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ORIGINAL PAPER

Dissolved extracellular polymeric substances (dEPS) dynamics and bacterial growth during sea ice formation in an ice tank study

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Abstract Extracellular polymeric substances (EPS) are known to help microorganisms to survive under extreme conditions in sea ice. High concentrations of EPS are reported in sea ice from both poles; however, production and dynamics of EPS during sea ice formation have been little studied to date. This investigation followed the production and partitioning of existing and newly formed dissolved organic matter (DOM) including dissolved carbohydrates (dCHO), dissolved uronic acids (dUA) and dissolved EPS (dEPS), along with bacterial abundances during early stages of ice formation. Sea ice was formed from North Sea water with (A) ambient DOM (NSW) and (B) with additional algal-derived DOM (ADOM) in a 6d experiment in replicated mesocosms. In ADOM seawater, total bacterial numbers (TBN) increased throughout the experiment, whereas bacterial growth occurred for 5d only in the NSW seawater. TBN progressively decreased within developing sea ice but with a 2-fold greater decline in NSW compared to ADOM ice. There were significant increases in the concentrations of dCHO in ice. Percentage contribution of dEPS was highest (63%) in the colder, uppermost parts in ADOM ice suggesting the development of a cold-adapted community, producing dEPS possibly for cryoprotection and/or protection from high salinity brines. We conclude that in the early stages of ice formation, allochthonous organic matter was incorporated from parent seawater into sea ice and that once ice formation had established, there were significant changes in the concentrations and composition of dissolved organic carbon pool, resulting mainly from the production of autochthonous DOM by the bacteria.

Keywords Sea ice · Extracellular polymeric substances · Frost flowers · Bacteria · Dissolved carbohydrate · Dissolved organic matter · EPS

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Introduction

During sea ice formation, inorganic and organic (both dissolved and particulate) constituents become concentrated in the brines present in ice channels and pores, the volume of which is primarily dependent on temperature (Petrich and Eicken 2010). Organisms entrained into ice also undergo very different chemical and physical constraints during the phase shift from open water to a semi-solid system where water and gas exchange can be limited (Mock and Thomas 2005). The microbial community composition in sea ice at early stages of ice formation reflects that of the parent seawater, but as the ice ages, there is shift in population and

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dominance of species leading towards the establishment of a psychrophilic community (Caron and Gast 2010; Collins et al. 2010; Deming 2010). Bacterial abundances and algae can be tightly coupled in young ice (Stewart and Fritsen 2004), although this relationship is not always so clear (reviewed by Brierley and Thomas 2002).

Studies have revealed that despite losses of dissolved organic matter (DOM) from consolidating sea ice due to brine drainage into the underlying water, there are generally significantly higher DOM concentrations in sea ice compared to seawater (Thomas et al. 2001; Dumont et al. 2009; Juhl et al. 2011; Norman et al. 2011). Within the ice, the distribution of DOM is unpredictable and dissolved organic carbon and nitrogen (DOC and DON) concentrations usually demonstrate a heterogeneous distribution (van der Merwe et al. 2009; Underwood et al. 2010).

A significant proportion of organic matter in sea ice can be present in the form of extracellular polymeric substances (EPS), predominantly polysaccharides containing uronic acids, sulphated sugars and some proteins (Hoagland et al. 1993; McConville et al. 1999; Meiners et al. 2003; Krembs et al. 2011), released by both bacteria and algae (Mancuso Nichols et al. 2005; Krembs and Deming 2008; Collins et al. 2010). EPS exist along a continuum of solubility, from dissolved to particulate states, that depends on both physical and chemical conditions, with EPS contributing up to 60% to particulate organic carbon (POC) and up to 99% to DOC (Herborg et al. 2001; Thomas et al. 2001; Riedel et al. 2006; Dumont et al. 2009; Underwood et al. 2010). Like DOC and DON, dissolved EPS (dEPS) concentrations in sea ice have a heterogeneous distribution pattern within ice core profiles, while particulate EPS (pEPS) are predominantly found in biomass-rich horizons in the ice (Krembs et al. 2002; Volkman and Tanoue 2002; Meiners et al. 2003; Dumont et al. 2009; van der Merwe et al. 2009; Krembs et al. 2011). EPS can modify the ice environment by changing ice microstructures, therefore increasing the sea ice habitability (Krembs and Deming 2008; Ewert and Deming 2011; Krembs et al. 2011). Presence of high concentrations of complex EPS (both particulate and dissolved forms) in sea ice is thought to be due to EPS playing an important role for sea ice microbial communities by providing buffer zones and cryo-protection against salinity and low temperatures (Bowman and Deming 2010; Underwood et al. 2010; Krembs et al. 2011). Both dEPS and pEPS may also be serving as important carbon sources in sea ice (Mock and Thomas 2005) as higher rates of bacterial activity have been reported to be associated with high molecular weight DOC fractions in other environments (Tranvik 1990). Similarly, pEPS are also reported as hot spots of microbial activity in sea ice (Meiners et al. 2008). However, it is likely that not all bacteria present can utilise all EPS types, as has been shown in estuarine sediments (Hofmann et al. 2009).

Both experimental and field studies have suggested that initial distribution of inorganic and organic matter in sea ice follows patterns of conservative enrichment (Clarke and Ackley 1984; Cota et al. 1987; Garrison et al. 1990) and that as the ice ages, microbial communities influence the subsequent distribution of DOM in sea ice (Stedmon et al. 2007). However, to understand the fate of organic matter in growing sea ice, systematic studies are needed. Giannelli et al. (2001) performed a mesocosm study to investigate the partitioning of DOM in young sea ice. Partitioning of inorganic nutrients and dissolved organic matter into brines, seawater and sea ice fractions were measured, but this study did not try to link DOM with microbial biomass and/or activity within the experimental systems. To the best of our knowledge, there have been no other detailed environmentally controlled sea ice tank studies that make simultaneous measurements of organic matter and bacterial dynamics during ice formation. The objective of the work presented here was to refine the experimental approach used by Giannelli et al. (2001) and to investigate the partitioning of organic matter, and changes in the dynamics and composition of dEPS during the early stages of ice growth in relation to bacteria numbers in ice and the underlying water. Furthermore, the addition of a complex algal-derived DOM source was included in the experimental matrix both to follow the partitioning of this additional organic carbon (both dissolved and particulate) and its effect on bacterial growth in the early stages of ice formation.

Materials and methods

General methods

The experiments were conducted between 30 September and 9 October 2009 at the HSVA environment test basin, Hamburg, Germany (http://www.hsva.de). Experimental polyethylene (PE) bags (total 18) supported by a floating frame were arranged in a random block design within the main test basin (Fig. 1a). On 30 September, each bag was filled with 1.2 m^3 of unfiltered seawater from the North Sea, and the temperature of the air was set to approximately 0°C to cool the water. The 21 m^3 of surface seawater had been pumped onto a ship close to the island of Helgoland (54°11′N, 7°55′E), transferred to a cleaned (food quality) road tanker and delivered to the experimental facility within 24 h of initial collection. The temperature of the seawater on collection was 13 ± 1°C. This temperature was maintained ±1°C during the transport to the test basin.

Each experimental bag was equipped with a PVC tubing in one corner (fixed on the floating frame) to allow for

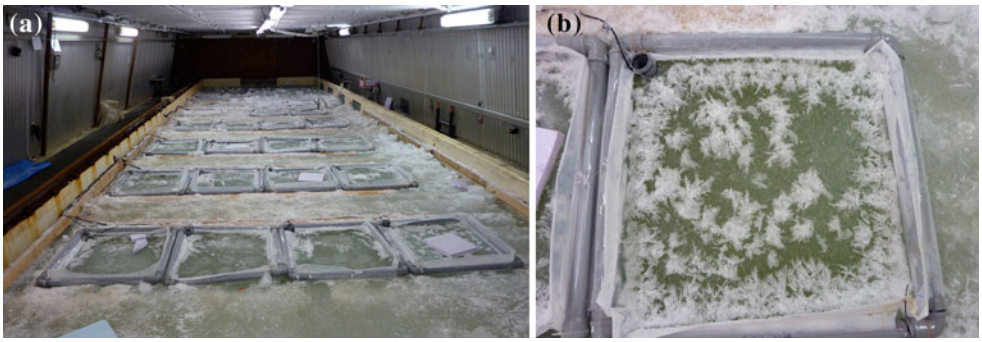

Fig. 1 **a** Experimental mesocosms in environmental test basin of HSVA, **b** Frost flowers on surface of one of the mesocosms on day 6. The surface area of each mesocosm was 1 m^2

water pressure equilibration during ice growth and to ensure that the ice was always in contact with the underlying water. These tubes were cleared of ice every day and also served as a portal for sampling under-ice water once an ice cover had developed. A simple circulation pump was set up, and continually running, in each of the experimental bags to ensure that mixing of the water was complete at all stages of the experiment.

On 2 October, 900 ml of a concentrated solution of algal-derived organic matter (ADOM) was added to 9 bags, which are referred to as the ADOM series, while remaining 9 bags filled with North Sea water were used as controls and are referred as NSW. The ADOM additive was produced as follows: Frozen paste of the freshwater green alga *Chlorella vulgaris* (supplied by Varicon Aqua Ltd, U.K.) was melted in synthetic seawater of a salinity of 34 (1 kg paste melted in 1 l seawater). The resulting suspension was sonicated in 500 ml batches with a Branson 450 Digital Sonifier (Branson Ultrasonics Corporation, Danbury, CT, USA). Samples were sonicated in a seawater/ice bath so that sample temperature did not rise above 0°C. The resulting suspensions were pooled, centrifuged at 12,235*g* in a Beckmann Coulter centrifuge using a J-LITE® JLA-8.1000 Rotor (Fixed Angle, Aluminum, 6 × 1,000 ml) and the supernatant was frozen at −18°C until used in Hamburg. This solution was pink in colour, presumably due to the chlorophyll breakdown products as described by Engel et al. (1991) and Hortensteiner et al. (2000). The dissolved organic carbon (DOC) concentration of this material was 550 mmol l^{-1}. Experimental set-up was designed to prevent autotrophic growth. The room was in darkness except during sampling periods (2–3 h per day) when the light levels were very low.

Initial samples (d0) were taken on the 2 October after >24 h of mixing of the water and ADOM. Freezing of the water in the mesocosms was then initiated by decreasing the air temperature to −13°C (±2°C), and spraying a fine mist of Milli-Q water over the surface of the mesocosms to provide surface ice nucleation points (Giannelli et al. 2001). This temperature was maintained throughout the experiment.

