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Exopolymer particles: microbial hotspots of enhanced bacterial activity in Arctic fast ice (Chukchi Sea)

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ABSTRACT: Sea ice is an important structuring element of Arctic marine ecosystems and provides a vast low-temperature habitat for ice-associated bacteria. While it is now known that sea ice sequesters large amounts of extracellular polymeric substances (EPS) contributing significantly to its particulate organic carbon pool, the ecological role of EPS in sea ice is poorly understood. Using *in situ* incubations combined with a newly developed triple-staining method (Alcian Blue, DAPI, CTC), we determined the number of CTC-reducing (i.e. actively respiring) sea-ice bacteria living freely or attached to gel-like exopolymer particles. Samples were collected at 6 depths from Chukchi Sea coastal fast ice in April, May and June 2003. Concentrations of exopolymer particles ranged between 1.8×10^6 and 149.1×10^6 particles l^{-1} (average 4.7×10^6 particles l^{-1}) and showed strong vertical gradients with maximum concentrations at the ice-water interface. Total bacterial numbers (TBN) ranged from 0.18×10^9 to 8.48×10^9 cells l^{-1} with an average fraction of 7.4% of actively respiring cells (range 3.0 to 17.2% of TBN). The attached bacterial fraction (range 4.6 to 28.5%, average 15.0% of TBN) showed a significantly, approximately 4 times higher proportion of actively respiring cells (average 19.6%, range 7.8 to 37.6%) when compared to the free-living fraction that had an average of 5.4% (range 1.1 to 11.2%) of actively respiring cells. In conclusion, exopolymer particles in sea ice are microbial hotspots of increased bacterial activity able to foster enhanced biogeochemical cycling.

KEY WORDS: Sea ice · Arctic · Exopolymer particles · Extracellular polymeric substances · Particle colonisation · Bacterial activity · CTC

INTRODUCTION

Sea ice is an important structuring component of Arctic marine ecosystems and provides a habitat for microbial communities consisting of bacteria, fungi, algae and heterotrophic protists (Gradinger 2002, Thomas & Dieckmann 2003). The biomass of the communities is generally dominated by diatoms, whereas bacteria dominate in terms of abundance (Mock & Thomas 2005). The inhabitable portion of sea ice is limited to liquid-filled brine inclusions which form a mostly interconnected brine-channel system. The ice-associated organisms are adapted to the harsh and highly variable environmental conditions of their habitat (Zhang et al. 1998, Krembs et al. 2000, Gradinger 2002). The concentration of salts and other solutes in sea-ice brine is controlled by the ice temperature; the brine volume fraction is a function of both sea-ice temperature and bulk solute concentration (Weeks & Ackley 1982). Sea ice in early spring is characterised by steep gradients in ice temperature and corresponding gradients in brine salinity, inorganic and organic solutes, and brine volume. Surface temperatures of Arctic winter sea ice (March) can be as low as –20°C with corresponding brine salinities of >150 (Junge et al. 2004), whereas the ice-water interface remains at the freezing point of seawater (–1.9°C). During spring/summer melt, sea ice shows isothermal vertical tem-

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perature profiles, it becomes increasingly permeable, and meltwater from the surface dilutes the brine and percolates through the ice.

Arctic sea ice harbours copious amounts of extracellular polymeric substances (EPS) occurring both in the dissolved fraction and as gel-like, so-called exopolymer particles (EP) in the particulate fraction (Krembs & Engel 2001, Krembs et al. 2002, Meiners et al. 2003, Riedel et al. 2006). EPS can contribute up to 30% of the particulate organic carbon pool of Arctic sea ice (Krembs et al. 2002, Meiners et al. 2003). Close relationships between sea-ice algal biomass and EPS concentrations as well as estimates of bacterial and algal cell-specific EPS production rates suggest that diatoms are the main EPS producers in the sea-ice habitat (Meiners et al. 2003, Thomas & Papadimitriou 2003). EPS may serve in the extracellular cryoprotection of sea-ice diatoms (Krembs et al. 2002, Krembs & Deming 2008), are a component of ice-associated biofilms and serve the organisms in attachment. Antarctic sea-ice EP are densely colonised by attached bacteria (Meiners et al. 2004). In the pelagic realm, EPS-rich particles such as transparent EP (TEP), organic microaggregates and marine snow are important substrates for bacteria and serve as microhabitats with highly increased bacterial abundance, cell volume and activity (Smith et al. 1992, Passow & Alldredge 1994, review by Simon et al. 2002).

