sta. 49 were included. The strength of this relationship across the dramatic environmental gradient from river to sea suggests a particle-driven connection among these archaeal populations, a possibility reinforced by the highly significant correlations of particles and SPM with PON (Fig. 4) and by the physical mechanisms that link particles in surface and bottom waters (including nearshore resuspension, particle settling, and the ultimate river source). This relationship with PON also raises the obvious question of whether Archaea play an important role in the nitrogen cycle of this Arctic shelf, especially given the significance of river plumes and benthic nepheloid layers to nitrogen cycling (particularly nitrification) in other coastal environments (e.g., Bianchi et al. 1999*a*,*b*).

Several lines of evidence in this study suggest that many of the Archaea along the Beaufort Shelf are allochthonous, including the large concentrations of Archaea found in the Mackenzie River (Table 2), the known dominance of the Mackenzie River as a particle source in this region, the enrichment of Archaea in nepheloid layers and particle-rich surface waters propagating from the river mouth, and the highly significant relationship between Archaea and PON, with the river representing the upper end member (Fig. 5A). If particles in Franklin Bay are largely derived from the Horton River (rather than the Mackenzie), then the abundance of Archaea in particle-rich waters also observed there sug-

gests that inputs of Archaea from terrestrial biomes might be common in the region. Although the few available quantitative studies indicate that Archaea are a small component of riverine bacterioplankton in other environments (usually ≤5%; Battin et al. 2001; Bouvier and del Giorgio 2002), our results are consistent with the high diversity of predominantly allochthonous Archaea associated with particles in the Columbia River estuary (Crump and Baross 2000) and the close phylogenetic affiliation between some marine benthic Crenarchaeal sequences and those from freshwater sediments (Vetriani et al. 1998). Given recent measurements of high archaeal fractions in tundra soil (up to 22% of detected cells; Kobabe et al. 2004), Arctic tundra is also a plausible seeding source of Archaea to the waters of this region, perhaps especially of methanogens. Although allochthony is the most parsimonious interpretation of our data, especially the relationships between Archaea and particulate variables (Fig. 5), our data do not exclude the possibility that different archaeal groups colonized the particle-rich waters in the ocean and in the river or that marine Archaea were stimulated by riverine input. We also note that the allochthonous scenario we propose implies an uncommon degree of euryhalotolerance.

Our deep profile of total cell and archaeal abundances (Fig. 3) showed trends similar to those reported for profiles in San Pedro Channel (Fuhrman and Ouverney 1998), at Sta. Aloha (Karner et al. 2001), and off the Antarctic peninsula (Church et al. 2003). In particular, the archaeal fraction increase with depth and peak at 22% off the bottom compares favorably with ~25% off Antarctica and 30–40% in the Pacific at similar depths, as reported by these other authors. In all three of these regimes, the concentrations of Archaea did not vary much between 100 and 1,000 m, hovering around $\sim 1 \times 10^4$ Archaea mL^{-1}. In general, the increased archaeal representation with depth seems largely to reflect decreasing concentrations of bacteria, an observation of currently unknown ecological import.

The higher archaeal concentrations and greater archaeal percentages recorded in 2002 than in 2000 probably reflect environmental more than methodological differences, given the less oligotrophic environment in 2002. Nearer the river mouth, the particle and PON concentrations important to describing archaeal distribution in both studies were also substantially greater in 2002. Although we used multiple archaeal probes rather than Arch915 alone (as in 2000), the detection in both years of similar, highly significant relationships between Archaea and particles (Fig. 5B), and especially Archaea and PON (Fig. 5A), argues for the ecological coherence of FISH data despite methodological differences.