The seawater was sampled from all mesocosms on d0 and d3; afterwards, underlying water was sampled along with ice in randomly chosen mesocosms on days 4, 5 and 6 in each of the two treatment series. Brines were collected on days 5 and 6 only, and once brines and ice were sampled from bags, no further sampling was done from those bags.

The seawater was sampled through the PVC pressure release tube. Firstly, ice (when present) was removed and then the water in the tube was sampled using 50 ml plastic syringes and small-bore Teflon tubes. At each sampling time, the temperature of the water was measured.

Ice was sampled by sawing ice cores from the ice sheet and sectioning the cores immediately (less than 1 min). The ice cores were floated until sectioning, minimising brine drainage from the skeletal layer. Ice temperatures, at 4 cm depth intervals, were measured on representative ice cores. The ice cores were melted at room temperature overnight in 5 l acid-washed PE drums. The melting was carefully monitored, and the temperature of the melt water did not rise above 0°C.

Brines were collected following sackhole drilling to a depth of 6 cm, using a Cherepanov ice ring of 20 cm diameter. Brine drained into the core rim left when the corer and core shavings were removed, but the partial core was left in place (see discussion by Papadimitriou et al. 2007). This approach enabled a minimum surface area of brine to come into contact with the air. Even so, due to the cold air and ice temperatures, only small volumes (<200 ml in 30 min) of brine were collected in each sackhole. Consequently, brines collected from several sackholes within each mesocosm had to be pooled to generate sample volumes necessary for subsequent analyses. Prior to sampling, the temperature of the brine was measured in each of the sackholes. The sackholes were drilled as far away from the location of ice core sampling as possible, so as not to compromise the samples.

Frost flowers developed on the surface of all of the mesocosms (NSW and ADOM, Fig. 1b) from day 2 onwards. On days 5 and 6, these were sampled by carefully scraping the frost flowers into acid-washed 1 l PE containers, using the rim of the container as the "scraper" and ensuring that the frost flowers fell straight into the container, thereby minimising contamination issues. The frost flowers were melted at room temperature (<1 h) and again the melt water did not rise above 0°C.

It has been shown that bacteria are capable of surviving short-term salinity reductions associated with direct melting without losing their viability (Helmke and Weyland 1995); therefore, ice cores were melted without the addition of seawater to avoid any potential dilution of samples and addition of allochthonous DOM or bacteria. Samples for determining major inorganic nutrients (nitrate, nitrite, phosphate and silicate), dissolved organic nitrogen (DON), dissolved organic carbon (DOC), dissolved carbohydrates (dCHO), dissolved uronic acids (dUA) and ethanol solubility-based fractionated dEPS were collected from seawater, melted ice, sackhole brine and melted frost flowers within 2 h, after filtering through pre-combusted (400°C for 2 h) Whatmann GF/F filters (20–500 ml). The filtrates were stored at −20°C until analysis. Salinity measurements were made on aliquots of these samples prior to freezing.

Temperature and salinity measurements

All ice (in the gradient across the ice), brine, frost flowers (base of frost flowers) and water temperatures were measured with a calibrated Testo 110 thermometer. Salinities of water, brines and melted ice were measured at laboratory temperature (17–22°C) using a SEMAT Cond 315i/SET salinometer with WTW Tetracon 325 probe.

Bacterial abundance and oxygen measurements

Samples for bacterial abundance determination were fixed with 0.2 µm filtered electron microscopy-grade glutaraldehyde (final concentration of 0.5%) and stored at 4°C. Cells were stained with SYBR Green I (Molecular Probes) at a final dilution of 1:10,000 for at least 10 min in the dark and analysed with an LSR II flow cytometer (BD Biosciences) using a 488 nm laser (essentially after Gasol et al. 1999) within 30 min of staining. CountBright beads (Molecular Probes) were added to each sample to calculate the volume of sample used in counting. Bacterial data were typically acquired until 50,000 events were recorded, and cell populations of high and low DNA bacteria identified from bivariate plots of green fluorescence vs. SSC (side scatter) using FACS Diva software (BD Biosciences). Cell abundance in cells ml^{-1} was calculated from sample flow rates and number of events recorded.

Dissolved oxygen concentrations of water samples (50 ml samples) were measured by Winkler titration with photometric end-point detection (HydroBios GmbH).

Organic carbon (POC and DOC) analyses

DOC was analysed by high temperature combustion on an MQ1000 TOC analyser (Qian and Mopper 1996), see Papadimitriou et al. (2007) and Norman et al. (2011) for further details. For POC measurements, samples (filters) were treated with 2N HCL to remove inorganic carbon if present and were kept overnight in the fume cupboard to dry. POC was measured on Shimadzu Solid Sample Module (SSM 5000A) linked with Shimadzu TOC-VCSH Analyser. TOC (total organic carbon) was calculated by adding DOC and POC together.

Carbohydrate analysis

Samples (in duplicate) were dialysed at room temperature for 8–15 h, depending on salinity, through 8 kDa dialysis tubing (VWR, USA) against Milli-Q water to a final salinity <1. The desalted samples were freeze-dried and stored at −20°C until further analysis. Subsequently, the samples were re-dissolved in 4 ml Milli-Q water (into four aliquots of 1 ml each). One aliquot was used for carbohydrate analysis (total dissolved carbohydrate dCHO), and other three were used for ethanol precipitation of dEPS. Carbohydrate concentrations in dissolved form (dCHO) and in dEPS (ethanol solubility-based EPS fractions) were determined by a modified method of Dubois et al. (1956) as described in Underwood et al. (2010). Glucose was used as a standard and so the carbohydrate concentrations are reported as glucose-carbon-equivalents. Uronic acids (dUA) concentrations in each fraction were determined by standard carbazole assay (Bitter and Muir 1962; Bellinger et al. 2005). Glucuronic acid was used as a standard, and all uronic acids are therefore expressed as glucuronic-carbon-equivalents. Fractions were calculated both as concentrations (µmol l^{-1} C) and as percentages of the total dissolved carbohydrate recovered.

EPS components with different solubility were precipitated with 30, 50 and 70% (v/v) ethanol overnight at 4°C. The precipitates for each were recovered by centrifugation (3,500*g*, 15 min), air-dried and re-dissolved in Milli-Q water (0.65 ml). These precipitated dEPS fractions were named as EPS-30, EPS-50 and EPS-70 (precipitated with 30, 50 and 70% ethanol, respectively). The terms EPS-30, EPS-50 and EPS-70 refer to the concentrations (µmol l^{-1} C) of highly complex, complex and less complex dEPS fractions and were used when carbohydrate concentration of each dEPS fractions were considered.

The term dCHO describes total dissolved carbohydrates (≥8 kDa) concentration (μmol l^{-1} C), which measures the carbohydrate concentrations of both dEPS (EPS-30 + EPS-50 + EPS-70) and the non-EPS (which did not precipitate with any ethanol) carbohydrate fractions. The terms $\%CHO_{0–30}$ (highly complex EPS), $\%CHO_{30–50}$ (complex EPS), $\%CHO_{50–70}$ (less complex EPS) and $\%CHO_{70–100}$ (non-EPS) refer to the percentage contribution of dEPS and non-EPS carbohydrate fractions relative to the total dissolved carbohydrate (dCHO) concentration and were calculated as described by Underwood et al. (2010) (Online Resource 1).

Inorganic nutrients and DON analyses

Major dissolved inorganic nutrients (nitrate, nitrite, phosphate and silicate) were analysed by standard colorimetric methodology (Grasshoff et al. 1983) as adapted for flow injection analysis (FIA) on a LACHAT Instruments QuickChem 8000 autoanalyser (Hales et al. 2004). Dissolved ammonium was determined by fluorimetric method of Holmes et al. (1999) using a Hitachi F2000 fluorescence spectrophotometer. DON was determined by the subtraction of nitrate and ammonium from the total dissolved nitrogen (TDN) analysed using online peroxodisulfate oxidation coupled with ultraviolet radiation at pH 9.0 and 100°C (Kroon 1993).

All measurements [for both inorganic (nitrate, nitrite, phosphate and silicate) and organic components (DOC, POC, dCHO, dUA and dEPS)] were obtained on all ice sections of each ice core collected for both NSW and ADOM series, but where the term 'bulk ice' is used it refers to average values of all ice sections of ice cores collected on that particular day for each of the NSW and ADOM mesocosms. In order to minimise the effect of dilutions or concentrations occurring during sea ice formation, all data (except when used for discussion of the data set) presented here were normalised to the starting seawater salinity of 33. All statistical analyses except permutational multivariate analysis of variance (PERMANOVA) analysis were conducted on SPSS® 18.0. PERMANOVA for EPS profiles were carried out in Primer 6, data were analysed using Euclidean Distance similarity measures. Significant differences between NSW and ADOM samples were calculated by *t* test. Significant differences between sample types were determined using analysis of variance (ANOVA, with Tukey post hoc tests). Normality of all groups of samples was checked, but the assumption of normality could not be rejected in any case. Standard error (SE) was used as measure of variability for all. Pearson's correlation analysis was used to investigate relationships between different variables. All statistically significant differences quoted are at $P \leq 0.05$.

Results

Temperature, salinity and inorganic nutrients

By the final sampling day (d6), the average ice thickness was 12 cm ± 0.4 (±SE) having grown at a rate of 1.8 cm d^{-1} (±0.1). Water temperatures before ice formation on the first sampling day ranged from 0.3 to 0.7°C, but once ice cover was established (d4); under ice water temperatures remained stable at −1.8 to −1.9°C. Average ice salinities decreased from d4 to d6 in both NSW (12–10.8) and ADOM (13–10.7) series with highest salinities always occurring in the bottom ice sections (Online Resource 2). Brine temperatures decreased from a range of −3.1 to −5.1°C when first measured (on d5) to a range of −5.3 to −6.0°C on the final day (d6). Over the same time interval, brine salinities increased from 71.2–81.2 (d5) to 85.4–90.4 (d6).

At the start of experiment (d0), concentrations of both phosphate and ammonium were significantly higher ($t_{16} = 14.7$ and 9.8 for NH_4^+ and PO_4^{3-}, respectively both at $P < 0.001$) in ADOM seawater in comparison with NSW seawater (Fig. 2). Higher concentrations of phosphate (4–15-fold) and ammonium (2–19-fold) were present in all samples collected from ADOM mesocosms compared to the NSW series, with phosphate and ammonium concentrations varying little in the NSW samples but with decreases in phosphate and increases in ammonium concentrations in the ADOM series over time (Fig. 2).