Determination of single-cell bacterial activity in natural environments remains challenging and can best be approached using proxies. Consequently, the number of active cells in environmental samples has been determined with different techniques, e.g. cultivation experiments (most probable number), microautoradiography, fluorescent *in situ* hybridisation and life stains. A stain used across many habitats is the soluble redox dye 5-cyano-2,3-ditolyl tetrazolium chloride (CTC). Bacteria able to take up CTC and reduce it to its fluorescent formazan have an active electron transport system. An active electron transport system is essential for respiration and CTC-reducing bacteria (CTC+) are therefore considered to be actively respiring (Rodriguez et al. 1992, Schaule et al. 1993). Although a number of studies criticised the method (Ullrich et al. 1996, Choi et al. 1999), the technique has been broadly applied in recent years and reveals important insights and comparisons between bacterial activities of different habitats (e.g. Jugnia et al. 2000 and references therein, Sherr et al. 2002, Davidson et al. 2004, Gasol & Arístegui 2007). Junge et al. (2002) using CTC incubations of melted ice samples reported an average of 4% (range 2 to 27%) actively respiring bacteria in Arctic pack ice during summer. In a more recent study on Arctic winter sea ice, the proportion of CTC+ cells in melted ice samples incubated at temperatures between –2 and –20°C varied between 0.5 and 4% (Junge et al. 2004). Using size-fractionation techniques the same study showed that almost all active cells were attached to particles retained on 3 µm pore-size filters (Junge et al. 2004).

In the present study, we used a novel non-invasive *in situ* approach to provide for the first time direct evidence for the existence of microbial hotspots on sea-ice EP, as evident from enhanced activity of attached bacteria. We avoided melting the ice samples before incubation with CTC and retained the distribution of bacteria and EP in the sea ice. The new method allowed us to estimate the number of active cells both in the free-living and EP-attached bacterial fraction of coastal Arctic sea ice during the spring–summer transition.

MATERIALS AND METHODS

Site and sampling. Land-fast ice was sampled in the eastern Chukchi Sea near Barrow, Alaska (71° 20′ N, 156° 40′ W) 300 m off the coast at a water depth of 6 m. Ice cores were collected on the 6 April, 2 May and 4 June 2003. On each sampling day, 2 ice cores (named A and B and taken <30 cm apart) were collected with a SIPRE ice auger (9 cm internal diameter) and, immediately after coring, six 1 cm segments were cut with a stainless steel saw from each of the 2 cores.

Temperature, salinity, dissolved organic carbon and chl *a*. Ice core A was used for the determination of temperature, salinity, dissolved organic carbon (DOC) and chl *a*. Ice temperature was measured with a digital thermometer (Control Company, accuracy 0.1°C) immediately after coring from small holes drilled into the core at 6 equidistant depths. Thereafter, the ice core was subsampled into 6 equidistantly spaced sections with a thickness of 1 cm (average sampling depths of sections were 0.5, 26.2, 55.0, 77.4, 103.1 and 129.2 cm). These ice sections were transferred into acid-washed glass containers, transported to the laboratory and melted in the dark at 4°C. After the ice was melted the salinity was measured with a YSI Model 30 conductivity probe and subsamples of the meltwater were taken for determination of DOC. Subsamples were filtered through precombusted Whatman GF/F filters and kept frozen (–30°C) until analysis by high-temperature catalytic oxidation with a Shimadzu TOC analyser after acidification with HCl. For the determination of chl *a* concentrations, subsamples were filtered onto Whatman GF/F filters and frozen (–30°C) until analysis with a Turner Designs 10-AU fluorometer according to Arar & Collins (1997). Brine salinity was calculated as a function of ice temperature (Assur 1958) and brine volume was inferred from ice temperature and ice bulk salinity according to Frankenstein & Garner (1967).

Biological parameters and EP. Ice core B was used for the determination of the abundance of actively respiring bacteria (CTC incubations), total bacterial numbers (TBN) and the concentration of EP. Careful attention was paid to maintain sterile conditions during sampling and processing of the core. All work was performed under dark foil to minimise the exposure of CTC to light.

Actively respiring bacteria: Our incubation technique was adopted from an *in situ* primary production method (Mock & Gradinger 1999) allowing for fine-scale *in situ* measurements in 1 cm vertical sections throughout the ice cover without severe disruption of ice morphology, geochemistry and distribution of bacteria and EP. The 6 equidistant 1 cm thick ice sections were sampled from core B and placed into cold and sterile Petri dishes. The samples were inoculated with 12 ml sterile-filtered (0.2 µm) seawater at a temperature of –1.9°C and adjusted volumes of a 50 mM CTC working solution resulting in a final concentration of 5 mM CTC in the brine of the incubated samples. The required additions of the working solution were inferred from brine volumes which were determined from temperatures measured in ice core A and from sea-ice bulk salinity measurements taken at the sampling location before the incubation. An additional section of the ice core (taken just above the lowermost incubation sample) was fixed with 2 ml of borax-buffered formalin (38%) before incubation with CTC and served as a blank. The Petri dishes were sealed watertight with a non-toxic silicon ring and electrical tape, and were wrapped in aluminium foil to keep samples in the dark. The remaining unused core sections and the Petri dishes were placed in PVC plastic tubing and re-inserted into the ice floe in their original position. The tube was secured with ice screws in the original bore hole holding the incubation vessels in their original depth horizon within the sea ice (Mock & Gradinger 1999). The core hole was covered with snow to the orignal snow depth and samples were incubated for 5 h at *in situ* temperature. Samples were retrieved from the ice and transferred from the Petri dishes to sterile plastic containers. Bacterial activity was stopped by addition of 100 ml sterile-filtered seawater (Garrison & Buck 1986) and 4 ml of a 0.2 µm prefiltered borax-buffered formalin solution (38%) resulting in a final formalin concentration of approximately 1%. The fixed ice samples were transported back to the laboratory and melted in the dark at 4°C. Replicate blanks of the filtered seawater were taken at each station and treated identically to the ice samples. The concentration of CTC-reducing bacteria, TBN and EP concentration in the blanks were insignificant (<1%) when compared to the experimental samples.

