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Importance of particle-associated bacterial heterotrophy in a coastal Arctic ecosystem

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ABSTRACT

The large quantities of particles delivered by the Mackenzie River to the coastal Beaufort Sea (Arctic Ocean) have implications for the spatial distribution, composition and productivity of its bacterial communities. Our objectives in this study were: (1) to assess the contribution of particle-associated bacteria (fraction ≥ 3 μm) to total bacterial production and their relationships with changing environmental conditions along a surface water transect; (2) to examine how particle-based heterotrophy changes over the annual cycle (Nov 2003–Aug 2004); and (3) to determine whether particle-associated bacterial assemblages differ in composition from the free-living communities (fraction < 3 μm). Our transect results showed that particle-associated bacteria contributed a variable percentage of leucine-based (BP-Leu) and thymidine-based (BP-TdR) bacterial production, with values up to 98% at the inshore, low salinity stations. The relative contribution of particle-associated bacteria to total BP-Leu was positively correlated with temperature and particulate organic material (POM) concentration. The annual dataset showed low activities of particle-associated bacteria during late fall and most of the winter, and a period of high particle-associated activity in spring and summer, likely related to the seasonal inputs of riverine POM. Results from catalyzed reporter deposition for fluorescence in situ hybridization (CARD-FISH) confirmed the dominance of Bacteria and presence of Archaea (43–84% and 0.2–5.5% of DAPI counts, respectively), which were evenly distributed throughout the Mackenzie Shelf, and not significantly related to environmental variables. Denaturing gradient gel electrophoresis (DGGE) revealed changes in the bacterial community structure among riverine, estuarine and marine stations, with separation according to temperature and salinity. There was evidence of differences between the particle-associated and free-living bacterial assemblages at the estuarine stations with highest POM content. Particle-associated bacteria are an important functional component of this Arctic ecosystem. Under a warmer climate, they are likely to play an increasing role in coastal biogeochemistry and carbon fluxes as a result of permafrost melting and increased particle transport from the tundra to coastal waters.

1. Introduction

The microbial characteristics of the coastal Arctic Ocean have been little explored, however studies over the Mackenzie Shelf in the Beaufort Sea, Western Canadian Arctic, have revealed diverse and active communities of Bacteria and Archaea that vary across salinity gradients (Galand et al., 2006; Garneau et al., 2006; Galand et al., 2008, in press; Vallières et al., in press). The Mackenzie region constitutes the largest shelf area on the North American Arctic, extending 100 km offshore with a total area of ~64 000 km^2 (Macdonald et al., 1995). The Mackenzie River discharges 249 km^3 of freshwater into the region each year, the fourth highest annual riverine discharge in the Arctic Basin after the Siberian rivers Yenisei, Lena and Ob (Telang et al., 1991). The Mackenzie also

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delivers about 120×10^6 tons of suspended particulate material into this shelf environment each year (Droppo et al., 1998; Macdonald et al., 1998) that could potentially provide physical and chemical substrates for bacterial colonization and growth.

Molecular studies have often revealed pronounced taxonomic differences between particle-associated bacteria and the community of free-living cells in the surrounding medium (Delong et al., 1993; Acinas et al., 1999; Riemann et al., 2000; Fandino et al., 2001; Moeseneder et al., 2001; Allgaier and Grossart, 2006; Grossart et al., 2006; Rink et al., 2007). However other studies have reported little difference (Murrell et al., 1999; Hollibaugh et al., 2000) or overlap between the free-living and particle-associated communities (Riemann and Winding, 2001; Stevens et al., 2005; Ghiglione et al., 2007). Particle-associated and free-living bacteria may differ in their capacities for processing organic matter, and resolving such discrepancy is critical for understanding carbon fluxes in the microbial food web, especially in particle-rich environments such as the Mackenzie Shelf ecosystem.

Particle-associated bacteria often show a higher specific metabolic activity than free-living communities (Crump et al., 1998, 1999; Murrell et al., 1999; Fandino et al., 2001; Ghiglione et al., 2007; Grossart et al., 2007). Phylogenetic groups known to be dominant colonizers of particles include Sphingobacteria/Flavobacteria (previously reported as *Cytophaga–Flavobacter–Bacteroides*), Gammaproteobacteria (Simon et al., 2002) and Planctomycetales (Delong et al., 1993), as well as phytoplankton-associated taxa such as *Roseobacter* (Riemann et al., 2000; Moran et al., 2007; Rink et al., 2007). Many of these taxa possess specific metabolic pathways for the degradation of complex polysaccharides (Cottrell and Kirchman, 2000; Moran et al., 2007), which are an important component of suspended particulate materials (SPM). Particle-associated bacteria have also been implicated in the production of dimethylsulfide (DMS; Scarratt et al., 2000), a climatically active biogenic gas (Malin and Kirst, 1997). The Archaea domain contains several groups of taxa, which may be involved in nitrogen cycling (Sinninghe Damsté et al., 2002). Archaea are also frequently found in association with particles (Crump and Baross, 2000a), and in the Mackenzie Shelf region, Archaea have been found to be more abundant in particle-rich waters (Garneau et al., 2006; Wells et al., 2006).

The greater part of the organic matter brought by the Mackenzie River to the coastal Arctic Ocean is land-derived, highly degraded and recalcitrant to further metabolic processing (Goñi et al., 2000). The remaining load of organic matter (~30%) is younger and more labile, and likely derived from vascular plant materials (Goñi et al., 2005). Arctic climate warming may be accompanied by an increase of aged carbon inputs as a consequence of permafrost melting (Payette et al., 2004), but with a concomitant increases in young organic carbon because of the northward treeline advance (Serreze et al., 2000; Guo et al., 2007). Given the likely differences in biological reactivity, the proportion of old and recalcitrant versus young and labile organic carbon will affect the suitability of particles for particle-associated communities.

The first objective of the present study was to assess spatial variability in the contribution of particle-associated bacteria to total bacterial production, and to evaluate relationships between particle-associated activity and environmental gradients across the Mackenzie Shelf. We hypothesized that the particle-associated contribution would likely be a function of the concentration of SPM. The second objective was to evaluate the seasonal changes in importance of particle-associated bacterial production in the coastal shelf environment. Our study was undertaken as a part of the Canadian Arctic Shelf Exchange Study (CASES) program, which included the freeze-in deployment of a research icebreaker on the Arctic shelf. This provided a unique opportunity to obtain a detailed record of seasonal variations in microbial and environmental variables. As a third objective, we addressed the question of whether particle-associated and free-living assemblages differ in phylogenetic community structure in this region of the coastal Arctic Ocean.

2. Methods

2.1. Sample collection

Samples were collected at eight stations within the Mackenzie River plume on the Mackenzie Shelf of the Beaufort Sea (Fig. 1), from 30 June to 11 July 2004, to investigate the spatial distribution and dynamics of microbial communities. The seasonal study was carried out in nearby Franklin Bay (70° 02′ N, 126° 18′ W; Fig. 1), from 4 November 2003 to 6 August 2004. This time-window included a 6-month period (December to June) where the icebreaker CCGS Amundsen was frozen into first-year pack ice. Microbial variables on the Mackenzie Shelf were collected from surface waters (≤3 m) from the CCGS Amundsen, or by Zodiac for coastal stations 915, 917 and 918 that were located inside the 20-m isobath positioned at ca. 70° N. Conductivity, temperature and depth were measured using a Seabird 911+CTD mounted on a Seabird Carousel rosette equipped with 24 Niskin type bottles of 12 L. Coastal stations were sampled with a 5-liter Go-Flow bottle and a SBE-19 CTD profiler. Samples from Franklin Bay were also collected from surface waters via the Amundsen during the open water season. During winter, subsurface samples were taken from a site located 450 m up current from the ship to avoid contamination. Samples from 3 m were collected below the sea ice interface to avoid frazil ice using a 5-liter Go-Flow bottle. The maximum ice thickness measured during the study was 1.8 m.