A second methodological issue pertains to recent reports questioning the specificity of Arch915 (Pernthaler et al. 2002; Brinkmeyer et al. 2004). An analysis of Arch915 against the Ribosomal Database Project II Version 9.0 (released September 2004; Cole et al. 2005) indicates that, unlike the other archaeal probes used in this study, Arch915 might legitimately recognize two sequences belonging to the candidate division OP11 within the Bacterial domain, although further verification is required. Additional discoveries of unintended specificity to members of the domain Bacteria are not unlikely as the database expands; indeed, many other probes in frequent use no longer appear specific to the taxa for which they were designed. (For example, the common α-Proteobacterial probes ALF1b and ALF968 each hit ~800 sequences not classified as α-Proteobacteria in the RDP-II.) Cross-hybridization should therefore be considered seriously in any FISH study, especially those using short oligonucleotides, and is consistent with the ~2.5 times greater archaeal fraction measured when Arch915 was included in our probe mix (the correlation between fractions probed with three vs. four probes then being fortuitous). Correcting the entire data set by this factor of 2.5 will not affect the correlation analyses, however, because such a correction does not alter ranks; moreover, examination limited to the data from three-probe hybridizations (i.e., those omitting Arch915) reveals similar ecological trends to those reported here (e.g., a correlation between Archaea and particles; $r_s = 0.83$, $p < 0.06$, $n = 6$). As for OP11 sequences, nothing is known about this division's quantitative distribution or importance because they have been detected so far only in clone libraries and primarily from reducing environments, including soils and aquatic sediments (Harris et al. 2004; to our knowledge, nepheloid layers and polar environments have yet to be examined). In the absence of such information, we cannot exclude or otherwise qualify the possible contribution of OP11 organisms to our archaeal counts.

Nonetheless, cross-hybridization is not the only or necessarily the best interpretation of the data. The greater detection of Archaea when four probes were used (with any Group I or II archaeal cell in theory being bound by three of them) could instead reflect a nonlinear relationship between detection threshold and the number of probes used, Arch915 binding to a more accessible region of the ribosome, or Arch915 recognition of more archaeal sequences. The latter is supported by the greater number of archaeal sequences in RDP-II Release 8.1 (Cole et al. 2003) recognized by Arch915 (830) than by any of the other three probes (maximum of 564; archaeal sequences are not yet included in the Probe Check function of Release 9.0). Regardless of the explanation, the internal consistency of our data set, its consistency with the work done in 2000, and, with reference to the deep profile, its consistency with other reports using disparate methods (Karner et al. [2001] and Church et al. [2003] used polyribonucleotide FISH; Fuhrman and Ouverney [1998] used multiprobe oligonucleotide FISH, but not probe Arch915) argue that Arch915 remains ecologically informative. Such a conclusion is also compatible with the data of Pernthaler et al. (2002, fig. 5), which show that Arch915 and polyribonucleotide archaeal probes measure similar ecological dynamics, despite disagreeing on absolute abundances. Brinkmeyer et al. (2004) likewise determined that Archaea were a small component (<1%) of bacterial populations in Arctic melt ponds, despite apparent cross-hybridization with members of the domain Bacteria.

Our more extensive sampling and analytical work in 2002 corroborated and strengthened many of the observations that we made in 2000, both about the distribution of bacterioplankton in general and of Archaea in particular. Bacterioplankton distribution was best explained by salinity, depth, organic freshness, and the concentrations of Chl *a* and TOC, consistent with a predominantly heterotrophic assemblage; total cell concentrations were not elevated in nepheloid lay-

ers with respect to adjacent waters. Unlike the bacterioplankton as a whole, Archaea were specifically associated with nepheloid layers and, additionally, particle-rich surface waters. Transmissivity profiles, the Chl *a* : POC ratio in bottom waters, and our review of the literature indicate a close link among surface particle-rich waters, nepheloid layers along the shelf, and the river discharge. This link, the detection of highest concentrations of Archaea in the Mackenzie River, and the predominance of the river as a particle source in the region favor an allochthonous origin for many Archaea in the particle-rich waters examined; a similar scenario could also pertain to distant Franklin Bay, where the Horton River is a likely particle source. If allochthonous, no information is yet available regarding the diversity or activity of Archaea in this environment. Their highly significant correlation with PON in particle-rich waters observed in both 2000 and 2002 might imply an important archaeal contribution to some aspect of nitrogen cycling in the region. Further study could productively focus on determining the phylogeny of Archaea in the Mackenzie River and along the shelf (in particular to assess how closely Archaea in the two environments are affiliated), and on in situ Archaea group-specific activity measurements that include nitrogen cycling across a range of salinities.

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