Polar Biol
DOI 10.1007/s00300-011-1041-y

ORIGINAL PAPER

Abundant dissolved genetic material in Arctic sea ice Part I: Extracellular DNA

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Received: 1 November 2010 / Revised: 15 May 2011 / Accepted: 24 May 2011

Abstract The porous medium of sea ice, a surface-rich environment characterized by low temperature and high salinity, has been proposed as a favorable site for horizontal gene transfer, but few measurements are available to assess the possibility of this mode of evolution in ice. Here, we report the first measurements of dissolved DNA in sea ice, measured by fluorescent dye staining of centrifugal-filter-concentrated samples of melted ice. Newly formed landfast and pack ice on the Canadian Arctic Shelf (ca. 71°N, 125°W) contained higher concentrations (scaled to volume of brine) of the major components of dissolved DNA—extracellular DNA and viruses—than the underlying seawater. Dissolved DNA was dominated by extracellular DNA in surface seawater (up to 95%), with viruses making up relatively larger fractions at depths below 100 m (up to 27%) and in thick sea ice (66–78 cm; up to 100%). Extracellular DNA was heterogeneously distributed, with concentrations up to 135 μg DNA L^{-1} brine detected in landfast sea ice, higher than previously reported from any marine environment. Additionally, extracellular DNA was significantly highly enriched at the base of ice of medium thickness (33–37 cm), suggestive of in situ production. Relative to underlying seawater, higher concentrations of extracellular DNA, viruses, and bacteria, and the availability of numerous surfaces for attachment within the ice matrix suggest that sea ice may be a hotspot for HGT in the marine environment.

Keywords Arctic · Sea ice · Extracellular DNA · Bacteria · Viruses · Horizontal gene transfer

Introduction

Horizontal gene transfer (HGT)—the incorporation of genetic material from one organism into another without reproduction—has played a pivotal role in the evolution of extant microbial genomes, as revealed by massive sequencing efforts over the past decade (Brown 2001; Gogarten et al. 2002; Lawrence and Hendrickson 2003; Beiko et al. 2005). This genomic evidence has led to the conclusions that HGT is both widespread among all three Domains of life (Bacteria, Archaea, and Eukarya) and intensive. A number of recent studies have implicated HGT in the transmission of freeze tolerance genes from one Domain of life to another, including ice-binding proteins (Janech et al. 2006; Raymond et al. 2007) and antifreeze proteins (Bayer-Giraldi et al. 2010; Kiko 2010). Sea ice is a very promising candidate site for the occurrence of HGT in these cases, but the environmental locale and gene transfer mechanisms responsible for these apparent transfers are not known, indicating a need to supplement gene sequence-based investigations with laboratory experiments using environmentally relevant microorganisms, and in situ experiments with natural populations.

The linked problems of identifying the location and mechanisms responsible for environmental HGT are not

This article belongs to the special issue "Circumpolar Flaw Lead Study (CFL)", coordinated by J. Deming and L. Fortier.

Electronic supplementary material The online version of this article (doi:10.1007/s00300-011-1041-y) contains supplementary material, which is available to authorized users.

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Published online: 18 June 2011

unique to sea ice—the relative importance of the known mechanisms of HGT on the evolution of microbial genomes is not known for any environment (Zaneveld et al. 2008), no less extreme environments and perennially cold environments. Each of the known mechanisms—transduction, natural transformation, and conjugation—utilizes a different form of exogenous DNA: transducing phage, free DNA, and mobile genetic elements like plasmids, respectively. The relative availability of these particular forms of transferable DNA in a range of environments may provide one comparative measure of the potential for HGT in those habitats.

Viruses can act as agents of HGT in a process called transduction (Zinder and Lederberg 1952). High abundances of viruses have been observed in both Arctic and Antarctic sea ice (Maranger et al. 1994; Gowing et al. 2002; Wells and Deming 2006; Collins and Deming 2011), but as yet no transducing phage are known from any sea ice. Viruses in sea ice are treated in more detail in a companion paper (Collins and Deming 2011). Natural genetic transformation involves the direct uptake, integration, and expression of exogenous DNA by a "naturally competent" host cell (Griffith 1928; Avery et al. 1944). We know of no previous studies of free DNA and the potential for transformation in sea ice. Conjugation is a biochemically complex system requiring direct contact between active cells (Lederberg and Tatum 1946). Gene transfer occurs between a donor cell, containing a mobile genetic element (e.g., a conjugative plasmid), and a recipient cell (usually lacking the mobile genetic element). In a unique study of plasmids in sea ice bacteria, Kobori et al. (1984) found plasmids more frequently in bacterial isolates from Antarctic sea ice than from Antarctic seawater.

Relatively few studies have investigated the frequency or even the potential for HGT in situ, which requires the presence of a critical raw material: DNA. As a first exploration into the cycling of DNA in sea ice and its potential use for HGT, we sampled several different types of first year sea ice in late autumn, including frazil, nilas, and pancake ice, as well as columnar ice of varying thicknesses. At each station, we measured the abundances of bacteria, viruses, and DNA in the ice and the underlying seawater, as well as various physical and chemical parameters providing environmental context. While we have not directly measured rates of HGT in sea ice, the results of this study support the hypothesis that sea ice is a potential hotspot for horizontal gene transfer.