Water samples were filtered through a 3 μm pore-size polycarbonate filter to separate particle-associated communities from free-living bacterial communities. For the bacterial analysis, the >3 μm fraction may also have included free-living filamentous bacteria which can vary from 8 to >100 μm in length (Schauer and Hahn, 2005). The <3 μm fraction may also have included some smaller aggregates of particle-associated bacteria or cells dislodged during prefiltration and therefore our estimates of particle-based heterotrophy presented here are likely to be conservative. The fractionation size was selected based on previous studies in estuarine waters (Vincent et al., 1996) and our preliminary results from the Beaufort Sea (Garneau et al., 2006). Total and small (<3 μm) fractions were also analyzed for suspended particulate materials (SPM), particulate organic matter (POM), chlorophyll *a* concentration (Chl *a*), leucine (Leu) and thymidine (TdR) incorporation rates to estimate bacterial production. The particle-associated fraction was calculated by difference. Analysis of community structure by catalyzed reporter deposition for fluorescence in

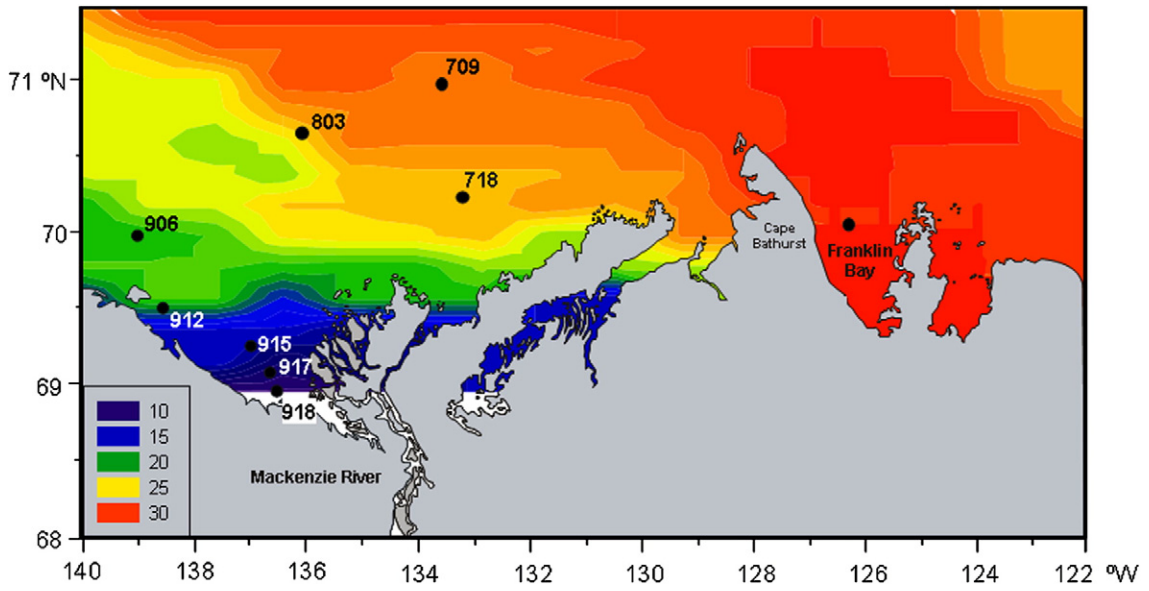

Fig. 1. Sampling stations and the salinity field on the Mackenzie Shelf and in Franklin Bay in summer 2004. Salinity values in psu.

situ hybridization (CARD-FISH) was made on the total and free-living fractions. For the denaturing gradient gel electrophoresis (DGGE), the free-living and particle-associated fractions were filtered sequentially through 3 µm and 0.2 µm pore-size filters.

2.2. Environmental variables

Samples for SPM were filtered onto pre-combusted, pre-weighed GF/F filters, rinsed with 10 ml of 1% ammonium formate to dissolve salts, and stored in petri dishes at −20 °C. The filters were subsequently dried at 60 °C for 24 h and weighed. Following SPM determination the filters were heated at 500 °C for 1.5 h to remove organic carbon and particulate inorganic material was determined. The difference between the two weights was used to estimate POM. The total and small fractions were measured from different filters, and the large fraction (>3 µm) was obtained by subtraction. At two stations, the technique led to negative values for POM>3 µm and these data were not used in subsequent analyses.

Absorption of colored dissolved organic matter (CDOM) was used as an index of the extent of riverine influence (Retamal et al., 2007). The samples were filtered through 47-mm 0.2 µm pore-size Nuclepore filters and absorbance was then measured in acid-cleaned, 10-cm quartz cells using a Varian Cary Bio 300 scanning spectrophotometer. Samples were scanned at 2 nm intervals between 250 and 820 nm (HP 8452A) and at 1 nm intervals between 200 and 850 nm (Cary Bio 300), against MilliQ pure water. The spectrum was corrected for the absorption offset using the mean value for wavelengths greater than 800 nm. The absorption coefficients (a) were calculated as:

$$a(\lambda) = 2.303 \times A(\lambda)/L$$

where $A(\lambda)$ is the optical density for wavelength λ and L is the cell path length in meters.

Phytoplankton biomass was estimated from Chl *a* extracted from the total community and the small <3 µm fraction. The large >3 µm fraction (Chl a_L) was obtained by subtraction. Samples were filtered onto Whatman GF/F filters and stored frozen (−80 °C) until pigment extraction in ethanol (Nusch, 1980). Concentrations were determined by fluorometry before and after acidification (Strickland and Parsons, 1972) using a Cary Eclipse spectrofluorometer standardized with *Anacystis* Chl *a*.

2.3. Bacterial abundance and diversity

Bacterial abundance was estimated from glutaraldehyde fixed samples (2% final v/v) filtered within 24 h onto 0.2 µm pore-size black polycarbonate filter and stained with 4′,6-diamidino-2-phenylindole (DAPI). This technique does not distinguish between Archaea and Bacteria; for convenience, we use the term bacteria when referring to DAPI counts and Archaea to refer specifically to the prokaryotes of this domain. A minimum of 400 DAPI stained cells were counted on each slide by fluorescence microscopy. Bacterial biomass was estimated using the bacterial cellular biomass of 10 fg C $cell^{-1}$ as recommended for oligotrophic marine systems (Bell, 1993).

2.3.1. CARD-FISH analysis

Cell identification for the two domains of prokaryotes was made by fluorescence in situ hybridization (FISH) using horseradish peroxidase (HRP)-labelled oligonucleotide probes combined with signal amplification by tyramide labelled with carboxyfluorescein, following the Pernthaler et al. (2002) protocol. Samples were fixed onboard the ship for 24 h at 4 °C in 2% formalin (final conc.), then filtered onto 0.2 µm pore-size polycarbonate filter and frozen at −20 °C until analysis. Permeabilization was done by incubation with lysozyme (10 mg ml^{-1}) in a solution (pH 8) of 50 mM EDTA and 0.1 M Tris–HCl for 60 min at 37 °C. The filter sections were

hybridized for 2 h at 35 °C with the HRP-oligonucleotide probe (Biomers.net, Germany) specific to Bacteria (Eub338) and Archaea (Arch915) (Pernthaler et al., 2002). After the hybridization, the filter sections were soaked for 5 min in pre-heated (37 °C) washing buffer (5 mM EDTA [pH 8], 20 mM Tris–HCl [pH 8], 0.01% [w/vol] SDS) containing 3 mM NaCl. The sections were then incubated in 50 ml of phosphate buffered saline solution (1× PBS, pH 7.6, catalogue #P3744; Sigma) for 15 min at room temperature in order to equilibrate the probe-delivered HRP. In the final step, the sections were put in a reaction vial containing 1000 parts of amplification buffer (2 M NaCl, 10% [w/vol] dextran sulfate, 1× PBS, 0.1% [vol/vol] blocking reagent, 0.0015% H_2O_2) and 2 parts of tyramide-carboxyfluorescein (1 mg ml^{-1}) and incubated for 15 min at 46 °C. The sections were mounted with 4:1 of Citifluor: Vecta Shield containing 1 μg ml^{-1} of 4′,6-diamidino-2-phenylindole (DAPI).