EP concentration and distribution: For the determination of the concentration and size distribution of EP, 2 to 30 ml subsamples of the meltwater (core B) were filtered onto 0.4 µm polycarbonate filters supported with backing filters at a pressure difference of <0.1 bar. Filters were stained with 0.2 µm pre-filtered Alcian Blue solution (Alldredge et al. 1993) and mounted on Cyto-Clear slides (Poretics) (Logan et al. 1994). Relative cover of total EP area on the filters was low (average: 6.4% of effective filter area). EP abundance and size were measured at 400× magnification with a Zeiss Axiovert 200M microscope, equipped with an AxioCam digital camera connected to a computer. A minimum of 500 random EP were measured using a semi-automatic function of Zeiss AxioVision software. Contour lines of digitised EP images were traced manually and the area of individual particles was determined with the image analysis system. Individual EP areas were converted to equivalent spherical diameters (ESD), which were assigned to 8 logarithmically increasing size classes ranging from 3 to 60 µm ESD.

Particle size distribution: Particle size distributions can be described by power functions of the type $N = kd_p^{-\beta}$, or $dN/d(d_p) = kd_p^{-(\beta+1)}$, where dN is the number of particles per unit volume in the size range d_p to $\{d_p+d(d_p)\}$ (e.g. McCave 1984). The constant k depends on the concentration of particles and β describes the size distribution of the particles; the smaller β is, the larger is the fraction of large particles. $\beta + 1 = 4$ denotes equal particle volumes in the logarithmically increasing size classes (McCave 1984). We estimated $\beta + 1$ values from the regressions of $\log\{dN/d(d_p)\}$ versus $\log\{d_p\}$ by plotting the relative frequency of EP (%) versus ESD of EP (µm). The magnitude of $\beta + 1$ allows comparison of EP size frequencies of different stations and environments.

Determination of free-living and EP-attached bacteria: For bacterial counts, subsamples of the meltwater (2 to 60 ml) were filtered onto 0.2 µm polycarbonate filters, double-stained with 4′6′-diamidino-2-phenylindole (DAPI) and Alcian Blue (Alldredge et al. 1993) and mounted on Cyto-Clear microscope-slides (Porter & Feig 1980, Logan et al. 1994).

Free-living bacteria: Only cells not attached to Alcian Blue-stained particles (observed by switching to bright field illumination) were counted to calculate the abundance of free-living bacteria stained by DAPI (DAPIfree) and free-living CTC-reducing cells (CTC+free). Cells counted as CTC+ were bacteria that were both stained by DAPI and showed formazan crystals. At least 1000 cells were enumerated on a minimum of 20 random fields at a magnification of 1250× using an Axiovert 200M microscope using UV excitation (for DAPI) and blue light excitation (for CTC+).

EP-attached bacteria (DAPI and CTC+): For each sample, both DAPI-stained and CTC+ bacteria associated with 24 EP were enumerated by switching between bright field (EP detection), UV (for DAPI-stained cells) and blue light illumination (for CTC+). The individual EP were sized (see section 'EP concentration and distribution' above), and associated bacteria were counted at a magnification of 1250×. Because EP are 3-dimensional, the entire volume of each EP was examined by changing the microscope's focal plane during the observation. Hence, both bacteria on the surface and embedded in EP were counted (Mari & Kiørboe 1996). A potential error source in this enumeration are free-living bacteria retained by the filter beneath the examined EP.

In order to estimate the fraction of total bacteria attached to EP, a relationship between EP size and the number of attached bacteria was calculated for each sample. The number of attached bacteria was fitted to a power law function $n = ad_p^b$, where n is the number of bacteria per EP, d_p is the ESD of the EP and a and b are constants for a given sample. Numbers of associated bacteria were plotted versus ESD in log-log coordinates to estimate a and b. The fraction of attached bacteria was calculated by combining this relationship, the size spectra of EP and TBN (Passow & Alldredge 1994, Mari & Kiørboe 1996). This was done separately for both DAPI-stained cells and CTC-stained cells. This evaluation gives results for the EP concentration and EP size distribution, DAPIfree abundance, the abundance of DAPI-stained bacteria attached to EP (DAPIatt), CTC+free abundance and the abundance of active bacteria attached to EP (CTC+att). Note that bacteria characterised as DAPIfree and DAPIatt include the CTC-stained cells because of the staining characteristics of our method. TBN was calculated as the sum of free and attached DAPI-stained cells (TBN = DAPIfree + DAPIatt), and the total number of active cells (CTC+) as the sum of CTC+free and CTC+att. Non-parametric Spearman's rank tests were used to explore correlations between variables. To compare mean values of normally distributed data, 1-way ANOVAs were performed. In cases of non-normally distributed data, the non-parametric Mann-Whitney *U*-test was used for analysis. All tests were computed using SPSS version 11.0.4.