Variations in the initial concentrations (c.f. d0) of nitrate, nitrite and silicate in the seawater were measured between individual mesocosms, but were not significantly different between the NSW and ADOM series (Fig. 2). Frost flowers had significantly higher concentrations of both nitrite and nitrate (at $P < 0.01$) than the underlying seawater (87- and 11-fold, respectively), sea ice (32- and 3-fold, respectively) and brines (53- and 11-fold, respectively) in both NSW and ADOM series.

Bacterial abundance and dissolved oxygen

Direct microscopic observation found no evidence of significant numbers of autotrophic protists (diatoms or flagellates). Abundances of both flagellates (278.1 ± 22.4 cells ml^{-1}) and ice algae (800 ± 188.6 cells ml^{-1}) were very low in the parent seawater (NSW at d0), and afterwards, the abundance of these taxa decreased progressively during the experiment, especially in the case of ice algae when only empty theca or broken cells were encountered after d3 (Online Resource 3).

The total bacterial numbers (TBN) were significantly ($P < 0.01$) higher in ADOM bags in comparison with NSW treatments, in all samples (seawater, sea ice, brines and frost

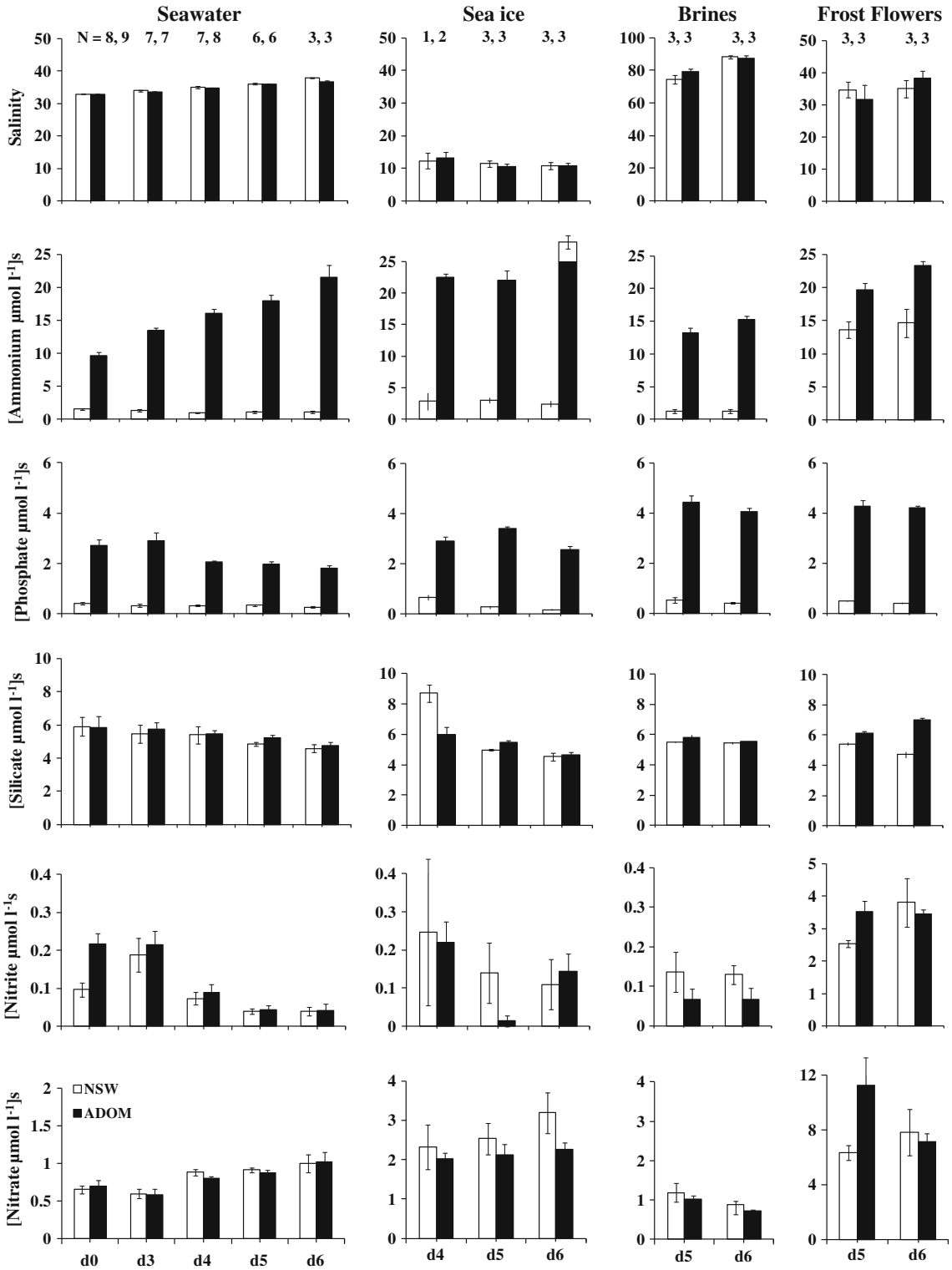

Fig. 2 Salinities and inorganic nutrients (normalised to salinity 33) in seawater, bulk sea ice, brines and frost flowers during experimental ice formation. The numbers (*N*) on the top of the *bars* represent sample number collected on that sampling day. No sampling was done on d1 and d2 (3rd and 4th Oct)

flowers; Fig. 3a–d). In ADOM seawater, TBN were still increasing after 6 days and the ratios of high-DNA/low-DNA bacteria doubled (2.7–6.6) during the last two sampling days (Online Resource 4). In NSW seawater, TBN (both high-DNA and low-DNA bacteria) declined after d5 (Fig. 3a). In both ADOM and NSW seawaters, TBN were negatively

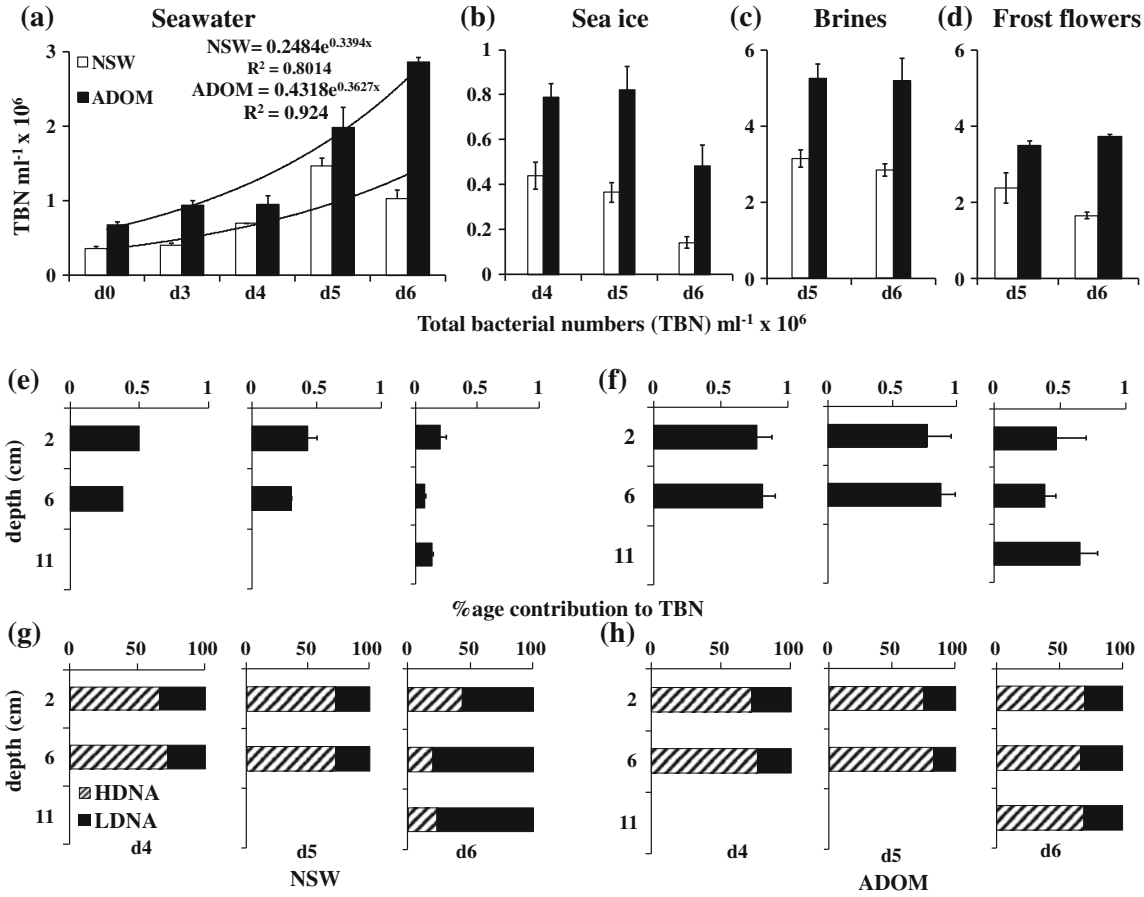

Fig. 3 Total bacterial numbers (TBN) in **a** seawater, **b** bulk sea ice, **c** brines and **d** frost flowers samples on each sampling day. Total bacterial numbers (TBN) along the vertical length of young sea ice (during growth from 0 to 14 cm thickness over sampling days) in **e** NSW and **f** ADOM mesocosms. Percentage contributions of high-DNA and low-DNA bacteria to TBN in **g** NSW and **h** ADOM ice. Except for ice cores collected on d4 ($N = 1$ and 2 for NSW and ADOM, respectively), N (number of samples) was ≥ 3 for all sample types collected from both NSW and ADOM mesocosm on each day

correlated with temperature ($r = 0.56$, $n = 23$ and $r = 0.56$, $n = 24$ at $P < 0.01$ NSW and ADOM seawater, respectively) and positively correlated with salinity ($r = 0.68$, $n = 23$ and $r = 0.91$, $n = 23$ at $P < 0.01$ NSW and ADOM seawater, respectively). In seawater, TBN in both series were also correlated with NO_2 and NO_3 concentration (Online Resource 5).