Total bacterial+archaeal cells were enumerated by DAPI after the CARD-FISH procedures (HP_{FISH}) and the results for each of the four probes were averaged for a given fraction at a given station. The relative error on absolute counts (expressed in cells ml^{-1}) varied from <1% at the offshore stations to >50% at river station 917 and 918 where the particle loads were highest. The overall mean relative error was 29%. The HP_{FISH} counts were compared with previous counts from DAPI staining of formalin fixed samples (2% final conc.) and filtered onto 0.2 μm black polycarbonate filter (without the CARD-FISH). On average, absolute counts were 1.8 times higher on filters that had not been CARD-FISH processed. This means that cells were lost during the procedure, but we considered that these losses would be the same across all taxa, and would not affect relative abundances. No DAPI samples were available for stations 803, 912, 917 and 918, and we multiplied the values from these sites by 1.8 to correct for cell losses.

2.3.2. DGGE analysis

Microbial biomass was collected by sequentially filtering up to 4 L of water through a 3-μm 47 mm polycarbonate filter and through a 0.2-μm Millipore Sterivex unit for the determination of DNA in the particle-associated and free-living fractions, respectively. Following a removal of excess liquid, buffer (50 mM Tris, 40 mM EDTA and 0.75 M sucrose) was added to the Sterivex units and to the cryovials containing the polycarbonate filter. The Sterivex units and cryovials were then stored at −80 °C. DNA extraction followed the protocol of Schauer et al. (2000). The cells were treated with lysozyme (or proteinase K for Archaea) and sodium dodecyl sulfate, followed by a phenol–chloroform–isoamyl alcohol extraction. Extracted DNA was concentrated with an Amicon filter device (Millipore Inc.).

DGGE was carried out as described elsewhere (Murray et al., 1996; Schauer et al., 2000) using a DGGE 2401-Rev B model (CBS Scientific Company, Inc.). The 16S rRNA gene fragments (approx. 550 bp long) were amplified by PCR. The specific bacterial primers used were 907r (CCGTCAATTCMTTTRAGTTT), complementary to positions 927 to 907 (*Escherichia coli* numbering); and 357f-GC (CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGC CCCCCTACGGGAGGCAGCAG; Muyzer et al., 1997), complementary to positions 341 to 358 and with a GC clamp (underlined). The PCR products were loaded on a 6% polyacrylamide gel with a linear gradient of DNA denaturing conditions ranging from 50 to 80% (100% denaturing agent is 7 M urea and 40% deionised formamide). The gel was run at 100 V and 60 °C for 16 h in 1×TAE (Tris–Acetate–EDTA) running buffer. DGGE gel images were analyzed using the Quantity One software (BIO-RAD). The denaturing gradient used gave excellent fingerprinting with the bands well separated down the gel. When PCR products were rerun, each set of samples showed a reproducible DGGE fingerprint.

2.4. Bacterial production

Bacterial production was estimated by incorporation of tritiated leucine (protein production) and tritiated thymidine (DNA synthesis) using the centrifugation method of Smith and Azam (1992). Under low light conditions, 1.2-ml sample volumes were dispensed into two sets of microtubes: a first set was inoculated with [4,5-^{3}H]-leucine (Leu; specific activity: 159 Ci $mmol^{-1}$) and a second set with [methyl-^{3}H]-thymidine (TdR; specific activity: 88 Ci $mmol^{-1}$), with final concentration of 10–15 nM for each radiolabelled substrate. Triplicate samples were then incubated in the dark for 4 h at temperatures close to in situ conditions (−1.4 to 4 °C). Incubations were terminated by addition of trichloracetic acid (TCA; 5% final conc.). All samples, including time zero controls, were centrifuged (12 000 rpm for 10 min) three times at low temperature (−1 °C) and the supernatant was removed. Between each centrifugation, the samples were rinsed with 1 ml of ice-cold 5% TCA. Finally, 1 ml of scintillation cocktail was added and samples were radio-assayed, or kept at −20 °C in 2-ml microtubes for later radio-isotopic analysis.

The incubation time was determined from experiments using surface (3 m) samples on two occasions during the study period. Samples inoculated with ^{3}H-leucine or ^{3}H-thymidine (10 nM final conc.) were incubated for 60, 120, and 150 to 480 min. Regressions between the DPM incorporated against time were linear for ^{3}H-leucine uptake for up to 480 min in October and in June ($r=94$, $p<0.05$, $N=4$), as well as for ^{3}H-thymidine uptake for up to 480 min in June ($r=85$, $p<0.05$, $N=4$), and up to 360 min in October ($r=65$, $p<0.05$, $N=3$). The use of a substrate concentration between 10 and 20 nM, as recommended for oligotrophic environments (Simon and Azam, 1989), was confirmed by substrate saturation curve experiments to be appropriate for both radio-isotopes, in which ^{3}H-leucine and ^{3}H-thymidine were added to samples at final concentrations of 15, 20, 25, 30, 40, 60 and 80 nM and incubated for 4 h.

Bacterial production rates were estimated from ^{3}H-leucine incorporation using the conservative carbon conversion factor of 1.5 kg C mol^{-1} of ^{3}H-leucine incorporated, as used for example in the Ross Sea, Antarctica (Ducklow, 2003). Our ^{3}H-leucine-based bacterial production rates provided the primary estimate of bacterial production. ^{3}H-thymidine-based bacterial production (BP-TdR) was used mainly to assess the metabolic status of bacteria by comparison with ^{3}H-leucine-based estimates (BP-Leu). To convert ^{3}H-thymidine incorporation into bacterial production, we used: (1) the common empirical carbon conversion factor for marine waters of 2.0×10^{18} cells mol^{-1} of ^{3}H-thymidine incorporated; and (2) the bacterial cellular biomass of 10 fg C $cell^{-1}$. The latter is recommended for oligotrophic marine systems, based on 97 experimental studies in the open ocean (Bell, 1993). It is

similar to the 11.5 fg C cell^{-1} used in the Greenland Sea (Boersheim, 2000) and is within the range of 7–15 fg C cell^{-1} estimated for oceanic regions (Ducklow, 2000). The Beaufort Sea region, specifically the Mackenzie Shelf area, is an oligotrophic system (Carmack et al., 2004), and thus the conservative value of 10 fg C cell^{-1} used in the present study was deemed appropriate.

On four occasions when ^{3}H-leucine measurements were not run (11, 16, 23 and 28 January), bacterial production was calculated from ^{3}H-thymidine incorporation rates. BP-Leu and BP-TdR of total and free-living bacterial communities were estimated directly from corresponding ^{3}H-leucine and ^{3}H-thymidine uptake rates. The bacterial production (BP) for particle-associated (PA) bacteria was estimated by subtraction for ^{3}H-leucine (% BP-Leu$_{PA}$) and ^{3}H-thymidine (% BP-TdR$_{PA}$). The resultant incorporation rates for particle-associated bacteria were converted to bacterial production rates as described above only if there was no overlap of the mean value ± SD of incorporation rates of the total and free-living fractions. The particle-associated bacterial production rate was otherwise assumed to be zero and bacterial production entirely due to the free-living fraction.