RESULTS

The ice showed a consistent and thin snow cover (range 2 to 5 cm) on all sampling dates. Ice thickness was relatively stable, measuring 128, 132 and 129 cm on 6 April, 2 May and 4 June, respectively. Ice temperatures varied between –12.0 and –0.1°C and increased with depth prior to the last sampling date (Fig. 1A). On 4 June the ice was almost isothermal with slightly higher temperatures at the surface indicating melting triggered by increasing air temperatures. At this time large areas of the sea ice were covered with melt-

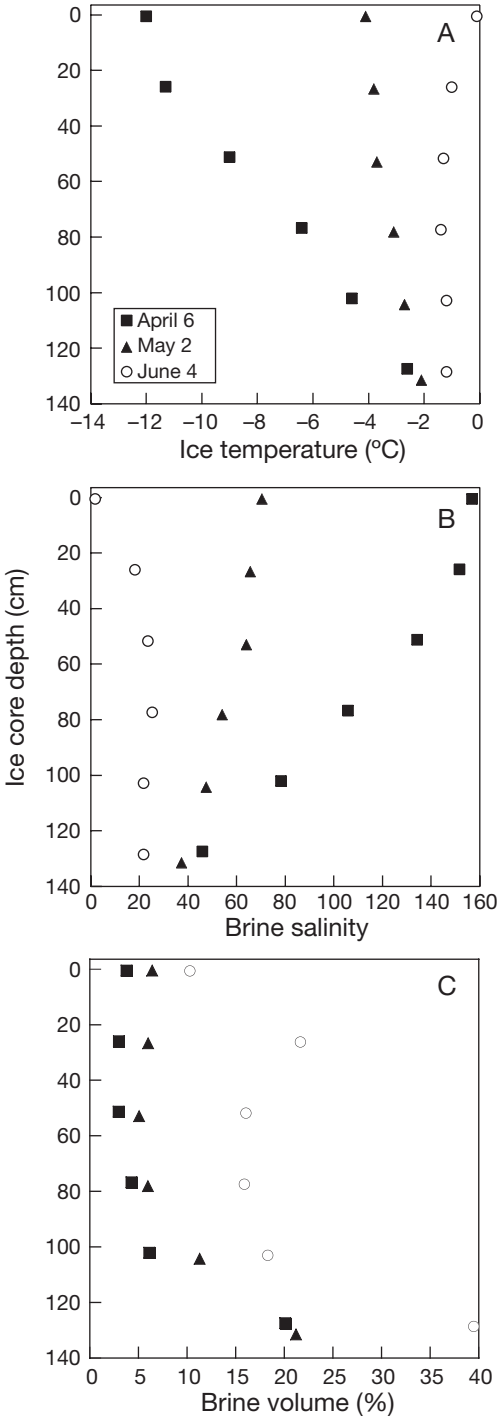

Fig. 1. Vertical profiles of (A) ice temperature, (B) brine salinity and (C) brine volume for sampling dates in April, May and June 2003

ponds. Corresponding to ice temperatures, brine salinities showed a wide range, with values between 1.8 and 156.9 (Fig. 1B). The maximum value was found at the surface of the ice on 6 April, the minimum at the ice surface on 4 June. Thus, both temperature and brine salinity were highly variable in the upper and interior parts of the sea ice, whereas thermodynamic conditions at the bottom of the ice remained relatively constant during the sampling period. Brine volume fractions (range 3 to 39%, average 12%) were small in the upper and interior parts of the sea ice on the first 2 sampling dates, with increasing values in the lower warmer parts of the sea ice. High brine volume fractions were found on 4 June (Fig. 1C). Chl *a* concentrations varied between 0.2 and 243.7 µg l^{-1} and were very low at the surface and in the interior of the ice and peaked in the lower-most centimeters of the ice cover, indicating well-developed bottom communities during the entire sampling period (Fig. 2A). DOC concentrations (range 0.04 to 3.29 mmol C l^{-1}) showed similar L-shaped vertical profiles and were positively correlated with chl *a* concentrations (Spearman's rank correlation: $\rho = 0.556$, $p = 0.022$) (Fig. 2B). EP concentrations ranged between 1.8×10^6 and 149.1×10^6 particles l^{-1} with maximum values always occuring at the ice/water interface (Fig. 2C). EP concentration was not significantly correlated with chl *a* concentration but was correlated with ice depth (Spearman's rank correlation: $\rho = 0.407$, $p = 0.041$), although slightly elevated values were observed in the uppermost centimeters of the ice cores on 6 April and 4 June (Fig. 2C). EP expressed as area per volume ranged between 1.1 and 82.3 cm^2 l^{-1} (data not shown).