In bulk ice, TBN significantly (at $P < 0.05$) decreased with decreasing temperatures (Online Resource 2) over the sampling period (Fig. 3b), but this decrease in TBN was greater (2-fold higher) in NSW ice than ADOM ice. On the first ice sampling day (d4), high-DNA bacterial numbers (3.0×10^5 and 5.8×10^5 cells ml^{-1} for NSW and ADOM, respectively) were significantly higher than low-DNA bacterial numbers (1.4×10^5 and 2.0×10^5 cells ml^{-1} for NSW and ADOM, respectively) in both cases, but on d6, there was 6-fold decrease in high-DNA bacterial numbers compared to low-DNA (9.5×10^4 cells ml^{-1}) in NSW ice, as a result the ratio of high-DNA/low-DNA decreased to 0.5. In ADOM sea ice, high-DNA/low-DNA ratios were still high (2.2) on the last sampling day (Online Resource 4).

TBN distribution in the depth layers of growing sea ice was also measured (Fig. 3e–h). In NSW sea ice, TBN were higher in surface ice (5×10^5 cells ml^{-1}), compared to lower sections (3.8×10^5 cells ml^{-1}) on d4 (Fig. 3e), whereas in ADOM sea ice, TBN were homogenously distributed (7.7×10^5 and 8×10^5 cells ml^{-1} for top and bottom ice sections, respectively) throughout the ice (Fig. 3f). The distribution pattern of TBN remained almost the same throughout the experiment in the NSW sea ice, i.e., highest cell numbers in surface ice (Fig. 3e), whereas in ADOM ice, there was a decrease in TBN in the surface sections of the ice on d6 (Fig. 3f).

There were no significant differences in dissolved O_2 concentrations in the seawater in NSW (291 ± 2 µmol l^{-1}) and ADOM (287 ± 2 µmol l^{-1}) mesocosms at the start of the experiment (Online Resource 3). Over the course of the experiment, the O_2 concentrations increased in the under-ice water by 55.9 ± 2 µmol l^{-1} in NSW bags, whereas in ADOM seawater, O_2 concentrations decreased by 17 ± 5 µmol l^{-1} (Online Resource 6).

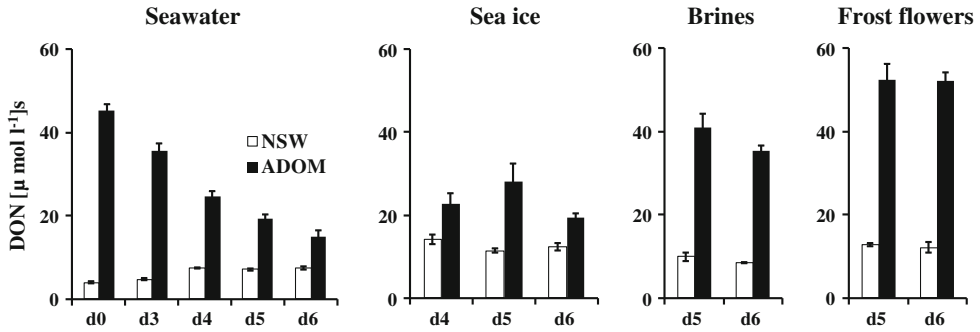

Fig. 4 Salinity-normalised (normalised to salinity 33) dissolved organic nitrogen (DON) in seawater, bulk sea ice, brines and frost flowers samples on each sampling day. Except for ice cores collected on d4 ($N = 1$ and 2 for NSW and ADOM, respectively), N (number of samples) was ≥3 for all sample types collected from both NSW and ADOM mesocosm on each day

Dissolved organic nitrogen (DON) and organic carbon (DOC and POC)

DON was significantly ($t_{12} = 40.8$ at $P < 0.01$) elevated at the start of the experiment (d0) in ADOM, compared to NSW mesocosms (Fig. 4). DON concentrations in the NSW mesocosms increased significantly (at $P < 0.01$) in the seawater by 4.0 ± 0.7 µmol l^{-1}, but changed little in the ice, brine and frost flowers over sampling period. In contrast, DON concentrations in seawater, bulk ice and brines decreased significantly (by 27.7 ± 0.4, 4.6 ± 1.0 and 5.6 ± 4.0 µmol l^{-1} for seawater, bulk ice and brine, respectively) in the ADOM mesocosms.

DOC constituted the major organic carbon fraction (74–85% of TOC) in the seawater and brine samples, whereas in frost flowers, DOC contributed only 48 and 36% of the TOC in NSW and ADOM samples, respectively. In comparison with NSW mesocosms, DOC and POC concentrations were significantly higher ($P < 0.01$) in all ADOM mesocosms at the start of the experiment (d0). Highest DOC levels (at $P < 0.01$) were measured in frost flowers (298.4 ± 12.9 and 520.2 ± 32.5 µmol l^{-1} from NSW and ADOM series, respectively) (Fig. 5a). For bulk ice samples, DOC concentrations decreased significantly (at $P < 0.05$) by 198 ± 21.2 and 161 ± 21.3 µmol l^{-1} in NSW and ADOM mesocosms, respectively, over the sampling period (Fig. 5a). There were also decreases in brine DOC concentrations from both NSW (decrease by 36.9 ± 6.9 µmol l^{-1}) and ADOM (decrease by 45.2 ± 3.6 µmol l^{-1}) series over the whole experiment (Fig. 5a). With these changes in DOC concentrations (except for brines), the ratios of DOC/DON also changed significantly in all groups of samples over the sampling period (Table 1).

POC concentrations were elevated in all samples taken from ADOM mesocosms with POC concentrations being higher ($t_{12} = 3.5$ at $P < 0.01$) in initial seawater (d0) by a factor of 2 (Fig. 5a).

Similar to the patterns showed by DOC, there was little variation in POC concentration in NSW seawater (5.1 ± 4.5 µmol l^{-1}), while there was a significant ($F_{4, 22} = 3.1$ at $P < 0.05$) increase in POC concentration (17.7 ± 12 µmol l^{-1}) in the ADOM water samples (Fig. 5a). POC concentrations increased by 31 ± 20.4 µmol l^{-1} in the ADOM ice, whereas there was a significant ($F_{2, 16} = 16.3$ at $P < 0.001$) decrease of 87 ± 6.4 µmol l^{-1} in the bulk ice from NSW mesocosms (Fig. 5a). In contrast to bulk ice, POC concentrations decreased in brines from both NSW (by 16.3 ± 3 µmol l^{-1}) and ADOM (by 34.5 ± 7 µmol l^{-1}) mesocosm (Fig. 5a).

Dissolved carbohydrates (dCHO and dUA) and different size fraction of dEPS

dCHO concentrations measured in seawater, brines and frost flowers were significantly higher in the ADOM mesocosms ($t = 5.0$–7.2 at $P < 0.05$). Highest concentrations of dCHO (at $P < 0.001$) were measured in bulk sea ice in comparison with all other sample types (Fig. 5b). Increases in dCHO concentrations were observed in bulk ice from both NSW (by 22.6 ± 3 µmol l^{-1}) and ADOM (by 37.0 ± 3.9 µmol l^{-1}) mesocosms (Fig. 5b). dCHO concentrations were correlated with temperature (negative for sea ice, but positive for brines and seawater) and salinity (negative for all samples types) in all samples collected from NSW tanks, whereas no such relationship was observed in samples taken from ADOM mesocosms (Table 2).

Dissolved uronic acids (dUA) concentrations were also significantly higher (at $P < 0.05$) in ADOM samples compared to NSW for all types of samples (Fig. 5b). Except for bulk ice from the NSW mesocosms, there was a gradual increase in dUA concentrations over the sampling period for all groups of samples (Fig. 5b). In seawater, the dUA contribution to dCHO increased significantly (at $P < 0.01$) up to 100% (for both NSW and ADOM) over the sampling period. In bulk ice, the dUA contribution to dCHO increased up to 83% in ADOM mesocosms, whereas in NSW treatments, it was reduced from 59 to 38% (Fig. 5b). In brines, there was also a significant ($P < 0.001$) increase