2.5. Statistical analysis

Association between pairs of variables was tested with the Pearson moment product correlation (r coefficient) given that there were no large deviations from normality (Zar, 1999). Partial correlations were run to examine the interactions between three variables in order to remove spurious correlations, and significance tests were made with Student's t test with $df = n - 3$ (Myers and Well, 2003). One-way ANOVA or the non-parametric Kruskal–Wallis test was used to test differences among sampling regions over the Mackenzie Shelf: marine (stations 709, 718 and 803), estuarine (stations 906 and 912) and river (stations 915, 917 and 918) areas. Stations were discriminated according to the surface water salinity.

The DGGE analysis was used to compare bacterial community structure among sites and between size fractions. A matrix was constructed for all DGGE lanes and the relative intensity of each band (in %) by comparison with the total intensity of the lane was calculated. A Euclidean distance matrix was computed and used to calculate a dendrogram with the unweighted pair-group average linkage method (UPGMA). The diversity of bacterial assemblages was assessed using the Shannon diversity index H' (Shannon and Weaver, 1963). Associations among environmental factors and bacterial assemblage structure (DGGE band patterns) were analyzed by Detrended Correspondence Analysis (DCA) and Canonical Covariate Analysis (CCA) using the Canoco software.

3. Results

3.1. Environmental conditions over the Mackenzie Shelf

The Mackenzie Shelf was free of ice at the time of the transect sampling, allowing the Mackenzie River plume to extend well out from the coast. The salinity field suggests that the influence of the river plume at surface extended up to 200 km north from the river mouth (station 918) and eastwards along the coast, possibly as far east as the tip of Cape Bathurst (Fig. 1).

The physico-chemical characteristics of the Mackenzie River freshwater (river stations: 915, 917 and 918) were slightly modified over the shelf when mixed with colder marine water (marine stations: 709, 718 and 803). There was a decrease in SPM, CDOM and phytoplankton (Chl a) concentrations (Table 1) along the river plume, with intermediate conditions at the estuarine stations 906 and 912. The contribution of larger cells to total chlorophyll a (% Chl a_L) was higher at river stations (mean 92%) than at marine stations (mean 52%; ANOVA, $P<0.05$, $N=8$). Partial correlations indicated that the CDOM content significantly decreased across the salinity gradient from the river freshwater to offshore marine sites ($r_{\text{Sal.CDOM|Chl } a} = -0.75$, $P<0.05$, $N=8$ and $r_{\text{Sal.CDOM|\%cChl } a\text{-L}} = -0.84$, $P<0.05$ $N=8$).

3.2. Bacterial abundance and production

DAPI estimated bacterial abundance varied little across the shelf, from 5.7 to 13.6×10^5 cells ml^{-1} with a mean value of 8.8×10^5 cells ml^{-1} (Table 1). Filamentous bacteria were observed at stations 709 and 906 but accounted for only a small fraction (<0.001%) of the total counts. Filamentous bacteria of 10–50 µm in size were observed at stations 709 and 906 but accounted for only a small fraction (<0.001%) of

Table 1
Environmental characteristics and biological properties of the Mackenzie River and shelf during sampling in July 2004

Stn	Temp	Sal	CDOM	SPM		POM	Chl a		Prok	Leu:TdR	BP leucine-based		BP thymidine-based	
	°C	psu	m^{-1}	mg L^{-1}	%	mg L^{-1}	µg L^{-1}	%	10^5 cells ml^{-1}		µg C L^{-1} d^{-1}	%	µg C L^{-1} d^{-1}	%
709	−0.22	27.4	0.9	10.5	80	1.9	0.10	41	6.1	3	0.58 (0.04)	39	2.65 (0.09)	35
718	6.2	25.5	1.9	5.8	25	1.5	0.30	73	12.1	9	0.58 (0.10)	0	0.90 (0.13)	0
803	8.5	24.0	2.6	1.8	2	0.3	0.18	43	12.0	4	0.28 (0.05)	40	0.87 (0.08)	0
906	7.9	14.8	6.2	6.2	60	3.1	1.38	88	5.7	5	1.61 (0.32)	51	3.92 (0.62)	0
912	7.2	8.7	9.3	6.0	61	0.4	2.37	80	7.0	6	0.49 (0.17)	0	1.10 (0.12)	24
915	6.7	3.9	9.1	7.6	19	0.7	2.18	94	6.5	4	0.22 (0.01)	33	0.76 (−)	86
917	14.0	7.9	9.7	15.1	97	2.6	3.86	93	7.8	7	2.25 (0.30)	92	4.05 (0.75)	78
918	16.8	0.1	8.8	143.7	98	12.2	2.94	90	13.8	3	0.55 (0.03)	98	2.37 (0.13)	94

Suspended particulate material (SPM), particulate organic material (POM) and chlorophyll a concentration (Chl a) are presented in absolute value for the total fraction, and as the percentage of the total amount attributed to the >3 µm fraction for SPM and Chl a. Bacterial production (BP) rates are presented in absolute values for the total fraction (standard deviation in parenthesis) and as the percentage of the total amount attributed to the particle-associated bacteria. A 0% value for BP attributed to particle-associated bacteria is given when BP attributed to free-living bacteria was not significantly different (Student t test) from the total BP. Temp: temperature; Sal: salinity; CDOM: colored dissolved organic matter absorption measured at 320 nm; Prok: prokaryotes (Bacteria and Archaea).

the total counts. If we assume a size of 0.2 µm for free-living bacteria, then the biggest filamentous bacteria we had in our sample was 250 fold bigger. Since in our samples one filament is equivalent to 250 free-living bacteria, their contribution to total biomass could be roughly estimated to be around 0.25%, which is still very low.

Incorporation rates of leucine and thymidine were correlated (r=0.88, P<0.005, N=8). Leucine incorporation rates ranged from 6.2 to 62.4 pmol^{-1} L^{-1} h^{-1} (mean 22.8) and thymidine rates from 1.6 and 8.5 pmol^{-1} L^{-1} h^{-1} (mean 4.3). The Leu:TdR ratios varied from 3 to 9, with an average of 5 (Table 1). Mean values of BP-Leu and BP-TdR were 0.82 and 2.09 µg C^{-1} L^{-1} d^{-1}, respectively. Production rate estimates based on both substrates were not significantly different among river, estuarine and marine stations, and showed no significant correlation with environmental variables. One exception was found between BP-Leu and % SPM_L, i.e., the relative contribution of large particles to total SPM (r=0.74, P<0.05, N=8).

The contribution of particle-associated bacteria to total bacterial production as estimated from the leucine (% BP-Leu_{PA}) and thymidine (% BP-TdR_{PA}) incorporation rates were highest at the river stations 915, 917 and 918 (Table 1). Mean values were 74% for BP-Leu_{PA} and 86% for BP-TdR_{PA}. Mean % BP-Leu_{PA} values were 26% at both estuarine and marine stations, whereas mean % BP-TdR_{PA} values were 0 and 12% for marine and estuarine stations, respectively. BP-Leu_{PA} increased with temperature (r=0.71, P<0.05, N=8) and POM (r=0.70, P<0.05, N=8). The relative contribution of the free-living fraction to total leucine-based bacterial production (% BP-Leu_{FL}) was correlated with POM<3 µm (r=0.76, P<0.05, N=8). Finally, the percent bacterial production in the free-living fraction decreased with increasing SPM in the >3 µm fraction for both BP-Leu (r=−0.75, P<0.05, N=8) and BP-TdR (r=−0.80, P<0.05, N=8). Thus, with increasing concentrations of large particles, there was a decreasing relative importance of free-living bacterial activity and increasing importance of particle-associated bacteria.