Materials and methods

Sample collection

Sampling took place aboard the CCGS Amundsen between November 10 and December 18 2007, as part of the Circumpolar Flaw Lead Systems Study (CFL), a project of the International Polar Year. Sea ice and seawater were collected in the vicinity of Amundsen Gulf and the Beaufort Sea on the Canadian Arctic Shelf (Fig. 1). At stations with ice less than 20 cm thick, samples were collected by scooping or sawing chunks from the floe using ethanol-sterilized tools. For ice greater than 20 cm thick, a full "physical" core was collected for measurements of temperature, taken in the field every 5 cm from the top (as before, see details in Collins et al. 2008), and bulk salinity, measured every 2.5 cm on melted samples. Sea ice microstructure analysis was conducted on many of the sections of the physical core by collaborators in CFL Team 2, led by Dr. David Barber (University of Manitoba). Three "biological" cores were cleanly drilled (Collins et al. 2008) using a 9 cm diameter Mark II coring system (Kovacs Enterprises). The three cores were collected at the corners of a 1 × 1 m square in conjunction with the physical core and sampled for later enumeration of bacteria and viruses and quantification of DNA, as described below. The top 10 cm and bottom 10 cm of each core were removed and aseptically transferred to separate sterile Whirl-Pak bags. Full-length "chemical" cores were collected within 5 m of the physical core for bulk measurements of suspended particulate matter (SPM), particulate organic carbon (POC), particulate nitrogen (PN), and chlorophyll *a*. Enough full-length cores were collected at each site to total at least 200 cm of ice core (>10 L when melted). At least 5 L of seawater was collected within 1 m of the base of the ice sheet at each station, at the same location as ice cores were collected, using a hand-held Niskin bottle. At Station D8, additional samples of seawater were collected at depths of 10, 30, 100, 150, 250, and 441 m using a rosette with a CTD. Ice cores and seawater were stored at 2–4°C until processed, within 24 h. The set of all seawater samples collected within the upper 10 m of the water column will be hereafter referred to as "surface seawater."

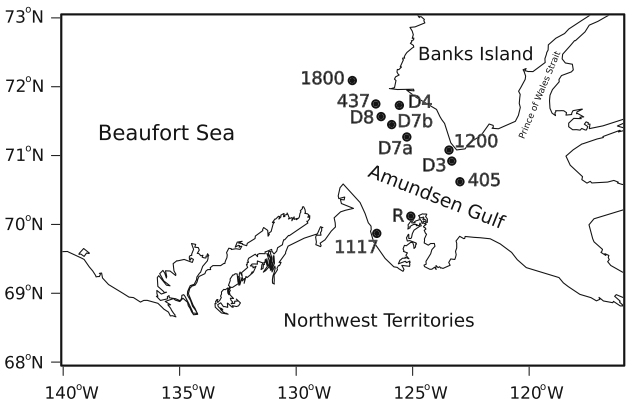

Fig. 1 Map of sampling stations in western Amundsen Gulf and the Canadian Shelf of the Beaufort Sea

"Chemical" core analysis

The chemical cores were mechanically crushed and transferred to 20 L polyethylene bags to melt in a water bath at room temperature. Immediately upon completion of melting, continuously stirred meltwater (and seawater) were prefiltered through 140 μm nylon mesh, then filtered with an inline filtration system driven by a peristaltic pump. For analyses of POC and PN, two or three 2 L aliquots of meltwater or seawater were filtered through separate precombusted inline 25 mm GF/F filters, then stored at −80°C in precombusted foil. Samples were later dried at 60°C, fumed with HCl, and dried again before combustion in a Leeman Labs Model CEC440 Elemental Analyzer, using acetanilide and caffeine as standards. Chlorophyll *a* was estimated on one or two 2–8 L samples of meltwater or seawater using standard techniques (Parsons et al. 1984). For SPM, a single aliquot of 2–16 L meltwater or seawater was filtered inline through precombusted preweighed GF/C filters and rinsed with 10 ml 1% sodium formate to remove salts, then stored at −20°C. Filters for SPM were dried overnight at 60°C and reweighed to a precision of 0.1 μg. For each procedure, ASW or distilled water (prefiltered by tangential flow filtration, TFF, with a 10 kDa cutoff) were used as negative controls. The chemical parameters were scaled to bulk melted ice volume because they were measured on whole cores rather than from specific depth horizons.

"Biological" core analysis

The biological core sections from each horizon of each core were mechanically crushed and aseptically transferred to individual sterile melt jars, to which two volumes of TFF prefiltered artificial seawater brine (made with ASW salts, Sigma Corp.) were added, so that after melting the final salinity was equivalent to the in situ brine salinity, calculated from the ice section temperature (as in Collins et al. 2008). The volume of melted ice was measured immediately upon melting. Concentrations of biological parameters in the ice were measured on individual cores from each horizon and were scaled to the volume of liquid brine in which they were presumed to be located in situ, as calculated from the equations of Cox and Weeks (1983, 1986). The assumption that bacteria reside in brine channels within ice (rather than being encased in the solid matrix) is well supported by microscopic observations (Junge et al. 2001); viruses and extracellular DNA have not been subjected to the same examination. Methodological details of bacterial and viral enumeration can be found in Collins and Deming (2011); briefly, bacterial and viral abundance were determined by epifluorescence microscopy using DAPI (bacteria) or SYBR Gold I (viruses). Extracellular polymeric substances were below detection limits as measured using the phenol-sulfuric acid method.

Extracellular DNA

Different pools of DNA can be measured as components of exogenous or total dissolved DNA in seawater. A recently devised method operationally defined dissolved DNA (D-DNA) as the DNA that passed through a 0.22 μm filter but not through a 10 kDa (about 15 bp)-cutoff filter and was quantifiable by a PicoGreen fluorescence assay (Brum et al. 2004). Thus, DNA from intact cells is excluded in this definition but viral DNA is included. Distinctions among the pools comprising total D-DNA have been defined as follows (Brum et al. 2004; Brum 2005): "viral DNA" is that fraction of the total D-DNA contained in viruses; "enzymatically hydrolyzable dissolved DNA" (eh-DNA) is the fraction of the total D-DNA degradable by DNAse; and "uncharacterized bound dissolved DNA" is that which is neither viral DNA nor eh-DNA, and which may be bound to particles or otherwise inaccessible to participate in HGT. Here, we report measurements of total D-DNA and estimates of viral DNA in sea ice, allowing an estimation of the total abundance of dissolved extracellular DNA (eh-DNA plus bound DNA), which we will refer to as extracellular DNA (eDNA).