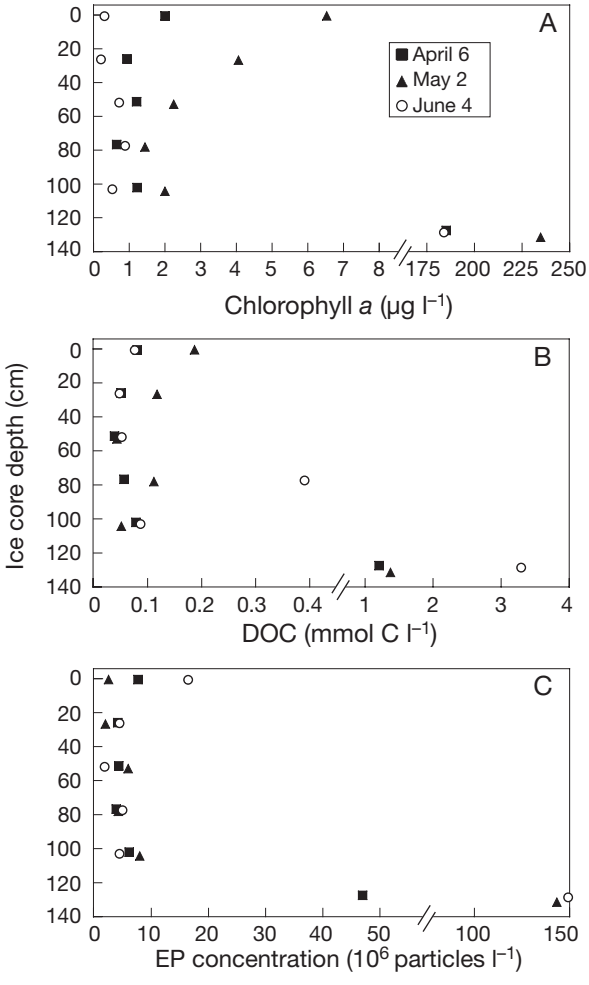

Fig. 2. Vertical distribution of (A) chl *a*, (B) dissolved organic carbon (DOC) and (C) exopolymer particle (EP) concentrations. Note scale breaks on *x*-axes

EP size distributions were fitted to power functions and the number of particles decreased with size in all samples (data not shown). The slopes of the EP size distributions in each ice core section were relatively flat with $\beta + 1$ values ranging between 1.2 and 2.0 (data not shown). EP size distributions for the pooled data from all ice sections of each sampling date (Fig. 3) showed a large contribution of relatively large particles in the sea ice as indicated by the low $\beta + 1$ values (range for pooled data 1.4 to 1.6). Size distributions of EP did not change considerably over the study period and the relative number of EP per size class was significantly negatively correlated with EP size (Fig. 3).

EP investigated in the present study did not contain any particles other than bacteria. The bacterial colonisation of EP from all sampled ice sections is shown in Fig. 4. EP were colonised by bacteria with a range of 0 to 93 DAPI-stained cells EP^{-1} and 0 to 28 CTC-stained cells EP^{-1}. Both groups were positively correlated with particle size (Fig. 4) and the number of DAPI-stained cells (average: 16.3 cells EP^{-1}) was significantly higher than the number of CTC-stained cells (average 3.2 cells EP^{-1}, ANOVA: $F = 18.439$, $p < 0.01$). The ratio of CTC-stained cells EP^{-1} to DAPI-stained cells EP^{-1} was not correlated with the size of individual particles (Spearman's rank correlation: $\rho = -0.048$, $p = 0.569$) which indicates that particle quality was constant and did not change along the size spectrum. The ratio DAPIatt:DAPIfree was positively correlated with both EP concentration and EP area (Spearman's rank correlations: EP concentration, $\rho = 0.510$, $p = 0.035$; EP area, $\rho = 0.478$, $p = 0.049$).

Fig. 5 shows vertical distributions of TBN, DAPIatt, CTC+ and CTC+att. TBN varied betwen 0.18×10^9 and 8.48×10^9 cells l^{-1}, correlated significantly with DOC concentrations (Spearman's rank correlations: $\rho = 0.476$, $p < 0.049$) and showed maximum numbers at the bottom of the sea ice. The relative contribution of DAPIatt ranged between 4.6 and 28.5% (average 15.0%) of TBN. The proportion of CTC+ averaged 7.4% (range 3.0 to 17.2%) of TBN. The abundance of

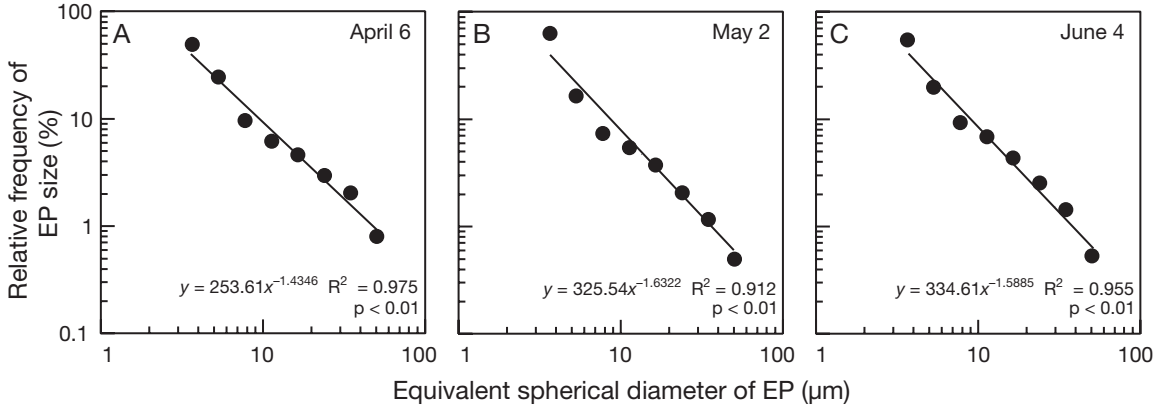

Fig. 3. Relative size frequencies of exopolymer particles (EP) in sea ice for the pooled data in (A) April, (B) May and (C) June 2003