3.3. Seasonal dynamics of SPM and particle-associated bacterial production in Franklin Bay

The concentration of SPM in the near-surface waters of Franklin Bay varied greatly over the annual sampling (Fig. 2). Highest values were recorded in late fall and winter, however there were large excursions, even between consecutive sampling dates. Similarly, the percent contribution of POM to SPM varied widely, from <5% to >80%, and with no evident seasonal pattern. The most stable period of SPM composition occurred from the beginning of January to early March, at around 20% POM (Fig. 2).

The particle-associated bacterial contribution to total BP-Leu also varied greatly, from 0 to 96% (Fig. 3). Over the sampling period, total bacterial abundance varied from 1.02×10^5 cells ml^{-1} on 17 December to 1.38×10^6 cells ml^{-1} on 16 July (mean of 1.02×10^5 cells ml^{-1}). Bacterial activity was low to non-detectable in the particle-associated fraction throughout most of winter, from 15 February to 11 April, except on 4 and 10 February, when particle-associated bacteria accounted for 36% of total bacterial production. There was no measurable activity by the particle-associated fraction during other periods, including late fall (19 November to 17 December) and spring (10 to 22 May). However during most of the spring and summer, particle-associated bacteria were active and contributed more than 50% of the total estimated production (Fig. 3). At some sampling dates, both leucine and thymidine uptake rates were measured. Leu:TdR ratios were ≤10 over most of the sampling period, from 4 March to 19 November. In winter, Leu:TdR ratios were >10 (mean=21), indicating a shift towards preferential leucine uptake for biomass

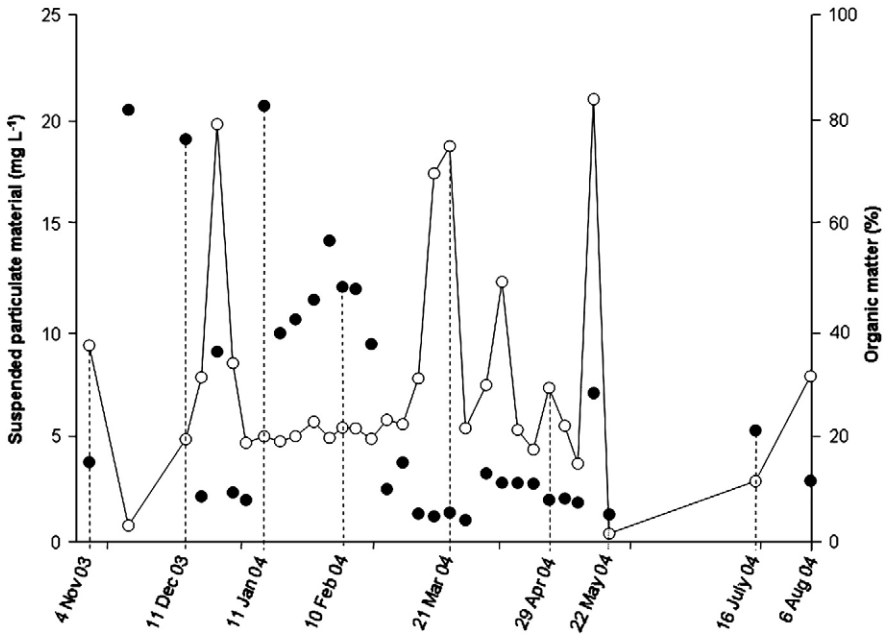

Fig. 2. Variations in suspended particulate matter (solid circles) and its percent organic composition (open circles) over a year at the Franklin Bay station. The values are for near-surface waters.

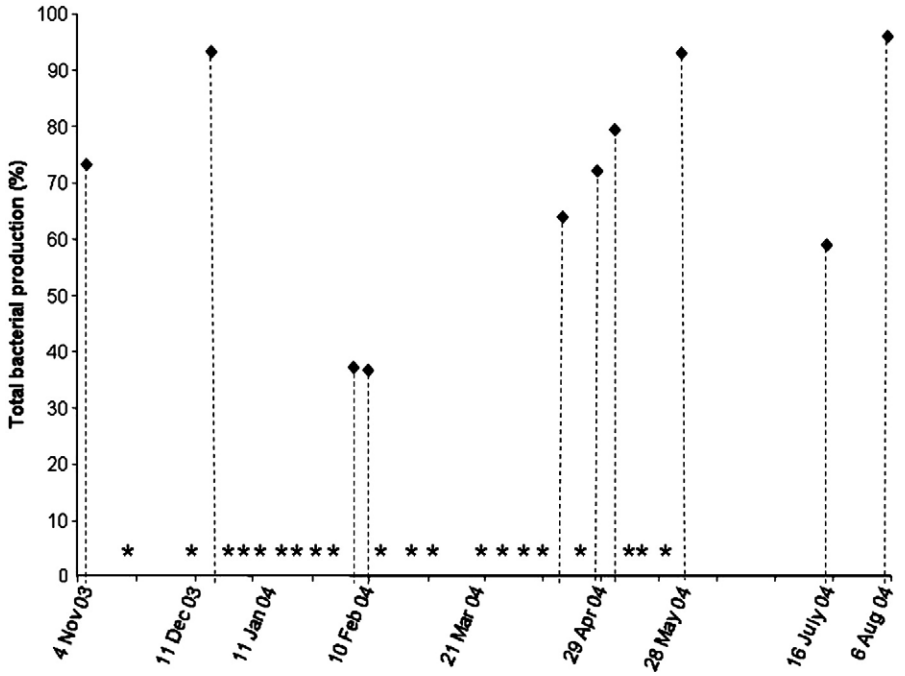

Fig. 3. Contribution of particle-associated bacteria to total bacterial production over a year at the Franklin Bay station. The values are % of bacterial production in the >3 µm size-fractionated leucine assays for samples from near-surface waters. The symbol * indicates that an assay was conducted but there was no significant difference between the total and <3 µm incubations.

production than thymidine uptake for cellular division. The annual dataset showed no relationship between bacterial variables and any of the particulate variables. However there were significant positive partial correlations between total bacterial abundance and temperature ($r_{Bact.Temp|CDOM}=0.86$, $P<0.0001$, $N=11$) and CDOM concentration ($r_{Bact.CDOM|Temp}=0.67$, $P<0.001$, $N=11$).

3.4. CARD-FISH analysis

The CARD-FISH results indicated the dominance of Bacteria and the presence of Archaea in the total and free-living cell fractions in the Mackenzie River and estuary, as well as in the offshore Beaufort Sea (Table 2). No significant differences in relative abundance of Bacteria (% Bact) and of Archaea (% Arch) were found among the marine, estuarine and riverine areas of the shelf for both particle-associated and free-living communities. The percent Bacteria in the free-living pool (% $Bact_{FL}$) was nearly equal to the total pool (% Bact), meaning that Bacteria were mainly found as free-living cells. The % Bact was negatively correlated with temperature ($r=-0.76$, $P<0.05$, $N=7$) and positively correlated with SPM<3 µm ($r=0.86$, $P<0.01$, $N=7$). There was a concomitant increase of % Bact and % BP-Leu_{FL} ($r=0.75$, $P<0.05$, $N=7$), while % $Arch_{FL}$ was negatively correlated with % BP-Leu_{FL} ($r=-0.76$, $P<0.05$, $N=7$). No significant correlation was found between Archaea and environmental variables.

3.5. DGGE fingerprinting

The DGGE analysis was applied to DNA samples from marine stations 709, 718, and 803, and estuarine stations 906 and 912 (Fig. 4). Each lane on the DGGE represents a different sample (Fig. 4a), for a specific site and fraction (free-living or particle-associated) and each band a separate operational taxonomic unit (OTU). The analysis yielded a total of 40 bands with a mean (±SD) of 16 (±1) bands per sample. Analysis of the DGGE fingerprints (Ward's clustering method) showed that there were two distinct clusters (Fig. 4b). One was composed of the large and small fractions of the marine stations 803 and 718, although with them clustered the large fraction of the estuarine station 912. The other cluster included the marine station 709 and the rest of the estuarine samples. Although there was no clear separation of marine and estuarine samples, normally both size fractions from the same station clustered together, with the exception of estuarine station 912.