Measurements of D-DNA followed broadly the method of Brum et al. (2004), using the fluorescent dye PicoGreen (Invitrogen) to quantify double-stranded D-DNA and a ^{35}S-labeled internal standard to determine yield. Several alterations were necessary, however, to account for the high salinity of the sea ice brines. For each sample, a 13 ml aliquot of the biological core filtrate was collected (having passed through a 0.22 μm Sterivex cartridge), 1.6 M tetrasodium-EDTA was added to an excess of 100 mM over the concentration of cations to disrupt viral capsids and inhibit protease activity, and the sample was stored at 2–4°C until shipboard analysis within 1 week. ^{35}S-labeled λ-DNA was constructed aboard ship, using a nick-translation labeling kit (Amersham #N5000) according to the manufacturer's instructions, and added to each sample to a final activity of 13000 dpm. Each sample was transferred to a Centricon Plus-20 centrifugal filter device (10 kDa nominal molecular weigh cutoff) and centrifuged for 60 min at 4,000×*g*. Each sample was washed by discarding the flow-through, adding 5 ml filtered 10 mM tetrasodium-EDTA (pH 10.5), and centrifugation for another 60 min at 4,000×*g*. This washing step was repeated if necessary with slowly filtering samples to ensure the removal of excess salts. Finally, 500 μL 10 mM tetrasodium-EDTA (pH 10.5) was added and concentrate was recovered by centrifugation at 600×*g* for 2 min. Total volume of concentrate was measured and transferred to a microfuge tube

for storage at 4°C. Aliquots of each sample were mixed 1:1 (v/v) with 0.5% PicoGreen and incubated for 15 min at room temperature in the dark. Fluorescence was measured on a Turner Designs TD-700 fluorometer at 75% sensitivity at an excitation of 480 nm and an emission of 520 nm. Standard curves containing 0–100 $ng\ ml^{-1}$ λ-DNA were measured on the same fluorometer at the same sensitivity. The remainder of the sample was combined with 20 ml scintillation fluid and ^{35}S radioactivity was measured on a liquid scintillation counter to correct for losses during processing. The mean ^{35}S recovery was 10% (range 1–72%), lower than previously reported values (Brum et al. 2004) despite repeated attempts to raise the recovery. Differences in recovery between our measurements and those of Brum et al. (2004) might be attributable to the higher centrifugation speed (4,000×g compared to 1,000×g), longer centrifugation time (60 min compared to 25 min), and more extensive washing steps we found were necessary to prepare our coastal seawater and sea ice samples compared to the oligotrophic seawater samples measured by Brum et al. (2004). Blanks, consisting of TFF-retentate from 240‰ artificial brine, were processed as samples and subtracted from the sample measurements. The viral component of D-DNA was calculated by multiplying viral abundance by an estimated genome mass of 55 ag per virus (Steward et al. 2000). The eDNA concentration was calculated by subtracting the viral component of D-DNA from total D-DNA. The DNA content in bacteria was estimated using a genome mass of 2.5 fg DNA per cell (Button and Robertson 2001).

Virally reduced production experiments were performed during three parallel incubations of sea ice brine collected from sackholes at Station D7b. Abundances of bacteria, viruses, and D-DNA were measured over the course of 60 h in brine at an in situ temperature of −7°C, with a corresponding salinity of 110‰ measured by refractometer. Methodological details on these experiments may be found in Collins and Deming (2011).

Enrichment index

A new enrichment index (E) was formulated for constituents in seawater that become entrained in sea ice. The main purpose of the new index is to incorporate the brine concentrating effect of sea ice and to assist in discriminating between passive entrainment, active entrainment, and in situ production. This index is scaled such that $E = 0$ when entrainment into the ice is equal to that of salt (i.e., passive entrainment, the equivalent of $I_s = 1$ in the enrichment index formulation of Gradinger and Ikavalko 1998), though salts can also be retained in ice due to inhibition of brine drainage when EPS is present (Krembs et al. 2011). Complete entrainment from seawater, represented by $E = 1$ (not distinguished by I_s), would only be expected by an "active" ice-affine solute that sorbs to the ice during brine expulsion and gravity drainage (e.g., EPS from *Colwellia psychrerythraea* 34H, Ewert and Deming 2011, or protein from ice algae, Raymond et al. 2009). Enrichment indices of $E < 0$ indicate loss relative to underlying seawater; e.g., by decay or mortality within the ice. Indices of $E > 1$ indicate gain within the ice relative to complete entrainment from the underlying seawater; e.g., by production or scavenging from depth in the water column. The equation is:

$$E = \frac{\frac{C_{ice}}{C_{sw}} - \frac{S_{ice}}{S_{sw}}}{1 - \frac{S_{ice}}{S_{sw}}} \quad (1)$$

where C_{ice} and C_{sw} are the concentrations of the given parameter measured in a bulk sea ice sample and seawater, respectively, and S_{ice} and S_{sw} are the salinities measured in the bulk sea ice sample and seawater, respectively. Enrichment indices were calculated for thin and bottom ice using seawater values from the same site. Note that both this enrichment index and that of Gradinger and Ikavalko (1998) are sensitive to differences in bulk salinity, so parametric comparisons should only be made among samples with similar bulk salinities.

Unless otherwise indicated, statistical analyses were conducted using nonparametric tests in R (R Development Core Team 2011) and an $\alpha < 0.05$ to indicate significance. Differences in enrichment indices from $E = 0$ or $E = 1$ were calculated with the Wilcoxon signed rank test (with test statistic W). Differences in concentrations of biological parameters were compared using the Mann–Whitney U test (with test statistic U). Pairwise correlations between parameters were calculated as Spearman's (ρ, nonparametric) or Pearson's (r, parametric) correlation coefficient.

Results

Temperature and salinity

Sea ice from a diversity of ice types was sampled from eight stations in the western Amundsen Gulf (Fig. 1; Table 1; Table S6). Air temperatures ranged from −14 to −22°C. Temperatures within the ice sections we sampled ranged from −1.7 to −11.3°C. Samples were separated into three groups by ice thickness: thin ice (3 cores, 4–9 cm), medium ice (3 cores, 33–37 cm), and thick ice (2 cores, 66–78 cm). Three stations had thin ice, including nilas (Stations 1800 and D3) and consolidated pancakes (Station 1200), with bulk salinities of 15–18.5, indicative of recent freezing. Stations R, 1117, and D4 had first year ice of medium thickness with bulk salinities of 6.0–10.3.