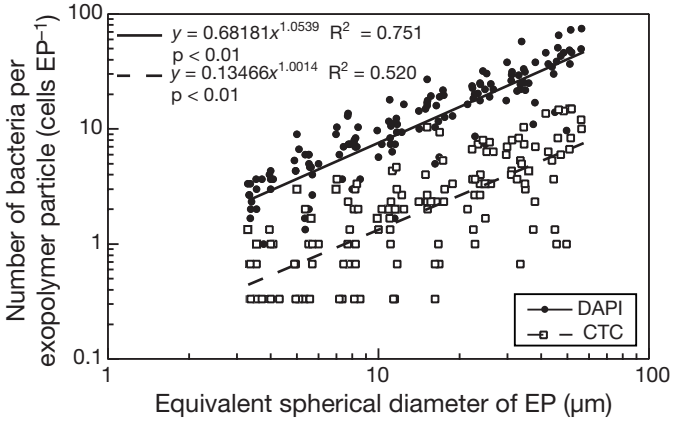

Fig. 4. Number of DAPI-stained (DAPI) and number of CTC-stained (CTC) bacteria per exopolymer particle (EP) versus size of respective EP. Each data point represents average of measurements on 3 individual particles

actively respiring bacteria was significantly correlated with the concentration of EP ($\rho = 0.511$, $p = 0.035$). Highest concentrations of CTC+ were always found in lower parts of the ice with the maximum occuring in the lower-most ice core sections on 4 June. The proportion of CTC+att ranged between 9.1 and 74.2% (average 39.3%) of CTC+ with maximum values ocurring in the upper and interior sections of the ice. Both the proportion of DAPIatt and of CTC+att were not significantly correlated with temperature (Spearman's rank correlation: $\rho = 0.194$, $p = 0.440$ and $\rho = 0.138$, $p = 0.570$ for DAPIatt vs. temperature and CTC+att vs. temperature, respectively). In contrast, TBN and CTC+ were both highly significantly correlated with temperature (Spearman's rank correlation: $\rho = 0.606$, $p = 0.008$ and $\rho = 0.657$, $p = 0.003$ for TBN vs. temperature and CTC+ vs. temperature, respectively). The proportion of CTC-stained cells in the free-living bacterial fraction ranged from 1.1 to 11.2% (average 5.4%) of DAPIfree and was significantly smaller than the CTC+ proportion of the particle-attached fraction (range 7.8 to 37.6%, average 19.6% of DAPIatt) (Mann-Whitney *U*-test: $p < 0.001$) (Fig. 6).

DISCUSSION

While concentrations of EPS in Arctic sea ice are high (Krembs & Engel 2001, Krembs et al. 2002, Meiners et al. 2003, Riedel et al. 2006), their ecological role remains poorly understood. Our newly developed triple-stain *in situ* method provides the first data on the number and activity of bacteria associated with EP in Arctic sea ice indicating the importance of these particles as microhabitat and sites of increased carbon turnover. Our results are consistent with previous observations indicating that sea ice harbours high numbers of surface-attached bacteria (Meiners et al. 2004) with a high proportion of actively respiring cells (Junge et al. 2002, 2004), but they also offer the first detailed quantitative information on the concentration, size distribution and colonisation of the EP serving as substrate for ice-associated bacteria.

Methods

Using a combination of planktological and sea-ice biological methods we determined vertical profiles of EP concentration and size distribution as well as the total and actively respiring number of free-living and particle-attached bacteria in Arctic sea ice. In contrast to other methods (Grossmann 1994, Junge et al. 2002), the new method allows incubation of bacteria with CTC within 1 cm vertical sections, minimising osmotic and thermal shock, and avoiding the destruction of ice morphology and sea-ice biogeochemical properties (Mock & Gradinger 1999). The final step of this method, however, involves melting of the ice, resulting