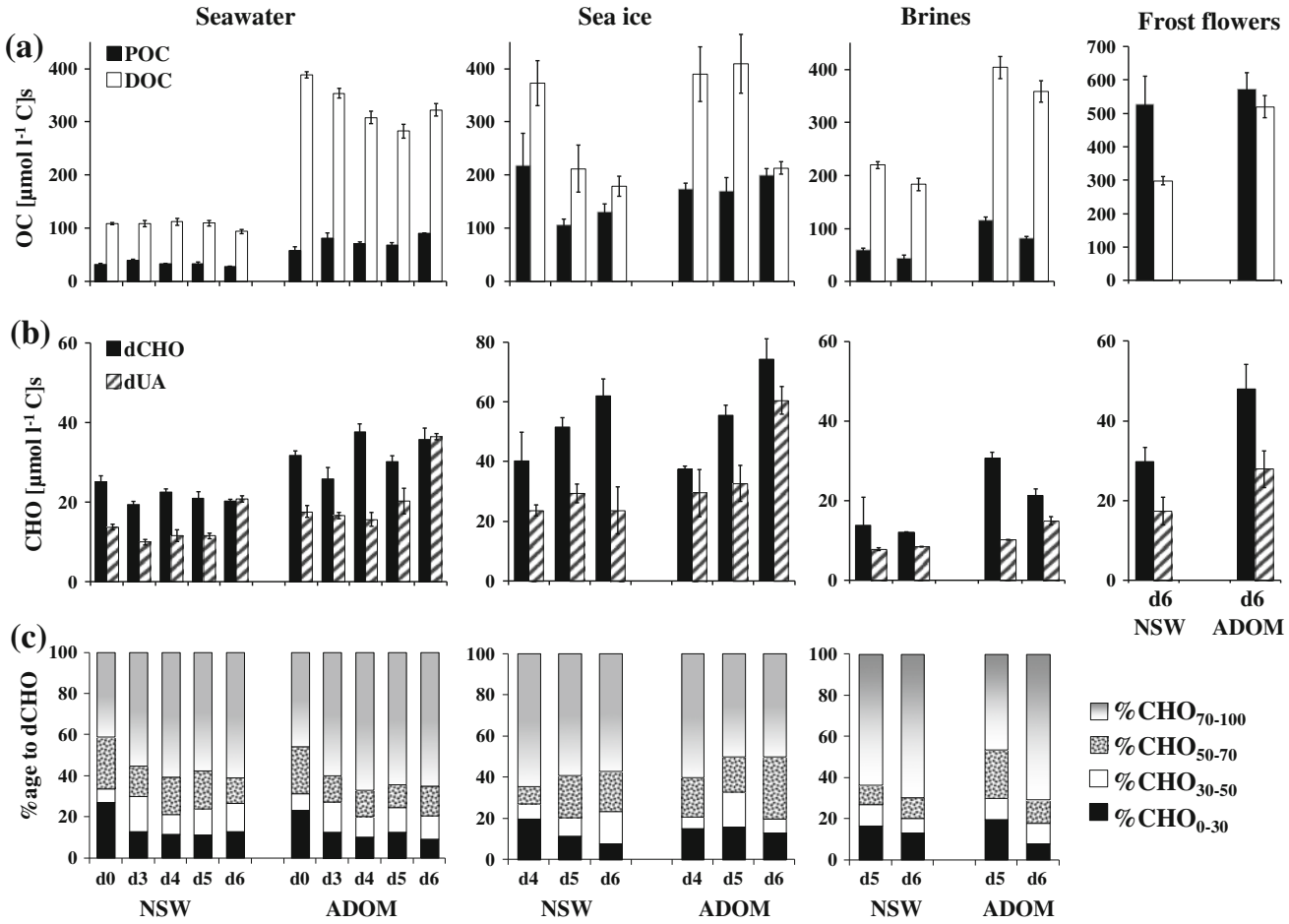

Fig. 5 Salinity-normalised (normalised to salinity 33) **a** organic carbon (POC and DOC), **b** carbohydrate (dCHO, dEPS and dUA) and **c** dEPS ($\%CHO_{0–30}$, $\%CHO_{50–30}$, $\%CHO_{70–50}$ and $\%CHO_{70–100}$) profile in seawater, bulk ice, brines and frost flowers. *Bars* on the left side of each graph represent concentrations in NSW samples, and bars on the right side are concentrations in ADOM mesocosms. Except for ice cores collected on d4 ($N = 1$ and 2 for NSW and ADOM, respectively), N (number of samples) was ≥ 3 for all sample types collected from both NSW and ADOM mesocosm on each day

Table 1 DOC/DON ratios of all groups of samples from sea ice generated in a 6d mesocosm experiment using North Sea water (NSW) and North Sea water enriched with additional algal-derived DOM (ADOM)

Sampling day	Seawater		Sea ice		Brines		Frost flowers	
	NSW	ADOM	NSW	ADOM	NSW	ADOM	NSW	ADOM
d0	27.9	9.0						
d3	23.1	10.0						
d4	15.1	12.5	27.5	17.7				
d5	15.3	12.6	18.0	15.0	22.2	9.8	14.2	10.8
d6	12.6	18.84	14.2	11.9	21.8	10.1	25.5	9.98

in dUA concentrations (34–69% to dCHO) over the sampling period in both NSW and ADOM mesocosms (Fig. 5b), whereas dUA contribution to dCHO was 57% (on day 6) in both NSW and ADOM frost flowers (Fig. 5b).

A significant proportion (>50%) of the dissolved carbohydrate present was composed of dEPS ($\%CHO_{0–30} + \%CHO_{30–50} + \%CHO_{50–70}$) in both NSW and ADOM seawater at the start of the experiment (d0), but dEPS levels decreased (from 59 to 39% and 54 to 33% in NSW and ADOM, respectively), and non-EPS ($\%CHO_{70–100}$) fraction increased (from 41 to 61% and 45 to 67% in NSW and ADOM, respectively) in both NSW and ADOM seawater over the sampling period (Fig. 5c).

Initially (d4), non-EPS carbohydrates ($\%CHO_{70–100}$) were proportionally abundant in bulk ice dCHO from both NSW (64%) and ADOM (60%) mesocosms. By d6, the

Table 2 Correlation between organic carbon (POC and DOC), dissolved carbohydrates (dCHO and dUA), dEPS fractions (EPS-30, EPS-50 and EPS-70), salinity and temperature in seawater, sea ice and brines

Variables	NSW							ADOM						
	DOC	POC	dCHO	EPS-30	EPS-50	EPS-70	dUA	DOC	POC	dCHO	EPS-30	EPS-50	EPS-70	dUA
Seawater														
Temperature	0.169 (29)	−0.181 (29	**0.631**** (29)	**0.877**** (29)	0.663 (29)	0.766 (29)	0.185 (28)	**0.691**** (27)	−0.505 (26)	0.066 (27)	**0.783**** (27)	**0.546**** (27)	**0.719**** (27)	−0.189 (26)
Salinity	−0.310 (31)	−0.309 (28)	**−0.414*** (29)	**−0.600**** (29)	**−0.522**** (29)	**−0.544*** (29)	0.374 (29)	**−0.749**** (33)	0.315 (26)	0.147 (27)	**−0.506**** (26)	**−0.424** (27)	**−0.463** (27)	**−0.574**** (26)
DOC		0.339 (28)	0.148 (29)	0.268 (29)	0.121 (29)	0.218 (29)	0.218 (29)		−0.206 (26)	−0.110 (27)	**0.557**** (27)	**0.527**** (27)	**0.576**** (27)	−0.077 (26)
POC			−0.270 (28)	−0.358 (28)	−0.325 (28)	−0.295 (28)	**−0.546**** (28)			−0.013 (26)	−0.558 (26)	**−0.468*** (26)	0.329 (26)	0.195 (25)
dCHO				**0.711**** (29)	**0.736**** (29)	**0.848**** (29)	0.07 (28)				0.288 (27)	**0.433*** (27)	**0.560*** (27)	0.166 (27)
TBN	−0.004 (23)	−0.100 (23)	0.354 (23)	**−0.430*** (23)	**−0.457*** (23)	**−0.479*** (23)	0.116 (23)	**−0.494*** (21)	**0.458*** (21)	0.103 (21)	**−0.439*** (21)	−0.409 (21)	−0.376 (21)	**0.858**** (20)
Bulk ice														
Temperature	−0.428 (16)	0.255 (15)	**−0.536*** (16)	0.227 (16)	−0.213 (16)	−0.336 (16)	0.174 (14)	0.221 (17)	−0.142 (18)	0.155 (18)	0.103 (18)	0.04 (18)	0.375 (18)	−0.028 (16)
Salinity	−0.072 (14)	0.066 (15)	**−0.739**** (16)	0.233 (16)	−0.322 (16)	−0.388 (16)	−0.102 (14)	0.246 (18)	−0.205 (18)	−0.302 (18)	−0.255 (18)	−0.452 (18)	−0.055 (18)	−0.231 (16)
DOC		**0.669**** (15)	0.113 (16)	0.183 (16)	0.044 (16)	0.202 (16)	−0.095 (14)		−0.266 (17)	−0.403 (17)	−0.397 (17)	−0.248 (17)	0.295 (17)	**−0.622*** (16)
POC			−0.45 (15)	0.105 (15)	0.351 (15)	−0.058 (16)	−0.078 (13)			0.168 (18)	−0.249 (18)	0.227 (18)	0.302 (18)	0.156 (16)
dCHO				−0.372 (16)	**0.563*** (16)	0.604 (16)	0.277 (14)				0.246 (18)	0.303 (18)	**0.608**** (18)	**0.739**** (16)
TBN	**0.635**** (16)	0.313 (15)	−0.256 (16)	0.342 (16)	−0.146 (16)	−0.367 (16)	−0.044 (14)	0.275 (16)	0.076 (18)	−0.400 (18)	−0.270 (18)	0.07 (18)	0.106 (18)	−0.301 (16)
Brines														
Temperature	**0.894*** (6)	0.593 (6)	**0.893*** (5)	**0.938*** (5)	**0.940*** (5)	0.981 (5)	−0.686 (5)	0.221 (6)	0.543 (6)	0.607 (6)	0.677 (6)	0.680 (6)	0.558 (6)	−0.553 (6)
DOC		0.625 (6)	0.815 (5)	**0.952*** (5)	0.809 (5)	0.880 (5)	−0.738 (5)		0.419 (6)	0.657 (6)	0.614 (6)	0.640 (6)	0.662 (6)	−0.639 (6)
Salinity	**−0.829*** (6)	−0.748 (6)	**−0.978**** (5)	**−0.906*** **(5)**	**−0.996**** (6)	**−0.910*** (6)	**0.829*** (6)	−0.560 (6)	−0.756 (6)	**−0.879*** (6)	**−0.896*** (6)	**−0.917*** (6)	**−0.892*** (6)	**−0.728*** (6)
POC			0.832 (5)	0.549 (5)	0.691 (5)	0.640 (5)	−0.732 (5)			**0.824*** (6)	**0.906*** (6)	**0.933**** (6)	**0.906*** (6)	**−0.92**** (6)
dCHO				0.873 (5)	**0.975**** (5)	0.866 (5)	−0.945 (5)				**0.979**** (6)	**0.935**** (6)	**0.951**** (6)	−0.786 (6)
TBN	−0.394 (7)	0.443 (6)	0.192 (5)	−0.102 (5)	0.018 (5)	0.241 (5)	0.205 (5)	−0.472 (6)	0.233 (6)	0.063 (6)	0.062 (6)	−0.047 (6)	0.006 (6)	−0.001 (6)

Values in bold are significant at $P < 0.05$* or < 0.01**

proportion of dEPS increased to almost parity with non-EPS fractions in both cases (Fig. 5c). In NSW sea ice, the percentage contribution of highly complex dEPS fraction ($\%CHO_{0–30}$) decreased to half (20–10%), whereas less complex EPS fraction ($\%CHO_{50–70}$) showed a significant increase (8–22%) on d5 (Fig. 5c). In general, there was an increase in dEPS contribution to dCHO over sampling period in both NSW and ADOM bulk ice, but these dEPS were mainly the less complex fractions ($\%CHO_{30–50}$ and $\%CHO_{50–70}$). Both dEPS ($\%CHO_{0–30}$, $\%CHO_{30–50}$ and $\%CHO_{50–70}$) and non-EPS ($\%CHO_{70–100}$) carbohydrate fractions did not show any significant temporal variation in NSW brines, whereas in ADOM brines contribution of highly complex EPS ($\%CHO_{0–30}$) decreased (3-fold) significantly and less complex EPS ($\%CHO_{50–70}$) and non-EPS ($\%CHO_{70–100}$) increased. Concentrations of dEPS fractions, in particular highly complex EPS (EPS-30) were significantly correlated with temperature and salinity in both seawater and brines (Table 2). Highly complex EPS (EPS-30) fraction was also negatively correlated with TBN in seawater (Table 2). In general, dEPS fractions (especially EPS-70) were correlated with dCHO for all sample types collected from NSW and ADOM mesocosms (Table 2).