Table 2
Relative abundance of Bacteria and Archaea for the total and the free-living fraction in the Mackenzie River and shelf

Station	Bacteria %		Archaea %	
	Total	Free-living	Total	Free-living
718	84	78	4.3	0.74
803	43	37	0.2	1.3
906	73	59	0.4	5.8
912	78	77	5.5	2.3
915	81	76	1.4	3.6
917	45	49	2.3	4.7
918	47	65	2.1	5.5
Average	64	63	2	3
SD	19	16	2	2

Percent Bacteria and Archaea were calculated from the total DAPI counts (prokaryotes) after CARD-FISH treatment with the appropriate probe.

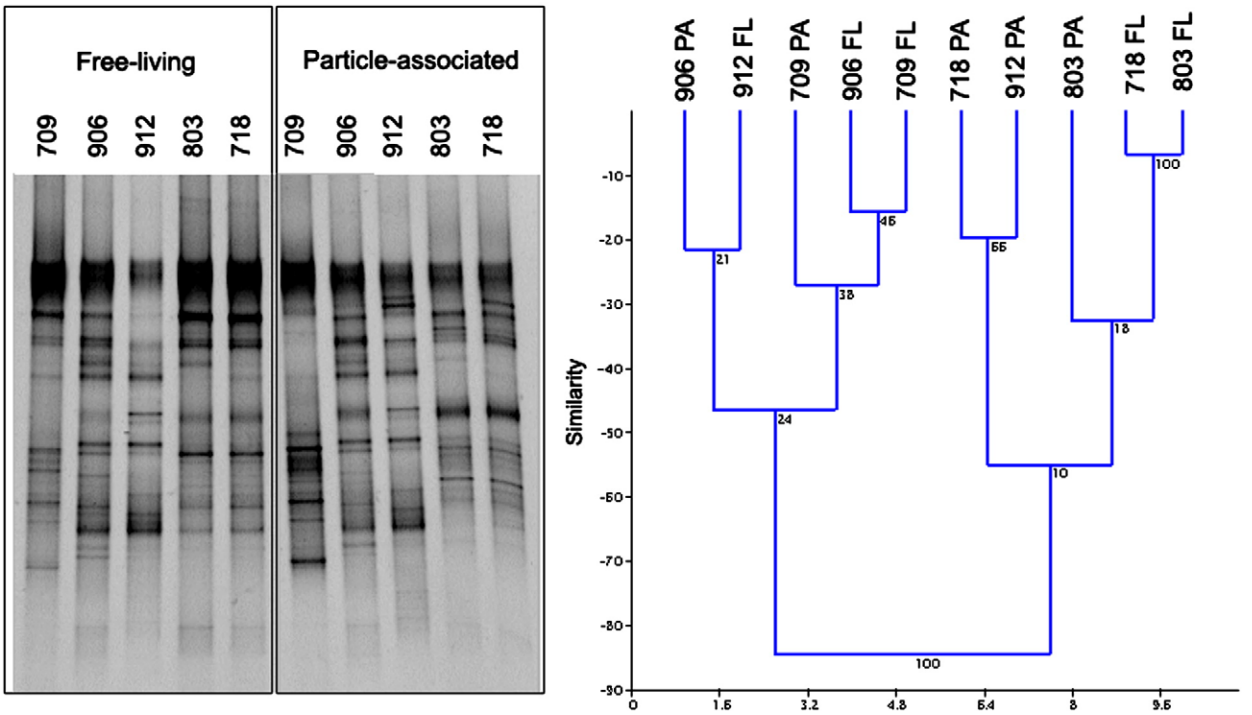

Fig. 4. (a) DGGE pattern of surface stations for free-living (FL) and particle-associated (PA) bacterial communities and (b) dendrogram classification (Ward's method).

CCA analysis (also called variance partitioning) was made according to the OTU composition of each site and for both size fractions (Fig. 5). The CCA analysis of the DGGE gel patterns showed that temperature, salinity and size fraction explained a total of 56% of the variation in the OTUs, with 45% of the variation in band composition explained by temperature and salinity alone and 12% explained by the particle-associated versus free-living fraction. Samples in the CCA diagram (Fig. 5) clustered together mostly as a function of the variables temperature and salinity. Marine stations 803 and 718 clustered together, for both fractions. Marine station 709 did not cluster with the rest of the marine stations, this station being strongly influenced by its water temperature. Station 709 also showed a greater difference between its free-living and particle-associated fractions compared to other marine stations. The CCA showed that five OTUs likely explained the difference between the two size fractions at station 709; three were characteristic of the particle-associated fraction and two of the free-living fraction. Estuarine stations 906 and 912 also clustered together, with a marked difference between their particle-associated and free-living community structure. These three stations (709, 906 and 912) had highest SPM values of the transect, and 906 and 709 had the highest POM (Table 1).

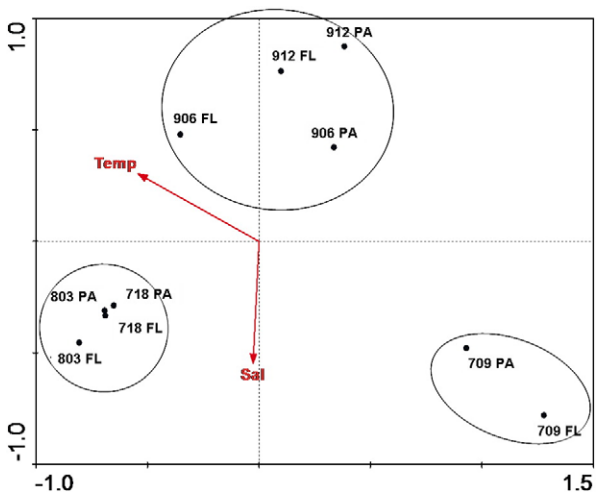

Fig. 5. Partial canonical clustering analysis of bacterial assemblage composition data from each site (DGGE banding patterns) for free-living (FL) and particle-associated (PA) bacteria. The *X*-axis represents the effect of salinity (Sal) and the *Y*-axis the influence of temperature (Temp) on the assemblage composition; the wider the angle relative to the corresponding axis, the larger the effect.

4. Discussion

The Mackenzie Shelf receives some 3 million tons of particulate organic carbon each year (Telang et al., 1991; Macdonald et al., 1998), and thereby receives high concentrations of allochthonous organic particles for bacterial attachment. We found that particle-associated bacteria dominated bacterial activity at river-influenced stations, contributing to up to 98% of the total measured bacterial production rate. This is in keeping with earlier reports where more than half of the total bacterial production was attributed to particle-associated bacteria in estuarine (Garneau et al., 2006) and riverine waters (Vallières et al., in press) of this region. Values up to ~90% were found in coastal waters of the Columbia River estuary (Crump et al., 1998, 1999; Crump and Baross, 2000b) and of the subarctic

Western North Pacific Ocean (Lee et al., 2001). The latter study considered the >1 μm fraction as particle-associated bacteria, which might have included other substrate types relative to those separated here. Elsewhere in the Arctic, enhanced bacterial production and activity has been associated with sinking particles (Huston and Deming, 2002), particles in sea ice (Junge et al., 2004), particle-rich bottom waters (Wells and Deming, 2006) and during a phytoplankton bloom (Yager et al., 2001). A global review by Simon et al. (2002) indicated that particle-associated bacterial production may exceed 30% of total bacterial production in riverine and estuarine systems, but in pelagic oligo- and mesotrophic environments it generally accounts for less than 14%. In one of the rare reports of a low contribution of particle-associated bacteria in coastal waters during a bloom, Fandino et al. (2001) speculated that this was mainly linked to the poor quality of the particulate material. It seems that although the SPM entering the Mackenzie Shelf has a high inorganic content, at least a subset of the particles is highly suitable for bacterial attachment and production.