Table 1 Summary of sampling stations and ice characteristics from western Amundsen Gulf and the Canadian Shelf of the Beaufort Sea

Station	Date (day of 2007)	Latitude	Longitude	Thickness (cm)	Ice type	Ice class (top)	Ice class (bottom)
D3	29 Nov (333)	70.922 N	123.317 W	4	Nilas	nd	nd
1200	20 Nov (324)	71.076 N	123.438 W	6	Consolidated pancakes	nd	nd
1800	26 Nov (330)	72.088 N	127.592 W	9	Nilas	Granular/columnar	nd
D4	02 Dec (336)	71.730 N	125.564 W	33	Medium first year pack	Granular	Columnar
1117	16 Nov (320)	69.872 N	126.542 W	35	Medium first year landfast	Granular	Columnar
R	14 Nov (318)	70.125 N	125.081 W	37	Medium first year landfast	nd	nd
437	22 Nov (326)	71.749 N	126.576 W	66	Thick first year pack	Granular/columnar	Columnar
D7a	10 Dec (344)	71.258 N	125.299 W	78	Thick first year pack	Granular	nd
D7b	14 Dec (348)	71.449 N	125.961 W	76	Thick first year pack	nd	nd
D8	16 Dec (350)	71.479 N	126.327 W	–	–	–	–

Only seawater was collected at Station D8

nd Not determined

Stations 437 and D7a had thicker first year ice with bulk salinities of 4.7–10.4. The calculated in situ brine salinities ranged from 41 (at −2.2°C) to 150 (at −11.3°C) in the sea ice, reaching maximal values in the colder surface layers of the thick ice. Thin ice and top ice sections were classified as "granular" or "granular/columnar" ice (also known as "transition" ice) by microstructural analysis, while bottom ice sections were classified as "columnar" ice (Table 1).

Seawater underlying sea ice was collected at nine stations and during a single full depth profile at Station D8 (Fig. 2). The water column at Station D8 was stratified, as previously reported for other sites in the Amundsen Gulf (Belzile et al. 2008; Garneau et al. 2008), with shallow Polar Mixed Layer water separated from the deeper, isothermal Halocline Arctic Layer by an intrusion of warm Pacific water at 20–40 m (Fig. 2a). The transition to warmer, more saline Deep Atlantic Layer water occurred around 200 m, as in Garneau et al. (2008).

Organic and inorganic particulate matter

The amount of SPM in seawater (1.6–8.9 mg L^{-1}) was higher than in sea ice (1.0–4.7 mg L^{-1} ice; Table 2). Measures of particulate organic matter (POM), however, even though similarly scaled (to bulk ice volume), were higher in ice relative to seawater. Values of POC (20–500 μg C L^{-1}), the percentage of organic matter (POC SPM^{-1} × 100%; 0.4–26%), and the molar ratio POC:PN (hereinafter C:N) ratio (8–32) increased from low endpoints in seawater to high endpoints in thick ice (Fig. S6). The abundance of chlorophyll *a* was generally very low (<0.3 μg L^{-1}; Fig. S6), except in the case of a visible algal band in bottom ice at Station 1117 (7.1 μg L^{-1}). Chlorophyll *a* concentrations in seawater decreased significantly over the course of the autumn ($r = -0.82$, $n = 6$, $P < 0.05$) while phaeopigment increased from 44 to 58% (Fig. S6). EPS concentrations were below detection limits (<10 μg xanthan gum equivalents L^{-1}) in all samples.

Sources of D-DNA

At Station D8 the concentrations of bacteria and D-DNA each exhibited a subsurface maximum within the Pacific-derived layer at 10–30 m (Fig. 2b, c); viral abundance decreased consistently through the water column. Bacterial abundance in Amundsen Gulf surface seawater decreased significantly over the course of the season ($\rho = -0.839$, $n = 10$, $P < 0.01$). While measured viral and eDNA abundances were generally greater at the end of the sampling period than at the beginning, differences were not significantly different (Fig. S6; see also Collins and Deming 2011). Bacteria were more highly concentrated in sea ice (scaled to brine volume) than in seawater ($U = 6.5$, $n = 45$, $P \ll 0.001$), and were more enriched in the ice relative to salts (median $E = 0.056$; $W = 125$, $n = 17$, $P < 0.05$; Figs. 3, 4; Table S6). Ice sections that had likely grown quickly (granular ice) were more enriched in bacteria than slower-growing (columnar) ice sections ($U = 60$, $n = 17$, $P \ll 0.001$; Table 1; Fig. 4). Viruses were more highly concentrated in sea ice compared to seawater ($U = 374$, $n = 45$, $P \ll 0.001$), reaching a mean concentration about 37× greater in upper ice sections than the mean of 1.2×10^7 viruses ml^{-1} observed in seawater (see Collins and Deming 2011, for more details on viral and bacterial enrichments). Enrichment indices for viruses were high (median $E = 1.05$, range 0.13–8.0, $n = 19$), indicating significantly greater enrichment into sea ice relative to salts ($W = 190$, $n = 19$, $P \ll 0.001$; Fig. 4) consistent with (not significantly different from) the complete entrainment ($E = 1$) of seawater viruses into the growing ice ($W = 121$, $n = 19$, $P > 0.05$).