Partitioning of POC, DOC, dCHO and dEPS within different depth layers of sea ice

Complete ice vertical sections (top to bottom) from each sampling day were analysed to study the partitioning of organic carbon (both DOC and POC), dissolved carbohydrates (dCHO and dUA) and ethanol solubility-based dEPS fractions in the growing sea ice (Fig. 6a–c). Except for ADOM sea ice on d5, DOC and POC showed identical distribution patterns in the ice. Initially (d4), dCHO concentrations were higher in surface ice sections in both series (Fig. 6b) and no significant changes were observed in NSW ice afterwards, while highest dCHO concentrations were measured in the middle ice sections in the ADOM ice on final sampling day (d6). The highest dUA concentrations were measured in bottom ice sections for both NSW (36 ± 13.6 µmol l^{-1}) and ADOM (62.1 ± 12 µmol l^{-1}) samples on the final sampling day. A 3-way PERMANOVA

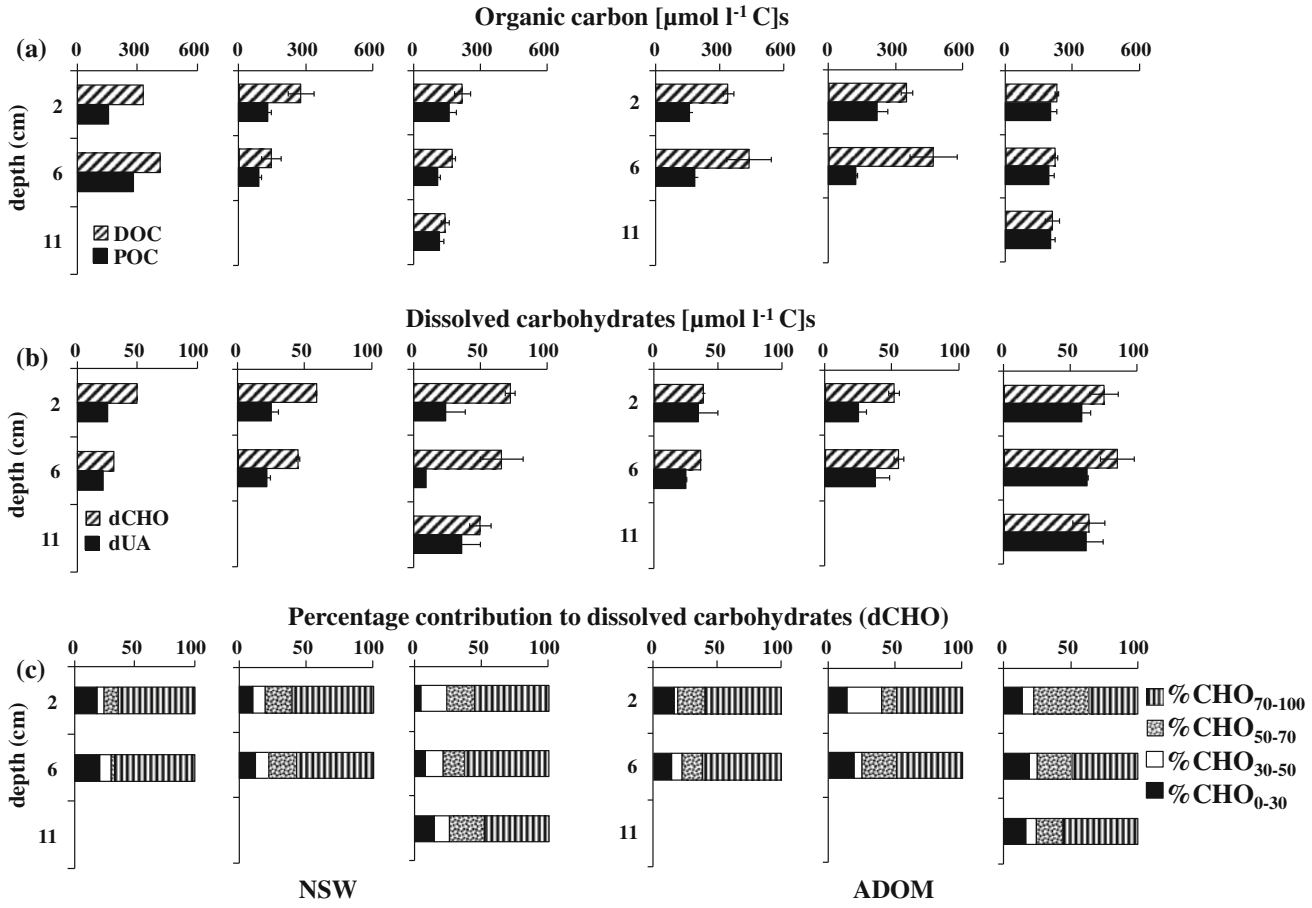

Fig. 6 Vertical profile of salinity-normalised (normalised to salinity 33) **a** organic carbon (POC and DOC), **b** carbohydrates (dCHO and dUA) and **c** dEPS profile in ice cores collected on each sampling day. Except for d4 ($N = 1$ and 2 for NSW and ADOM, respectively on d4), 3 ice cores were collected on each sampling day for both NSW and ADOM series and average of 3 ice cores was used for all graphs

analysis revealed that the profile of dEPS fractions in sea ice underwent significant changes over the sampling period ($F_{2,\ 26} = 3.2$ at $P < 0.05$) in both NSW and ADOM ($F_{1,\ 26} = 3.7$ at $P < 0.05$) treatments (Online Resource 5). SIMPER (Percentage similarities) revealed that these changes in dEPS profile were mainly due to increase in less complex EPS ($\%CHO_{50–70}$) and decrease in non-EPS ($\%CHO_{70–100}$) fractions in both NSW and ADOM series.

Physicochemical effects of changing salinity on the partitioning of organic carbon (DOC and POC) and carbohydrates (dCHO, dUA and dEPS fractions) in growing sea ice were also studied by plotting the salinity-normalised data against changing salinity over the sampling period (c.f. Gleitz et al. 1995). Partitioning of all the parameters studied deviated from the expected physical conservative concentration due to freezing (Online Resource 7). Overall (except for frost flowers from ADOM series), DOC concentrations declined below the levels expected due to physical concentration, while POC, dCHO, dUA and EPS-70 were enriched in both NSW and ADOM series (ESM_7).

Discussion

Bacterial abundance and activity

The aim of this experiment was to follow the partitioning of different organic carbon fractions together with the abundances of bacteria incorporated during the early stages of sea ice formation, using both unmodified seawater and seawater with a substantially enhanced DOC loading. The natural bacterial population in the water from the North Sea (which does not experience any extensive winter freezing) was able to grow in seawater at freezing point and were also presumably active in the newly formed sea ice as indicated by constant ratios of high-DNA/low-DNA bacteria with average temperatures of −7.5°C, particularly when additional organic substrates were added (ADOM treatment). This result is not surprising since in seasonally ice-covered non-polar seas (e.g. Baltic, Caspian Seas), ice forms over parent water that had reached the temperatures as high as +20°C during summer, yet psychrophilic ice communities emerge from that parent water (Kaartokallio et al. 2005, 2008). Although the North Sea does not have a direct history of ice, presumably, there are psychrophilic or psychrotolerant members in the bacterial communities that thrive in cold water during the winter months.

Decreases in bacterial abundance during the initial phases of ice formation in a natural sea ice system have been reported by Kaartokallio (2004) and have been considered to be a result of a gradual community shift towards an ice-adapted bacterial community (Collins et al. 2010; Deming 2010). Such a shift may be a possible explanation for observed decrease in ice bacterial abundances in the end of the experiment. Underlying seawater oxygen concentrations decreased significantly in ADOM bags, indicating high bacterial respiration rate (exceeding inputs of O_2 due to overlying ice formation). In NSW, increases in seawater O_2 concentrations, probably due to sea ice degassing and brine drainage, exceeded any decreases due to bacterial respiration.

Temperature and substrate concentrations were suggested by Pomeroy and Wiebe (2001) to act as interactive limiting factors for bacterial growth in cold environments, with abundant substrate compensating for cold temperature to a certain extent. This concept seems to be corroborated by our results, as substrate addition led to increased bacterial abundance as well as higher fractions of metabolically active, high-DNA bacteria in the bacterial population in the ADOM water. The increases in TBN in seawater were coupled to increases in NH_4^+ (due to the active metabolism of DON) and decreases in DOC concentration (Arrigo et al. 1995; Gleitz et al. 1995; Kattner et al. 2004), strongly indicating that the ADOM served as substrate and fuelled the growth of microbial assemblages in seawater (Junge et al. 2002; Brinkmeyer et al. 2003).

Negative correlation between TBN and temperature could be due to the temporal development of an ice-adapted bacterial community with the cooling of water. TBN were elevated in the ADOM treatments, with overall highest bacterial numbers present in the ADOM brines. These results reflect the spatial partitioning of cells into the brine channels (Junge et al. 2001), which in general occupy 8–10% of ice volume depending on temperature (Petrich and Eicken 2010). Bacterial abundance was lower in the forming ice than underlying seawater, with evidence of the establishment of heterotrophic bacterial community in the bottom ice segments (Deming 2010). There were gradual decreases in POC and DOC concentrations and changing characteristics of the EPS, along with highest TBN being recorded in bottom ice sections in the ADOM series. Greatest biological activity in bottom layers of ice is due to the close proximity to seawater, high rates of nutrient exchange with the underlying seawater, warmest temperatures and lowest fluctuations in salinity, making this zone a favourable habitat for microbial growth (Horner et al. 1992). Biological activity in bottom layers of ice is usually largely associated with ice algae and other protists (Arrigo et al. 2010), but probably because the experimental system was kept in darkness (as part of the experimental design), there was no evidence of any ice algae or protists growth in sea ice or seawater.

Partitioning and transformation of organic carbon

DOC concentration decreased with a subsequent increase in POC concentration in ADOM seawater, and these changes

in organic carbon were a result of increase in bacterial biomass, as indicated by the significant correlations of TBN with organic carbon (negative correlation with DOC and positive correlation with POC). An opposite trend was observed in samples collected from NSW mesocosms where a decrease was observed in both DOC and POC levels over the sampling period, which is indicative of decline in net heterotrophy (Deming 2010).