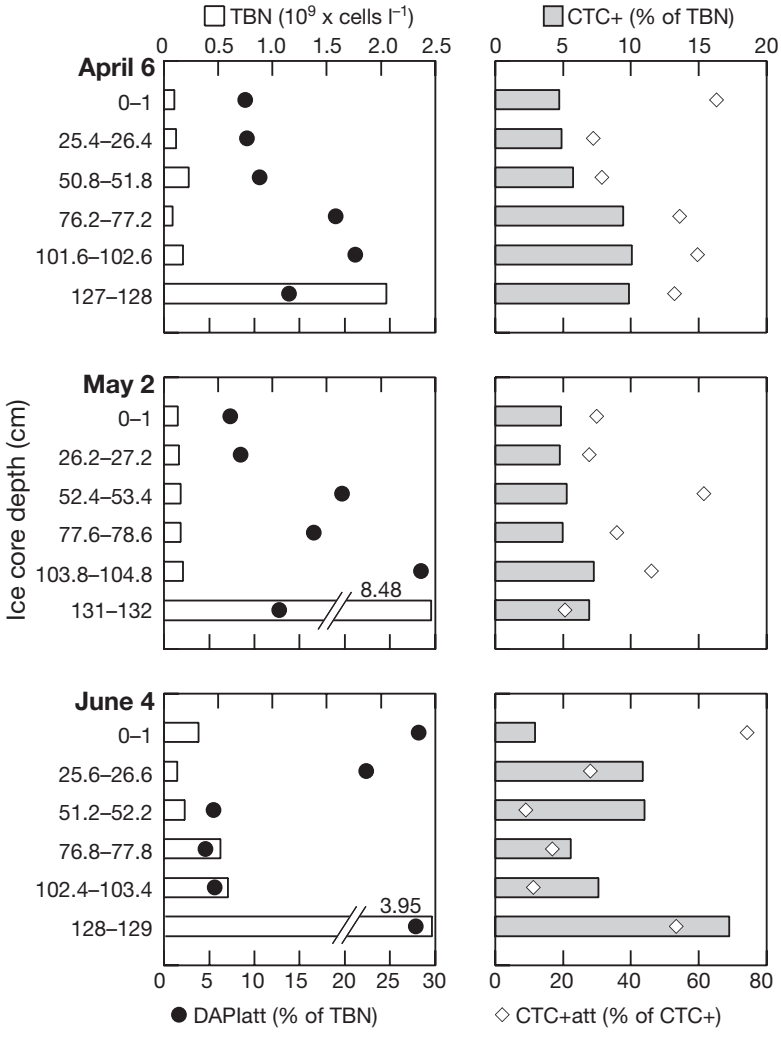

Fig. 5. Vertical profiles for total bacterial number (TBN, 10^9 cells l^{-1} melted sea ice), fraction of bacteria attached to exopolymer particles (DAPIatt), total number of actively respiring bacteria (CTC+) and fraction of actively respiring bacteria attached to EP (CTC+att) for April, May and June 2003

in changes in salinity, DOC concentration and particle interactions in the meltwater, potentially inducing artefacts in particle concentrations, size distribution and bacterial colonisation. Addition of sterile-filtered seawater during melting minimises these effects but artefacts cannot be totally ruled out (Garrison & Buck 1986, Krembs et al. 2002, Meiners et al. 2003).

Smith & del Giorgio (2003) noted that rather than dividing natural bacterial concentrations into an active and inactive fraction, single-cell activity should be viewed as a continuum of physiological states. CTC has been widely used to determine the concentration of actively respiring bacteria in different aquatic habitats and is considered to label oxygen-reducing bacteria with high metabolic activity which is necessary to reduce CTC to fluorescent formazan crystals. Several studies indicated toxic effects of CTC at high concentrations (Ullrich et al. 1996, Choi et al. 1999), while others reported no toxic effects and a good agreement between the number of CTC-labelled cells and bacterial respiration and production (Lovejoy et al. 1996, Smith & del Giorgio 2003), resulting in opposing views on the utility of the method. Sherr et al. (1999) reported that CTC is non-toxic at concentrations up to 5 mM, which was the final concentration used in the incubations in the present study. In a recent re-examination of the method Gasol & Arístegui (2007) found that high CTC concentrations and long incubation times can result in breakup of bacteria due to intracellular CTC-formazan crystal formation and thus may cause problems in flow-cytometric studies, but that epifluorescence microscopical counting of CTC- and DAPI-labelled cells — as done in the present study — provides sound ecological

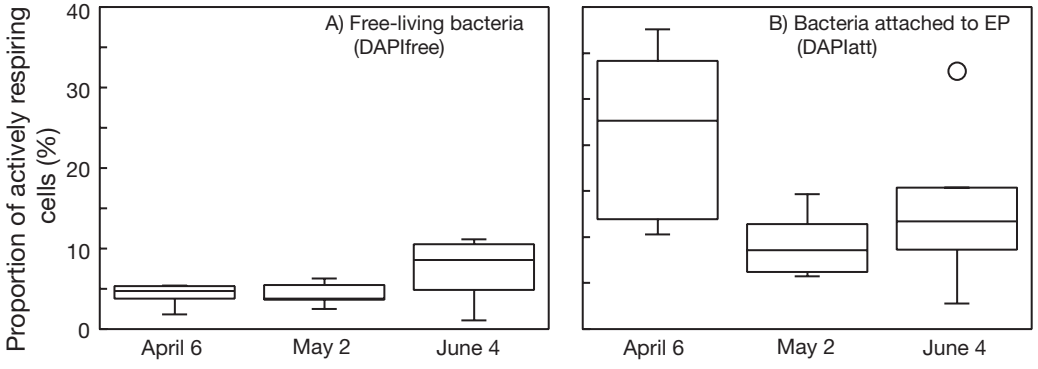

Fig. 6. Box plots for the proportion of actively respiring cells in (A) the free-living bacterial fraction and (B) exopolymer particle (EP)-attached bacterial fraction on the 3 sampling dates in 2003. Box plots show total data range, 25 to 75% quartile range, and median. Single data points were marked as outliers when they exceeded a value of V = UQ + 1.5 × IQD (where UQ is upper quartile and IQD is interquartile distance)

results on the number of actively respiring bacteria. We therefore conclude that the CTC-labelled bacteria observed in the present study represent the highly metabolically active bacterial fraction in the natural sea-ice environment.