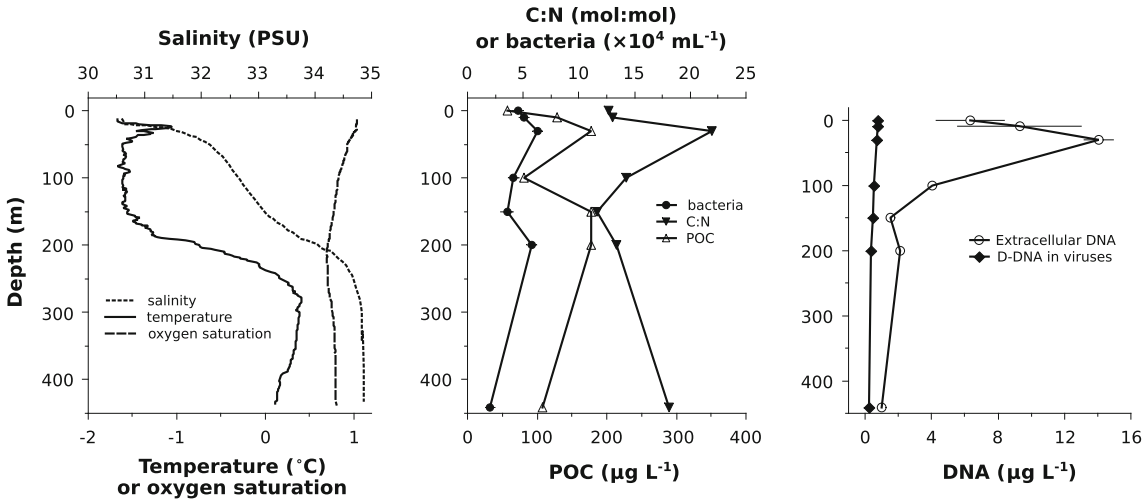

Fig. 2 Seawater depth profile taken at Station D8 showing **a** temperature, salinity, and oxygen saturation, **b** bacterial abundance, particulate organic carbon (POC), and C:N ratio (mol:mol), and **c** concentrations of eDNA (*open circles*) and D-DNA in viruses (*filled diamonds*)

Table 2 Summary of parameters measured on bulk "chemical" ice cores and seawater from western Amundsen Gulf and the Canadian Shelf of the Beaufort Sea

Sample type		POC (µg L^{-1})	C:N	SPM (mg L^{-1})	% Organic	Chl *a* (µg L^{-1})	% Phaeo.
Surface seawater	Mean	47	12	3.7	1.9	0.10	54
	SEM	4.5	1.1	0.9	0.4	0.02	0.9
	N	12	12	9	9	9	9
Sea ice	Mean	192	14	2.5	11	1.0	52
	SEM	36	1.4	0.4	3	0.9	1.5
	N	8	8	8	8	8	8

% Phaeo. 100% × phaeopigment/(phaeopigment + Chl *a*), *SEM* standard error of the mean

The total D-DNA was composed primarily of eDNA in surface seawater (23 ± 23%), thin ice (35 ± 14%), and ice of medium thickness (29 ± 23%), whereas viruses made up a significantly greater fraction of the total in thick ice (66 ± 28%) than any other sample type (combined

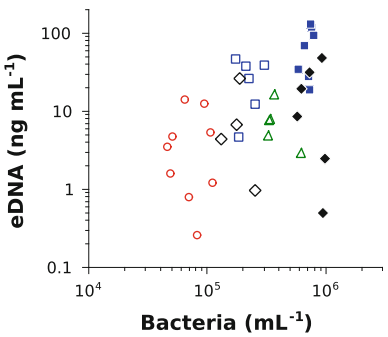

Fig. 3 Concentrations of bacteria and eDNA in sea ice (scaled to brine volume) and seawater from western Amundsen Gulf and the Canadian Shelf of the Beaufort Sea. Symbols represent sample type: seawater, *circles*; thin ice, *triangles*; bottom of medium thickness ice, *open squares*; top of medium thickness ice, *closed squares*; bottom of thick ice, *open diamonds*; top of thick ice, *closed diamonds*

$W = 307$, $n = 43$, $P < 0.001$). Concentrations of both eDNA and D-DNA in viruses were consistently greater than the amount of DNA estimated to be present within bacterial cells, which was less than 10% of the total DNA in every sample type. No correlation was observed between bacterial abundance and eDNA concentration in seawater ($\rho = 0.257$, $n = 8$, $P > 0.05$) or sea ice ($\rho = 0.088$, $n = 35$, $P > 0.05$).

The range of D-DNA concentrations in surface waters was 1.3–13.0 µg L^{-1}, of which 0.3–12.7 µg L^{-1} was in the form of eDNA (Figs. 3, S6; Table S6). In the depth profile at Station D8, the concentrations of D-DNA were 1.0–5.6 µg L^{-1}, of which 0.8–4.9 µg L^{-1} was eDNA, with the exception of a strong peak in D-DNA of 15.0 µg L^{-1} (14.2 µg L^{-1} eDNA) at a depth of 30 m (Fig. 2c). Overall, the concentrations of eDNA were significantly higher in sea ice (scaled to brine volume) compared to seawater ($U = 276$, $n = 46$, $P < 0.01$; Fig. 3), reaching a mean concentration about 13× greater in upper ice sections than the mean of 3.1 µg L^{-1} observed in surface seawater. When separated by ice type, the bottom of medium ice had significantly higher eDNA concentrations than seawater

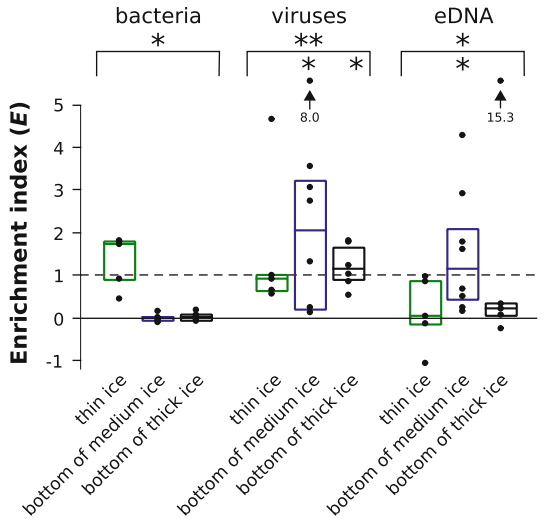

Fig. 4 Enrichment indices (E, see text for calculation of this newly formulated index) of bacteria, viruses, and eDNA in sea ice from western Amundsen Gulf and the Canadian Shelf of the Beaufort Sea. A value of $E = 0$ (equivalent to $I_s = 1$) indicates entrainment into the ice equal to that of salt; a value of $E = 1$ indicates entrainment into the ice equal to complete entrainment from seawater. All data points are plotted; lower and upper limits of boxplots indicate 25 and 75% quartiles, respectively; center line indicates median. *Asterisks* indicate significant ($^*P < 0.05$) or highly significant ($^{**}P < 0.001$) differences from passive entrainment ($E = 0$) as given by the Mann–Whitney test; *asterisks above bracket* indicate significance level for all samples combined, *asterisks below bracket* indicate significance levels for each ice group

($U = 62$, $n = 16$, $P < 0.001$; Fig. 5), but the highest concentrations of eDNA (124 and 135 μg L^{-1}; Table S6) were observed at the top of 35 cm thick landfast sea ice collected at the outflow of the Horton River (Station 1117; Fig. 1).