The turbidity maximum within an estuary is created by several physical processes such as gravitational circulation, tidal pumping and trapping (Mitchell et al., 1998), deposition and resuspension (Wellershaus, 1981) and by chemical processes such as salinity-induced flocculation of dissolved organic material (Horne and Goldman, 1994). These mechanisms may significantly affect the behavior of heavy metals and organic materials, and augment the availability of particles for bacterial colonization and growth. However, on the Mackenzie Shelf, dissolved organic matter composition is highly conserved across the salinity gradient (Retamal et al., 2007; Emmerton et al., in press), suggesting no loss to particle formation. Contrary to expectation, the inorganic content of suspended particulate material was found to increase substantially over the shelf (Emmerton et al., in press). This contrasts with the Lena River estuary, where particles become organically enriched, and perhaps more conducive to bacterial growth, as living and dead biogenic riverine particles replace the inorganic sediments (Dittmar and Kattner, 2003).

Photodegradation of humic matter, which promotes the microbial utilization of this otherwise refractory material, may also play a role in the bacterial dynamics of estuarine waters, and prior exposure of Mackenzie River water samples to full sunlight has been shown to increase the biolability of its dissolved organic carbon (Vallières et al., in press). For the water column over the Mackenzie Shelf, however, photochemical processes are severely constrained by the strong attenuation of UV radiation by CDOM and suspended particulate material, and by the ice cover that persists through much of the year (Bélanger et al., 2006).

The relative abundance and production of particle-associated bacteria are often related to total SPM or aggregate concentration (Crump et al., 1998; Simon et al., 2002; Selje and Simon, 2003), but POM composition may have a stronger influence on the success of particle-associated bacteria (Fandino et al., 2001). In accordance with this latter conclusion, the contribution of particle-associated bacteria to total activity (% BP-Leu_{PA}) on the Mackenzie Shelf did not correlate with the total SPM but rather increased with the concentration of particulate organic component of the total SPM pool. Our results concord with those reported by Wells et al. (2006) in nearby Franklin Bay who found that bacterial abundance correlated with POM but not SPM. A correlation between the bacterial production by free-living cells (% BP-Leu_{FL} and % BP-TdR_{FL}) with organic particles in the small <3 μm fraction suggests that bacterial production depended on POM. There was no significant correlation between bacterial abundance or production and CDOM on the Mackenzie Shelf, implying no relationship with river-derived dissolved organic substrates. Similar results were reported from the coastal Alaskan Arctic Chukchi Sea where bacterial abundance showed no relationship with either dissolved organic carbon or inorganic nutrients, but was positively correlated with POM (Hodges et al., 2005). Those results contrast with other observations in the Chukchi Sea (Yager et al., 2001) and elsewhere in the Arctic in the highly productive North Water area (Middelboe et al., 2002) where the bacterial communities apparently responded to autochthonous DOM released during phytoplankton blooms. It is likely that bacterial communities are more specialized in metabolizing POM in turbid coastal Arctic areas compared to offshore sites where microbial assemblages are more frequently exposed to inputs of labile DOM from the phytoplankton production rather than to particle concentration. The relative stability of the metabolic index Leu:TdR over the Mackenzie Shelf suggests that the microbial communities were well adapted to their local conditions.

4.1. Seasonal variations in the contribution of particle-associated bacteria

The importance of particle-associated bacteria in global biogeochemical cycles is well documented for marine systems (Simon et al., 2002). However few studies have addressed seasonal variations in the particle-associated bacteria contribution to bacterial production (Griffith et al., 1994; Lee et al., 2001). Most of the bacterial production analyses have been conducted during the periods of active phytoplankton production (Crump and Baross, 2000b; Fandino et al., 2001; Yager et al., 2001; Ghiglione et al., 2007), with little information from other times of year. The ensemble of studies indicates that particle-associated bacteria are important consumers of freshly produced substrates (Fandino et al., 2001; Yager et al., 2001; Ghiglione et al., 2007), but the broader annual significance of this component is less understood.

The present study focused on the temporal variations of particle-associated bacterial activity. The seasonal variations in total bacterial production in Franklin Bay reported by Garneau et al. (2008) indicated that there was a continuous low level of activity in winter, rising to a maximum in July. The present results of Franklin Bay winter community revealed substantial variations in SPM concentrations and composition, and % BP-Leu_{PA} was also highly variable, ranging from nil to 96%. The latter is consistent with the large seasonal fluctuations in total bacterial production rates, which exceed the fluctuations that have been reported at many other marine sites (Garneau et al., 2008). This large variation contrasts with % BP_{PA} in subarctic coastal surface waters reported by Lee et al. (2001) which varied over a smaller range (26–68%, mean of 41%), and showed no seasonal increase during the spring bloom/post-bloom period relative to the rest of the year. This difference in seasonal patterns may be the result of relatively stable environmental conditions in subarctic coastal waters compared to the strongly seasonal, coastal polar environment,

where there are severe fluctuations in irradiance, in situ photosynthesis, sediment resuspension and transport by hydrodynamic processes, and in the terrestrial inputs of riverine freshwater, nutrients and organic carbon.

Coastal aquatic systems display complex circulation patterns punctuated by short-term hydrodynamic events. The Mackenzie Shelf system showed short-term variations in particulate organic carbon fluxes during the CASES study period, especially during fall and winter, which coincided with episodes of resuspension and advection of shelf bottom particles by thermohaline convection, windstorms, and/or current surges and inversions (Forest et al., 2006). These mixing events were also accompanied by fluctuations in total bacterial production (Garneau et al., 2008), and the entrainment of bottom particles associated with such events likely explains some of the short-term variations that we observed in SPM, %POM and particle-associated bacterial production (Figs. 2 and 3).

We found that total bacterial abundance increased with increasing temperature and CDOM concentration, considered here to be an indicator of riverine inputs. However our dataset showed no statistical relationships between % BP_{PA} and the measured environmental variables. The seasonal dataset showed the peak activity of particle-associated bacteria in the summer-fall period when riverine discharge was maximal, which underscores the importance of allochthonous organic matter in supporting Franklin Bay bacterial communities. Conversely, low riverine inputs of particles and complex terrestrial organic material during wintertime likely explain the low % BP_{PA} at that time of year, with a switch to free-living bacterial communities utilizing the remaining, less labile DOC. Microbial degradation is likely to decrease the lability of particles as the season progresses, with a possible generation of more refractory DOC by bacterial enzymatic activity (Azam, 1998). In winter, after the productive phytoplankton period, Leu:TdR ratios for the entire bacterial community (free-living and particle-associated) reflected preferential biomass accumulation over cell division. This implies survival in difficult conditions, such as a poor supply of available substrates in winter darkness, as well as the persistent cold temperature. Temperature was shown to be key factor affecting seasonal patterns of bacterial abundance, production and % BP_{PA} in the Chesapeake estuary, but contrary to our results, highest % BP_{PA} was recorded in winter (25–50%) when BP_{FL} was lowest (Griffith et al., 1994). However during summer, particle-associated bacteria appeared to be less influenced by changing temperature, and more influenced by particulate protein quantity, than were free-living bacteria (Crump et al., 1998).