Dissolved carbohydrate (dCHO) concentrations were higher in sea ice than in the other types of samples, including brines and frost flowers, indicating that dCHO were retained in sea ice as described by other studies (Ewert and Deming 2011; Juhl et al. 2011). A major proportion of the dCHO was composed of acidic sugars (uronic acids), similar to field samples of Antarctic sea ice (Underwood et al. 2010). Dissolved uronic acid (dUA) concentrations showed large increases during the experiment, contributing up to 100% of the dCHO in seawater in both NSW and ADOM series. dUA are a major constituent of bacterial extracellular polysaccharides (Sutherland 1990), and production of uronic acids has also been reported to increase with increasing cold conditions (Mancuso Nichols et al. 2005). Hence, increases in uronic acid concentrations in this study could be evidence of bacterial adaptation to survive under extreme temperature condition in sea ice by the production of uronic acid-rich EPS. Our data support the hypothesis that developing microbial populations in the ice matrix utilised available DOM and produced new, chemically different DOM (Riedel et al. 2007). This interpretation is supported by the strong coupling of TBN with dUA concentrations in ADOM seawater and especially in the bottom ice layers where dUA concentrations increased up to 95% of the total dCHO pool. Changes in the DOM profiles observed in this study are related purely with bacterial activity as there was no evidence of either ice algal growth generating primary productivity or a sufficiently dense flagellate population whose grazing could control bacterial growth or create significant organic carbon transfer from bacteria to flagellates.

It is possible to fractionally precipitate a charged polymer (e.g. DNA and polysaccharides) based on its degree of polymerisation (complexity) by controlling the polarity of the solvent. A highly complex polymer precipitates in solvent at low polarity, while less complex polymers require high solvent polarity for precipitation. Hence, highly complex EPS are less soluble in a polar solvent (ethanol used for this study) and will precipitate with low concentration of solvent (30% ethanol in this case) in comparison with less complex EPS, which require high concentration of solvent (70% ethanol in this case) for precipitation (Aspinall 1982; Underwood et al. 2004, 2010). Sea ice bacteria are known to produce EPS (Mancuso Nichols et al. 2005), and such production is evident from the increases in dCHO and the significant increases in the proportions of dEPS (and decreases in $\%CHO_{70\text{-}100}$) in the bulk ice samples. In addition to production by bacterial communities, the increased dEPS concentration in sea ice could also be linked with cell lysis as TBN progressively decreased in growing sea ice. As a result of cell lysis, both intracellular and extracellular polysaccharide might have added to the dEPS pool in growing sea ice.

Higher concentrations of dEPS were measured in colder ice (surface ice sections). This increased production of dEPS in colder ice can be interpreted as an effort of psychrophilic bacteria to maintain a buffered environment to protect them against extreme salinity and temperature (Krembs and Deming 2008; Bowman and Deming 2010; Underwood et al. 2010; Ewert and Deming 2011; Krembs et al. 2011). Complex EPS do not easily drain into brine samples (Krembs et al. 2002; Underwood et al. 2010); hence, the brine samples contained predominantly $\%CHO_{70\text{–}100}$ and also DOM < 8 kD in size. This latter material was excluded from the carbohydrates (measurements during the course of study due to the necessary dialysis step (Underwood et al. 2010), which explains the low EPS and CHO concentrations measured in the brines, despite high DOC concentrations.

Frost flowers

Frost flowers developed on the surface of the developing sea ice, as is common on actively forming cold ice (Rankin et al. 2002; Kaleschke et al. 2004; Shaw et al. 2010). Frost flowers are formed by the upward transport of brine from the ice interior towards the surface (Style and Worster 2009) with subsequent evaporation of water. TBN in frost flowers were in the same order of magnitude as in the brines, but were several fold higher than in the bulk ice mainly because bacteria present in brines were concentrated into frost flowers as described by Bowman and Deming (2010). Upward transportation of nutrients from within sea ice to frost flowers was evident given the higher concentrations of NH_4^+, NO_3^- and NO_2^- measured in top ice sections. High nitrate and nitrite levels would have some implications to aerosol release from ice surfaces or melting sea ice (Shaw et al. 2010) as the formation of bromine nitrates can substantially modify the availability of reactive bromine (Piot and von Glasow 2007).

In frost flowers, both DOC (up to 3-fold) and POC (up to 18-fold) were elevated in comparison with seawater, sea ice and brines. These elevated levels of organic carbon and very high TBN were as recorded for frost flowers by Bowman and Deming (2010). Cell lysis is a non-probable source for the observed elevated DOC concentrations as all bacterial carbon present in frost flowers equals approximately 6–8 µmol C l^{-1} (calculated with a conservative

estimate of 35–40 fg C cell^{-1}), which is approximately 50–65 times lower than measured DOC concentrations (Fukuda et al. 1998). It would appear that the physical supply of organic and inorganic nutrients to frost flowers establishes them as potential sites of significant biogeochemical activity.

Conclusions

The addition of biolabile DOM in the ADOM treatment stimulated bacteria, supporting the view that bacteria in sea ice systems can be either limited or co-limited (Pomeroy and Wiebe 2001; Kuosa and Kaartokallio 2006) by the availability of biolabile organic substrate. In the early stages of ice formation, allochthonous organic carbon (DOC and POC) was incorporated from parent seawater into sea ice. Once ice formation was established, there were significant changes in the concentrations of organic carbon and the production of autochothonous DOM. This DOM appeared to be produced by a cold-adapted bacterial community that became established in sea ice (indicated by the production of dUA and dCHO) as well as the degradation of the NSW and algal-derived DOM (as indicated by the inorganic nutrient dynamics). Addition of algal-derived DOM continued to fuel the bacterial communities in ADOM seawater; as a result, bacterial communities were able to thrive in comparison with NSW treatment where bacterial growth declined after the first 5d. In ADOM sea ice, the complexity of dEPS produced by the bacteria was clearly linked with low temperature and high salinity since the highest concentrations and greatest contribution of the most complex fractions of dEPS were in the coldest and most saline surface ice sections. These results suggest that a psychrophilic community was established in growing sea ice possibly structured differently than the community in NSW that produced high concentrations of complex dEPS, suggesting that EPS play important roles of buffering and cryo-protection for microorganisms against harsh environmental conditions (low temperature and high salinity) in sea ice.

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References

Arrigo KR, Dieckmann G, Gosselin M, Robinson DH, Fritsen CH, Sullivan CW (1995) High resolution study of the platelet ice ecosystem in McMurdo Sound, Antarctica: biomass, nutrient, and production profiles within a dense microalgal bloom. Mar Ecol Prog Ser 127:255–268. doi:10.3354/meps127255

Arrigo KR, Mock T, Lizotte MP (2010) Primary producers in sea ice. In: Thomas DN, Dieckmann GS (eds) Sea ice, 2nd edn. Blackwell, Oxford, pp 283–325

Aspinall GO (1982) The polysaccharides, vol 1. Academic Press, New York

Bellinger BJ, Abdullahi AS, Gretz MR, Underwood GJC (2005) Biofilm polymers: relationship between carbohydrate biopolymers from estuarine mudflats and unialgal cultures of benthic diatoms. Aquat Microb Ecol 38:169–180

Bitter T, Muir HM (1962) A modified uronic acid carbazole reaction. Anal Biochem 4:330–334. doi:10.1016/0003-2697(62)90095-7

Bowman JS, Deming JW (2010) Elevated bacterial abundance and exopolymers in saline frost flowers and implications for atmospheric chemistry and microbial dispersal. Geophys Res Lett 37:L13501. doi:10.1029/2010GL043020

Brierley AS, Thomas DN (2002) Ecology of Southern Ocean pack ice. Adv Mar Biol 43:171–276. doi:10.1016/S0065-2881(02)43005-2

Brinkmeyer R, Knittel K, Jürgens J, Weyland H, Amann R, Helmke E (2003) Diversity and structure of bacterial communities in Arctic versus Antarctic pack ice. Appl Environ Microbiol 69:6610–6619. doi:10.1128/AEM.69.11.6610-6619.2003

Caron DA, Gast RJ (2010) Heterotrophic protists associated with sea ice. In: Thomas DN, Dieckmann GS (eds) Sea ice, 2nd edn. Blackwell, Oxford, pp 327–356

Clarke DB, Ackley SF (1984) Sea ice structure and biological activity in the Antarctic marginal ice zone. J Geophys Res 89:2087–2095

Collins RE, Rocap G, Deming JW (2010) Persistence of bacterial and archaeal communities in sea ice through an Arctic winter. Environ Microbiol 12:1828–1841

Cota GF, Prinsenberg SJ, Bennett EB, Loder JW, Lewis MR, Anning JL, Watson NHF, Harris LR (1987) Nutrient fluxes during extended blooms of arctic ice algae. J Geophys Res 92:1951–1962. doi:10.1029/JC092iC02p01951

Deming JW (2010) Sea ice bacteria and viruses. In: Thomas DN, Dieckmann GS (eds) Sea ice, 2nd edn. Blackwell, Oxford, pp 247–282

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356

Dumont I, Schoemann V, Lannuzel D, Chou L, Tison JL, Becquevort S (2009) Distribution and characterization of dissolved and particulate organic matter in Antarctic pack ice. Polar Biol 32:733–750

Engel N, Jenny TA, Mooser V, Gossauer A (1991) Chlorophyll catabolism in *Chlorella prototheocoides*—isolation and structure elucidation of a red bilin derivative. FEBS Lett 293:131–133

Ewert MEM, Deming JW (2011) Selective retention in saline ice of extracellular polysaccharides produced by the cold-adapted marine bacterium *Colwellia psychrerythraea* strain 34H. Ann Glaciol 52:111–117

Fukuda R, Ogawa H, Nagata T, Koike I (1998) Direct determination of carbon and nitrogen contents of natural bacterial assemblages in marine environments. Appl Environ Microbiol 64:3352–3358

Garrison DL, Close AR, Gordon LI (1990) Nutrient concentrations in Antarctic pack ice during the austral winter. In: Ackley SF, Weeks WF (eds) Sea ice properties and processes. Proceedings of W. F. weeks sea ice symposium, CRREL, Monograph 90–91, pp 35–40

Gasol JM, Zweifel UL, Peters F, Fuhrman JA, Hagstrom A (1999) Significance of size and nucleic acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria. Appl Environ Microbiol 65:4475–4483