A wide range of enrichment indices was observed for eDNA, from −1.0 to 15.3 (Fig. 4), with an overall positive enrichment into ice (median $E = 0.42$) that was statistically inconsistent with passive entrainment ($E = 0$; $W = 149$, $n = 18$, $P < 0.01$). Half of the newly formed sea ice sections had eDNA enrichment indices suggestive of active accumulation ($E > 0$), particularly within samples from the bottom 10 cm of medium-thickness ice (mean $E = 1.5$; $W = 36$, $n = 8$, $P < 0.01$; Fig. 4). Thin ice and ice sections from the bottom of thick ice contained concentrations more consistent with passive accumulation ($E = 0$) or depletion ($E < 0$) from seawater (mean $E = 0.1$ excepting an extreme outlier of $E = 15.3$; Fig. 4).

Production experiments indicated no consistent trend in eDNA production over the course of 60 h (Fig. S6), with different bottles exhibiting different responses. In one bottle, a large spike in eDNA concentration was observed between the last two time points of the experiment (24 h apart); the other bottles showed no change in eDNA concentration over the course of the incubation.

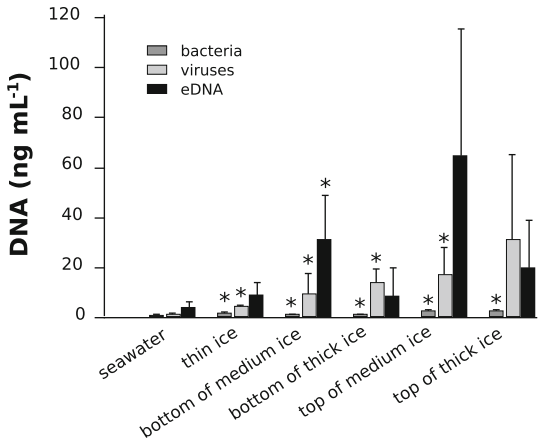

Fig. 5 Concentrations of DNA in bacterial cells (*dark gray*), viruses (*light gray*) and eDNA (*black*) in sea ice of a variety of types (scaled to brine volume) and seawater from western Amundsen Gulf and the Canadian Shelf of the Beaufort Sea. *Asterisks* indicate concentrations that were significantly different from surface seawater by two-tailed Student's t tests ($\alpha < 0.05$)

Discussion

Sea ice is a microbiological habitat that combines physical, chemical, and biological characteristics that, individually, have been shown to elevate the frequency of HGT in experimental settings. The purpose of this study was to examine the potential for HGT in sea ice as determined by the availability of exogenous DNA, a critical piece of information missing from the sea ice literature. During the course of this exploratory work, we obtained the first measurements of eDNA in sea ice, but also of viral entrainment into newly formed sea ice. Overall, we found high concentrations of bacteria, viruses, and eDNA in first year Arctic sea ice, some of which showed evidence for entrainment into sea ice by active processes. These investigations may help constrain the evolutionary mechanisms responsible for apparent HGT events involving ice-binding (Janech et al. 2006; Raymond et al. 2007) and antifreeze proteins (Bayer–Giraldi et al. 2010; Kiko 2010) and lend support to the hypothesis that these events occurred within sea ice rather than in the underlying seawater.

Dissolved DNA in Arctic seawater

Seawater underlying first year sea ice was sampled throughout the Amundsen Gulf during the autumn freeze-up period, allowing a consideration of seawater (versus sea ice, see below) as a site for HGT. Concentrations of total D-DNA observed among all seawater samples (1.0–15.0 μg L^{-1}) were comparable to the available measurements for other cold waters (≤16 μg L^{-1}; Karl and Bailiff 1989; ≤16 μg L^{-1}; Bailiff and Karl 1991), though most D-DNA measurements have been made on

subtropical waters near Florida (Paul and Carlson 1984; DeFlaun et al. 1987; Paul et al. 1988), Hawaii (Karl and Bailiff 1989; Brum et al. 2004), and Italy (Dell'Anno et al. 1998; Turk et al. 1992), where concentrations range from 1 to 31 μg L^{-1} seawater and median values fall near 10 μg L^{-1}. We detected no correlation between D-DNA and microbial abundance in Arctic seawater, echoing the findings of Bailiff and Karl (1991), who found that water mass characteristics better predicted the D-DNA concentrations in Southern Ocean seawater. Their hypothesis that the presence of sea ice influences the D-DNA and particulate DNA (P-DNA) dynamics is supported by our findings of high enrichment indices of D-DNA in some sea ice, discussed below.

Subsurface maxima in POC, C:N molar ratio, bacterial abundance, and eDNA concentration were observed between 10 and 50 m at Station D8 (Fig. 2b, c). The peak in D-DNA observed at 30 m (14.1 ± 1.2 μg L^{-1}) was the largest value we observed in seawater, but no increase in viral abundance occurred at that depth, indicating that eDNA concentration accounted for the peak. The increase in eDNA was concomitant with a spike in C:N to 22, a value more suggestive of ice algal exopolymers (Meiners et al. 2003; Forest et al. 2007) than Arctic river terrigenous matter, which has end member C:N ratios of 12–15 (Krishnamurthy et al. 2001). One possible source of high-C:N matter was multiyear ice, which was observed to flow south into Amundsen Gulf through Prince of Wales Strait (unpublished observations). C:N ratios of 12–50 have been observed in multiyear ice (attributed to the accumulation of exopolymer particles in older ice; Meiners et al. 2003) and thus melting of multiyear ice and release of particulate matter in Amundsen Gulf might have contributed to the high C:N ratio POM we observed in the water column.