The Beaufort Sea region, specifically the Mackenzie Shelf, is an oligotrophic system (Carmack et al., 2004). It is highly stratified throughout the year (Garneau et al., 2008), resulting in low nutrient availability (Carmack et al., 2004) and low phytoplankton stocks, as observed in the present study. This limits the supply of autochthonous organic particles for bacterial colonization. We have estimated that the annual bacterial production in Franklin Bay is equivalent to 37% of phytoplankton production (Garneau et al., 2008), which is large compared to the usual 10–20% found in systems elsewhere (Ducklow, 2000). The higher value for Franklin Bay would be consistent with a large subsidy to bacterial production from riverine carbon inputs, much of it entering in particulate form during summer and fall, and the relatively low primary production rates.

4.2. Spatial distribution of bacterial communities on the Mackenzie Shelf

In theory, the Bacteria and Archaea counts in our CARD-FISH analyses should add up to 100% since all non-eukaryotic cells in the samples were targeted by one of the two probes. We found that Archaea and Bacteria occurred at all stations, however the total percentage of DAPI-positive cells for the sum of the two domains was on average 65%. This may be the result of specific taxa that are not detected with the Eub338 probe, notably the bacterial phyla Planctomycetales and Verrucomicrobia which would be better detected by the mix of the probes Eub338, Eub338-II and Eub338-III (Daims et al., 1999). The Actinobacteria group, known to be common in lakes and rivers (Zwart et al., 2002; Sekar et al., 2003), might not be detected because they have gram-positive cell walls that may not be adequately be permeabilized by lysozyme during the CARD-FISH procedure (Sekar et al., 2003).

Previous results based on correlations with SPM suggested that Archaea were especially associated with particles (Wells and Deming, 2003; Garneau et al., 2006; Wells et al., 2006). Our results show the presence of Archaea, along with Bacteria, in both the total and free-living fractions. There was an apparent inconsistency in some of our Archaea counts, in that more Archaea were reported in the free-living fraction than in the total community (Table 2). This might be a consequence of visual obstruction by particles during microscopic examination, making it impossible to count bacteria located within or behind an aggregate. It is also possible that the CARD-FISH filtration procedures broke up particles and dislodged particle-associated cells (Crump et al., 1998). Nonetheless, more Archaea were counted in the free-living fraction at river-influenced, particle-rich stations 906, 915, 917 and 918. We also found a significant correlation between % Bact and % $BP\text{-}Leu_{FL}$ and the negative correlation between % $Arch_{FL}$ and % $BP\text{-}Leu_{FL}$, indicating dominance of the free-living fraction by Bacteria rather than Archaea.

Our spatial study of bacterial communities on the Mackenzie Shelf showed that free-living and particle-associated bacterial community structure can either be similar or different, depending on site. Similarly in the Columbia River estuary, the free-living and particle-associated clone libraries in the river portion contained the same phyla and subphyla, whereas free-living and particle-associated clone libraries of coastal samples were very different (Crump et al., 1999). Estuarine circulation and coastal hydrodynamic processes create a complex, heterogeneous environment in the Mackenzie Shelf ecosystem (Carmack and Macdonald, 2002). Estuarine particle retention (e.g. Frenette et al., 1995), bottom resuspension (Forest et al., 2006), ice barriers (Galand et al., 2008) and upwelling effects on the river plumes (Garneau et al., 2006) are among the factors that can considerably modify particle distribution and microbial communities in this environment.

The environmental variables salinity and temperature, as well as organic content of the suspended material, appear to play important roles in shaping bacterial community structure of the Mackenzie Shelf. The dendrogram of the DGGE

band profile matrix (Fig. 4) grouped the marine stations 718 and 803, but excluded station 709, which was included on the estuarine stations cluster. Station 709 was defined as a marine station according to salinity and low Chl *a* and CDOM concentration. The lack of clustering of marine samples is because they are from different water masses, with different bacterial communities. Stations 803 and 718 displayed similar temperature and salinity while the station 709 sample came from a much colder water mass. Bacterial assemblages at station 709 were likely similar to estuarine assemblages because they shared high particulate material content and high $\%BP_{PA}$. This high particle content might explain why the CCA analysis revealed differentiation of free-living and particle-associated bacterial assemblages at station 709, but not at the other marine stations.

Free-living and particle-associated bacterial communities were different at station 906, which was far (540 km) from station 709 but shared the characteristic of high concentrations of organic particles. Particle abundance and composition (lability) at a given location not only affect the partitioning of total bacterial production between the free-living and particle-associated components, but have also been identified as factors explaining similarity or dissimilarity between particle-associated and free-living bacterial communities (Hollibaugh et al., 2000). We found increasing dissimilarity between free-living and particle-associated bacterial community assemblages in areas that were rich in organic particles, which suggests that the particle habitat selects for specialized particle-associated bacteria that can attach to and use such substrates. Our results are consistent with the selection for particle-associated bacteria producing extracellular enzymes that degrade particles, as hypothesized by Chróst (1991). Huston and Deming (2002) also concluded that POM was responsible for changes in communities by selecting specialized particle-associated bacteria that excrete certain extracellular enzymes. Riemann and Winding (2001) have further argued that the nature of particulate matter acts as a selective force, which drives community succession inside of particle microenvironments towards a phylogenetic composition that is distinct from free-living communities in the surrounding waters. The longevity of the microenvironment may also be important since we found that at other stations there was no difference between particle-associated and free-living communities, consistent with evidence indicating that there can be an exchange between the two types of bacterial habitat, in incubations (Kiorboe et al., 2002) as well as in natural environments (Stevens et al., 2005; Ghiglione et al., 2007).

5. Conclusions

We found large variations over time and space in the contribution of particle-associated bacteria to bacterial production. Much of the short-term variations may be the result of hydrodynamic events, and the resuspension and entrainment of sediments into the water column. Maximum particle-associated activity was observed at stations with highest POM concentrations, and seasonally during summer river flow. Molecular analysis showed greatest differences between the free-living and particle-associated communities when POM concentrations were highest, implying some selection for particle-associated bacterial specialists. These particle-based communities likely play a major role in the respiration of allochthonous organic carbon, and may be a food source for higher trophic levels.

Ongoing climate change in the region is likely to have a broad range of impacts on particle-based bacterial production in the coastal ocean, with implications for biogeochemical processing and net carbon fluxes to the atmosphere. Increased melting of sea ice and prolonged open water conditions may stimulate phytoplankton production and the availability of high quality biogenic particles for bacterial production. However, this effect may be offset by increases in river discharge (Prowse et al., 2006) that would enhance stratification, and thereby reduce the nutrient supply for primary production. This negative effect may be compounded by increased riverine inputs of turbidity and CDOM, exacerbating the light limitation of deep phytoplankton populations (Retamal et al., 2008). There may also be effects of decreased salinity on bacterial community structure, since the relative abundance of Alphaproteobacteria, Betaproteobacteria and picocyanobacteria has been shown to change markedly along the salinity gradient over the Mackenzie Shelf (Garneau et al., 2006). Global warming is also likely to increase the particle loading into the coastal Arctic Ocean because of permafrost melting (Payette et al., 2004) that releases aged soil organic carbon (Frey and Smith, 2005), and northward treeline advance, which supplies young, mobile organic carbon to the drainage basins (Serreze et al., 2000; Guo et al., 2007). If enhanced respiration exceeds in situ primary production, the coastal Arctic Ocean would be a heterotrophic ecosystem (i.e., a net source of CO_2 to the atmosphere), with feedback effects on warming. Higher losses of organic matter through respiration could arise from the metabolism of aged particles found in the permafrost, since the growth efficiency of bacteria is reduced on such particles (Grossart and Ploug, 2000). Northern permafrost soils are estimated to contain 30–50% of the global soil pool (Dixon et al., 1994), which is equivalent to twice the carbon present in the atmospheric CO_2 reservoir. With ongoing warming and permfrost erosion, particle-associated bacteria in the coastal Arctic Ocean may play an increasing role in the transfer of this tundra organic carbon to the atmosphere.

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