Giannelli V, Thomas DN, Haas C, Kattner G, Kennedy H, Dieckmann GS (2001) Behaviour of dissolved organic matter and inorganic nutrients during experimental sea ice formation. Ann Glaciol 33:317–321. doi:10.3189/172756401781818572

Gleitz M, von der loeff MR, Thomas DN, Dieckmann GS, Millero FJ (1995) Comparison of summer and winter inorganic carbon, oxygen and nutrient concentrations in antarctic sea-ice brine. Mar Chem 51:81–91

Grasshoff K, Ehrhardt M, Kremling K (1983) Methods of seawater analysis. Verlag Chemie, Weinheim

Hales B, van Geen A, Takahashi T (2004) High-frequency measurement of seawater chemistry: flow-injection analysis of macronutrients. Limnol Oceanogr Meth 2:91–101

Helmke E, Weyland H (1995) Bacteria in sea-ice and underlying water of the eastern Weddell Sea in midwinter. Mar Ecol Prog Ser 117:269–287

Herborg LM, Thomas DN, Kennedy H, Haas C, Dieckmann GS (2001) Dissolved carbohydrates in Antarctic sea ice. Antarct Sci 13:119–125

Hoagland KD, Rosowski JR, Gretz MR, Roemer SC (1993) Diatom extracellular polymeric substances—function, fine-structure, chemistry, and physiology. J Phycol 29:537–566

Hofmann T, Hanlon ARM, Taylor JD, Ball AS, Osborn AM, Underwood GJC (2009) Dynamics and compositional changes in extracellular carbohydrates in estuarine sediments during degradation. Mar Ecol Prog Ser 379:45–58

Holmes RM, Aminot A, Kerouel R, Hooker BA, Peterson BJ (1999) A simple and precise method for measuring ammonium in marine and freshwater ecosystems. Can J Fish Aquat Sci 56:1801–1808. doi:10.1139/cjfas-56-10-1801

Horner R, Ackley SF, Dieckmann GS, Gulliksen B, Hoshiai T, Legendre L, Melnikov IA, Reeburgh WS, Spindler M, Sullivan CW (1992) Ecology of sea ice biota. Polar Biol 12:417–427

Hortensteiner S, Chinner J, Matile P, Thomas H, Donnison IS (2000) Chlorophyll breakdown in *Chlorella protothecoides*: characterization of degreening and cloning of degreening-related genes. Plant Mol Biol 42:439–450

Juhl AR, Krembs C, Meiners KM (2011) Seasonal development and differential retention of ice algae and other organic fractions in first-year Arctic sea ice. Mar Ecol Prog Ser 436:1–16. doi:10.3354/meps09277

Junge K, Krembs C, Deming J, Stierle A, Eicken H (2001) A microscopic approach to investigate bacteria under in situ conditions in sea-ice samples. Ann Glaciol 33:304–310. doi:10.3189/172756401781818275

Junge K, Imhoff F, Staley T, Deming JW (2002) Phylogenetic diversity of numerically important arctic sea-ice bacteria cultured at subzero temperature. Microb Ecol 43:315–328

Kaartokallio H (2004) Food web components, and physical and chemical properties of Baltic Sea ice. Mar Ecol Prog Ser 273:49–63

Kaartokallio H, Laamanen M, Sivonen K (2005) Responses of Baltic Sea ice and open-water natural bacterial communities to salinity change. Appl Environ Microbiol 71:4364–4371

Kaartokallio H, Tuomainen J, Kuosa H, Kuparinen J, Martikainen PJ, Servomaa K (2008) Succession of sea-ice bacterial communities in the Baltic Sea fast ice. Polar Biol 31:783–793

Kaleschke L, Richter A, Burrows J, Afe O, Heygster G, Notholt J, Rankin AM, Roscoe HK, Hollwedel J, Wagner T, Jacobi HW (2004) Frost flowers on sea ice as a source of sea salt and their influence on tropospheric halogen chemistry. Geophys Res Lett 31:L16114. doi:10.1029/2004GL020655

Kattner G, Thomas DN, Haas C, Kennedy H, Dieckmann GS (2004) Surface ice and gap layers in Antarctic sea ice: highly productive habitats. Mar Ecol Prog Ser 277:1–12

Krembs C, Deming JW (2008) The role of exopolymers in microbial adaptation to sea-ice. In: Margesin R, Schinner F, Marx J-C, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, pp 247–264

Krembs C, Eicken H, Junge K, Deming JW (2002) High concentrations of exopolymeric substances in Arctic winter sea ice: implications for the polar ocean carbon cycle and cryoprotection of diatoms. Deep Sea Res I 49:2163–2181

Krembs C, Eicken H, Deming JW (2011) Exopolymer alteration of physical properties of sea ice and implications for ice habitability and biogeochemistry in a warmer Arctic. Proc Natl Acad Sci USA 108:3653–3658

Kroon H (1993) Determination of nitrogen in water—comparison of a continuous-flow method with online UV digestion with the original kjeldahl method. Anal Chim Acta 276:287–293

Kuosa H, Kaartokallio H (2006) Experimental evidence on nutrient and substrate limitation of Baltic Sea sea-ice algae and bacteria. Hydrobiologia 554:1–10

Mancuso Nichols C, Bowman JP, Guezennec J (2005) Effects of incubation temperature on growth and production of exopolysaccharides by an Antarctic sea ice bacterium grown in batch culture. Appl Environ Microbiol 71:3519–3523

McConville MJ, Wetherbee R, Bacic A (1999) Subcellular location and composition of the wall and secreted extracellular sulphated polysaccharides/proteoglycans of the diatom Stauroneis amphioxys Gregory. Protoplasma 206:188–200

Meiners K, Gradinger R, Fehling J, Civitarese G, Spindler M (2003) Vertical distribution of exopolymer particles in sea ice of the Fram Strait (Arctic) during autumn. Mar Ecol Prog Ser 248:1–13

Meiners K, Krembs C, Gradinger R (2008) Exopolymer particles: microbial hotspots of enhanced bacterial activity in Arctic fast ice (Chukchi Sea). Aquat Microb Ecol 52:195–207

Mock T, Thomas DN (2005) Recent advances in sea-ice microbiology. Environ Microbiol 7:605–619

Norman L, Thomas DN, Stedmon CA, Granskog MA, Papadimitriou S, Krapp RH, Meiners KM, Lannuzel D, van der Merwe P, Dieckmann GS (2011) The characteristics of dissolved organic matter (DOM) and chromophoric dissolved organic matter (CDOM) in Antarctic sea ice. Deep Sea Res II 58:1075–1091

Papadimitriou S, Thomas DN, Kennedy H, Haas C, Kuosa H, Krell A, Dieckmann GS (2007) Biogeochemical composition of natural sea ice brines from the Weddell Sea during early austral summer. Limnol Oceanogr 52:1809–1823

Petrich C, Eicken H (2010) Growth, structure and properties of sea ice. In: Thomas DN, Dieckmann GS (eds) Sea ice, 2nd edn. Blackwell, Oxford, pp 23–77

Piot M, von Glasow R (2007) The potential importance of frost flowers, recycling on snow, and open leads for ozone depletion events. Atmos Chem Phys Discuss 7:4521–4595. doi:10.5194/acpd-7-4521-2007

Pomeroy LR, Wiebe WJ (2001) Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. Aquat Microb Ecol 23:187–204

Qian JG, Mopper K (1996) Automated high performance, high-temperature combustion total organic carbon analyzer. Anal Chem 68:3090–3097

Rankin AM, Wolff EW, Martin S (2002) Frost flowers: implications for tropospheric chemistry and ice core interpretation. J Geophys Res 107:4683. doi:10.1029/20021JD002492

Riedel A, Michel C, Gosselin M (2006) Seasonal study of sea-ice exopolymeric substances on the Mackenzie shelf: implications

for transport of sea-ice bacteria and algae. Aquat Microb Ecol 45:195–206

Riedel A, Michel C, Gosselin M, Leblanc B (2007) Enrichment of nutrients, exopolymeric substances and microorganisms in newly formed sea ice on the Mackenzie shelf. Mar Ecol Prog Ser 342:55–67

Shaw PM, Russell LM, Jefferson A, Quinn PK (2010) Arctic organic aerosol measurements show particles from mixed combustion in spring haze and from frost flowers in winter. Geophys Res Lett 37:L10803. doi:10.1029/2010GL042831

Stedmon CA, Thomas DN, Granskog M, Kaartokallio H, Papadimitriou S, Kuosa H (2007) Characteristics of dissolved organic matter in Baltic coastal sea ice: Allochthonous or autochthonous origins? Environ Sci Technol 41:7273–7279

Stewart FJ, Fritsen CH (2004) Bacteria–algae relationships in Antarctic sea ice. Antarct Sci 16:143–156

Style RW, Worster MG (2009) Frost flower formation on sea ice and lake ice. Geophys Res Lett 36:L11501. doi:10.1029/2009GL037304

Sutherland IW (1990) Biotechnology of microbial exopolysaccharides. Cambridge University Press, Cambridge

Thomas DN, Kattner G, Engbrodt R, Giannelli V, Kennedy H, Haas C, Dieckmann GS (2001) Dissolved organic matter in Antarctic sea ice. Ann Glaciol 33:297–303. doi:10.3189/172756401781818338

Tranvik LJ (1990) Bacterioplankton growth on fractions of dissolved organic-carbon of different molecular-weights from humic and clear waters. Appl Environ Microbiol 56:1672–1677

Underwood GJC, Boulcott M, Raines CA, Waldron K (2004) Environmental effects on exopolymer production by marine benthic diatoms: dynamics, changes in composition, and pathways of production. J Phycol 40:293–304

Underwood GJC, Fietz S, Papadimitriou S, Thomas DN, Dieckmann GS (2010) Distribution and composition of dissolved extracellular polymeric substances (EPS) in Antarctic sea ice. Mar Ecol Prog Ser 404:1–19

van der Merwe P, Lannuzel D, Nichols CAM, Meiners K, Heil P, Norman L, Thomas DN, Bowie AR (2009) Biogeochemical observations during the winter-spring transition in East Antarctic sea ice: evidence of iron and exopolysaccharide controls. Mar Chem 115:163–175

Volkman JK, Tanoue E (2002) Chemical and biological studies of particulate organic matter in the ocean. J Oceanogr 58:265–279. doi:10.1023/A:1015809708632