In the surface seawater we sampled, eDNA made up the majority of the total D-DNA (68–98%, with an outlier of 16% at Station 437), with the rest composed of D-DNA in viruses. The primary form of D-DNA in the water column at Station D8 was also eDNA (73–95%), with viral DNA representing larger fractions of the total in waters collected below 100 m (Fig. 2c). This result contrasts with Station ALOHA: when viruses were considered there, they made up about 55% of the D-DNA throughout the water column (Brum 2005). Overall, similar amounts of D-DNA were observed in viruses at Station D8 and Station ALOHA, but several times as much eDNA was found in the water column at Station D8 compared to Station ALOHA.

DNA is a readily available source of phosphorus for microorganisms (Paul et al. 1988), but unlike at Station ALOHA, productivity in the Beaufort Sea is not limited by the availability of phosphorus except inshore (<20 m; Carmack et al. 2004). Concentrations of eDNA did not change significantly over the course of the autumn as bacterial concentrations decreased, but maximum concentrations were observed at Station D8 in mid-December (Fig. S6). The lower temperatures and more readily available dissolved phosphorus probably allowed longer turnover times and greater concentrations of eDNA to persist in Amundsen Gulf than at the warm, oligotrophic Station ALOHA.

Dissolved DNA in Arctic sea ice

Temperature and salinity

Physical, chemical, and biological dynamics were observed within Arctic sea ice during the fall freeze-up period. Temperatures within the ice sections we sampled ranged from −1.7 to −11.3°C, with strong temperature gradients expected within the newly formed ice. When it has been tested in experimental settings, decreasing temperature has usually led to decreasing frequency of natural transformation because DNA uptake mechanisms depend on cellular activity, though transformation has been observed at temperatures more than 20°C below the optimal growth temperature of the host (Frischer et al. 1993; Lorenz and Wackernagel 1992). As temperature in sea ice decreases during winter, the fraction of active bacteria decreases as well (Helmke and Weyland 1995; Junge et al. 2004), suggesting that, based on temperature alone, the optimal location for transformation and conjugation in autumn and winter sea ice is near the base of the ice sheet, where the temperature approaches that of underlying seawater.

The high salinity in sea ice brine—between 1.3 and 4.5× the salinity of the underlying seawater in this study—might influence the frequency of HGT in a number of ways. Cations bind to the negatively charged phosphate backbone of DNA and bridge the gap between it and other negatively charged surfaces, including mineral grains (Lorenz and Wackernagel 1987) and potentially phage particles (Kokjohn 1989) and EPS (Beveridge et al. 1997; Steinberger and Holden 2005). Adsorption of eDNA to minerals increases with cation concentration (Paget et al. 1992; Romanowski et al. 1991), though this adsorption does not necessarily render the eDNA inaccessible to uptake via transformation (Lorenz and Wackernagel 1987, 1992). The increased salt concentration in sea ice brines likely affects the physical interactions between microorganisms and extracellular DNA.

Organic and inorganic particulate matter

Because attachment can induce pathways for HGT in marine bacteria (Meibom et al. 2005), we measured a number of parameters relating to the presence of surfaces available for attachment in first year sea ice. During autumn freeze-up in Amundsen Gulf, we observed higher

concentrations of POC in older ice than in medium or thin ice, both in absolute terms and relative to particulate nitrogen and the total particle load (Fig. S6; the limited number of samples precluded statistical analysis). These results contrast with the decrease expected had POC drained with brine during sea ice growth. The observed increase in POC implies de novo production in older ice or higher values in the seawater from which it froze. We have no measurements of seawater POC high enough to support the latter explanation, but the higher C:N ratios in older ice are consistent with the in situ production of carbon-rich material of high molecular weight, like EPS. Although EPS was below the detection limits of the method applied to this autumn ice, previous work has demonstrated the production of EPS in sea ice from the same region during winter (Collins et al. 2008) and spring (Riedel et al. 2006). The presence of EPS has also been shown to increase the surface area within the sea ice matrix (Krembs et al. 2011) and bacteria have been observed attached to the ice walls of the matrix (Junge et al. 2001). In one microcosm study, transformation was shown to be 50× more efficient at the solid-liquid interface than in the liquid alone (Lorenz et al. 1988); the several square meters of surface area available within a single kilogram of sea ice (Krembs et al. 2000) provides ample opportunity for surface-associated HGT.

Bacteria and viruses

Bacteria have previously been shown to be more highly concentrated within sea ice brine than in seawater due to the brine concentrating effect (Riedel et al. 2007; Collins et al. 2008), as also observed in this study, where concentrations in ice brines averaged 3.3× those in underlying seawater. Additionally, we found that bacteria were more highly enriched relative to salt in nilas and pancake ice, with an average enrichment index of 1.4 (equivalent to $I_s = 2.0$ in Gradinger and Ikavalko 1998), lower than observed by others in thin sea ice ($I_s = 7$, Gradinger and Ikavalko 1998; $I_s = 5.5$, Riedel et al. 2007) but suggestive of active entrainment, e.g., by scavenging of bacteria attached to frazil ice or large phototrophs (Grossmann and Gleitz 1993; Riedel et al. 2007). Bacteria were not highly entrained into newly formed ice at the base of thicker columnar ice (mean E =0.03, mean $I_s = 1.1$), indicative of a more passive mechanism of entrainment into slowly growing ice. A number of previous reports on the frequency of conjugative gene transfer in environmental microcosms suggest that the frequency of recombination is proportional to the concentration of donor cells (Frischer et al. 1994; Dröge et al. 1998). The significantly higher concentrations of bacteria we observed in autumn sea ice relative to the underlying seawater may favor the occurrence of conjugation there relative to underlying seawater.

Viruses, discussed in greater detail in Collins and Deming (2011), were also present and highly enriched in thin sea ice relative to salts (median $E = 1.24$, median $I_s = 6.6$; Fig. 4). These first measures of viral enrichment indices in sea ice suggest that viruses become entrained into the ice via an active pathway, rather than by passive incorporation as with bacteria in columnar ice.

eDNA

Concentrations of D-DNA and eDNA in our Arctic sea ice samples were significantly greater than in the underlying seawater, with enrichment indices consistent with the effects of passive brine concentration from seawater (Figs. 3, 5). The maximum values detected (to 135 μg eDNA L^{-1}) were higher than any previously reported from the marine environment (Table S6). Possible mechanisms of DNA release and accumulation in sea ice include sloppy feeding by grazers (Proctor and Fuhrman 1990; Turk et al. 1992), release of DNA-containing EPS (Steinberger and Holden 2005; Allesen–Holm et al. 2006; Bockelmann et al. 2006), abiotic concentration from source seawater, or bacterial production followed by excretion or cell lysis (Paul et al. 1988). We collected no data on grazing within sea ice, but in another late-autumn study bacterivorous protists were thought to exert control over the microbial community within sea ice from the Greenland Sea (Gradinger et al. 1999). Below, we discuss the other mechanisms of eDNA dynamics within sea ice.

The greater enrichments of eDNA observed in medium ice than in thick ice suggest biotic factors played a role in the entrainment process, as also suspected for viruses. The estimated pool of DNA within living bacterial cells was always less than 10% of the total eDNA (Fig. 5), so it is unlikely that the excess eDNA in highly enriched samples was produced exclusively by lysis of a static bacterial community. Rather, the enrichment indices suggested continued production over a period of time, for example by growth and lysis of an active bacterial community, as part of a biofilm matrix, or in response to stress or competence development. We were not able to detect the production of eDNA in brine from thick sea ice at −7°C (Fig. S6), but future studies of D-DNA dynamics could benefit by focus on the base of medium thickness ice, where the greatest mean enrichment indices were observed (Fig. 4).

The general dynamics we observed in this study resemble those reported by Grossmann and Dieckmann (1994) for a bottom-ice microbial community during freeze-up in the Antarctic, albeit using different suites of measurements. They describe an evolving community in which bacteria are actively entrained into thin ice, followed by a period of adaptation in ice of medium thickness during which bacterial activity slows and abundance decreases, at

least partially due to grazing within the ice sheet. We also observed entrainment of bacteria, and of viruses, into recently formed thin ice, followed by a divergence between the entrainment of bacteria (which decreased) and viruses (which increased) as the ice became thicker (Fig. 4). During this period of adaptation in ice of medium thickness, we observed large enrichment indices for both virus and eDNA, implying their active production in the ice at the expense of bacterial cells. By the time the ice grew to thicknesses greater than 50 cm, the enrichment indices of bacteria and eDNA in bottom ice had stabilized at low values, while the enrichment indices of viruses centered around a new, higher value, possibly indicating arrival of the community at a new equilibrium point between bacterial growth and viral lysis.

Abiotic mechanisms of entrainment, like attachment to particles, might account for an increased abundance of D-DNA within sea ice but our protocol involved filtering particles greater than 0.22 μm so we are not able to directly address this possibility. Sediment grains and particulate matter, discussed above (Fig. S6), bind DNA that can still act as a suitable agent of transformation (Lorenz et al. 1981; Lorenz and Wackernagel 1987, 1990; Stewart et al. 1991), a finding that may be particularly important for coastal sea ice that contains large amounts of resuspended sediments (Stierle and Eicken 2002).

The frequency of transformation in experimental studies is often directly proportional to free DNA concentration (Frischer et al. 1993; Sikorski et al. 1998), such that high concentrations of eDNA should favor transformation in sea ice relative to the underlying seawater. However, because of the long, flexible nature of free DNA, a large fragment of eDNA ($\sim$50 kbp) has a predicted diffusivity at least an order of magnitude lower than a comparable genetic complement of highly efficiently packed viral DNA within a protein capsid (calculations, not shown, used a diffusion-based model; Murray and Jackson 1992; calculations, not shown, used a diffusion-based model; Wells and Deming 2006). Thus, it is possible that a sea ice bacterium would have contacted more viral DNA than eDNA per unit time, especially at lower temperatures, despite the fact that eDNA made up a larger fraction of the total DNA in most ice types. Transformation may be of limited importance in colder parts of the ice sheet.

The quality of DNA and its suitability for HGT are related to its age, as older eDNA is more likely to have been degraded by extracellular DNases and is thus less suitable for transformation (Lorenz and Wackernagel 1994). Results from our productivity estimates on natural sea ice brine incubated at −7°C were inconclusive with regards to the dynamics of eDNA in sea ice, but, combined with the presence of high concentrations of eDNA in upper sections of thick sea ice (likely several weeks old) suggest that eDNA can persist in sea ice at temperatures down to −11°C (Fig. 3). Previous studies provide a wide range of half lives for D-DNA in warmer marine environments, from 3.4 h in estuaries to 3.5 days in P-replete open ocean regions (DeFlaun et al. 1987). These half lives, measured in subtropical waters, are surely shorter than in colder polar waters and especially sea ice, where the activity of extracellular nucleases may be limited by both temperature and salinity.

Despite not having directly measured HGT in sea ice, the results of this study can be used to refine hypotheses regarding the relative importance of different mechanisms of HGT in sea ice. Other factors influencing HGT, including lysogeny, nutrient availability, and cell-to-cell communication (quorum sensing), likely occur in sea ice as well and may be usefully investigated in future studies. If sea ice is in fact a hotspot for HGT, the dispersal of recombinant microorganisms from sea ice could be significant over large geographic and temporal scales. Arctic sea ice can entrain, transport and distribute particulates (Nurnberg et al. 1994; Stierle and Eicken 2002; Eicken et al. 2005) throughout the Arctic Ocean faster than ocean currents alone and could be expected to do the same for recombinant microorganisms. The rapid and widespread distribution of a new lineage could promote the rapid fixation of new genes into populations of marine bacteria, a route of evolution that might be at threat in a warmer future characterized by dwindling volumes of Arctic sea ice.

Acknowledgments We thank the captain, crew, and scientific party of the CCGS *Amundsen* for a successful cruise. We gratefully acknowledge M. Pucko, W. Walkusz, P. Galand, B. Else, N. Sutherland, and M. Gupta for field assistance, C. Marrasé for assistance with chlorophyll *a* measurements, J. Islefson, D. Barber and CFL Team 2 for ice microstructure data and the use of ice-coring equipment, and S. Carpenter for help with laboratory analyses. We thank the three referees, whose contributions improved the manuscript.

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