Exopolymer particles

Our study shows that large amounts of EPS occur throughout Arctic sea ice during the spring–summer transition. Using similar methods, i.e. microscopic determination of EP, as in the present study, Krembs & Engel (2001) report high EP concentrations for the bottom 10 cm of Laptev Sea pack ice during summer. A study on pack ice in Fram Strait reported 0.43×10^6 to 14.93×10^6 particles l^{-1} in ice of varying age during autumn (Meiners et al. 2003). A combination of different processes has been proposed to explain the high amount of EPS in sea ice including incorporation and accumulation of water column EPS during ice formation, formation of EPS due to destruction of organisms within the sea ice, physico-chemical formation from dissolved precursors and biological production (Krembs et al. 2002, Meiners et al. 2003, Riedel et al. 2007a, Collins et al. in press). The currently available information points towards biological EPS production by sea-ice diatoms dominating the accumulation of EPS in the ice matrix. This is supported by the present study, as the maximum concentrations of EP were associated with the high chl *a* levels found at the bottom of the ice cover. Our 3 sampling events over a 2 mo period do not provide detailed temporal information on the seasonal dynamics of the sea-ice algae and EPS, but overall point towards the same conclusion as a better seasonally resolved study of Beaufort Sea fast ice in which EPS peaks occurred at the same time as the chl *a* maxima in May (Riedel et al. 2006).

Using an EP size to carbon conversion developed for laboratory-made TEP, we calculated crude estimates for EP carbon according to the formula TEP-C (µg C TEP^{-1}) = 0.25×10^{-6} $r^{2.55}$, where r is the equivalent spherical radius and 2.55 the fractal dimension of the (transparent) EP (Mari 1999). Estimates for sea-ice EP carbon ranged between 0.04 and 2.46 mg C l^{-1} (average 0.39 mg C l^{-1}) and were equivalent to 1.4 to 33.5% (average 13.6%) of the measured DOC, illustrating that EP contribute a significant fraction to the total sea-ice carbon pool. While DOC and EP are separate carbon pools, DOC can be physically converted to EP by gel formation (Chin et al. 1998). The ratio of estimated EP carbon to DOC was correlated neither to ice depth nor to ice temperature (data not shown), indicating that physical transformation of DOC into EP, induced by temperature-driven changes in brine salinity, ion concentration and brine volume, fostering physical formation of EP from precursor material, did not significantly contribute to EP formation.

Like diatoms, bacteria can also produce large amounts of EPS which can accumulate as thick gels around the cells (Cooksey & Wigglesworth-Cooksey 1995, Stoderegger & Herndl 1999, Deming & Baross 2001) and thus may significantly contribute to the biological formation of EP in sea ice. As no cell-specific production rates are available for cold or sea-ice adapted bacteria, we calculated bacterial EPS production rates in our samples using rates determined for temperate planktonic bacteria (i.e. 4 amol C cell h^{-1}, Stoderegger & Herndl 1999). Estimated bacterial EPS production rates in our samples ranged between 0.1 and 4.6 µg C l^{-1} d^{-1}. These values might be overestimated, as our calculation did not include any correction for the lower ice temperatures but still only accounts for a daily bacterial EPS production of 0.3% of the sea-ice EP standing stock. This suggests that bacteria did not significantly contribute to EP accumulation in the sea ice sampled in our study. However, Mancuso Nichols et al. (2004) measured higher EPS production rates for bacterial strains isolated from Antarctic sea ice at cold temperatures compared to 20°C. This shows that temperature does have a measurable effect on bacterial EPS production and that the above calculated bacterial EPS production must be considered to be a very crude estimate. In contrast to the present study, Collins et al. (in press) investigated temporal dynamics of bacteria and particulate EPS in Arctic winter sea ice (January to March) in Franklin Bay, Northwest Territories, Canada, and reported increasing EPS concentrations in the upper horizons of the sea ice and suggested that bacteria can contribute significantly to the EPS pool in sea ice.

EP numbers decreased with particle size and EP size distributions showed $\beta + 1$ values between 1.2 and 2.0 indicating a relatively large proportion of larger EP typical for sea ice (Meiners et al. 2003, 2004). This is in contrast to $\beta + 1$ values of 4, often reported from the pelagial, which indicate similar distributions of particle volume in the different size classes (McCave 1984). Thus, although small EP dominated the numbers of EP in sea ice, the total EP volume was dominated by large particles. Large EP may physically block brine channels and thus structure the sea-ice habitat affecting trophic interactions as well as movement of brine. However, EP observed in the present study were generally smaller than the average diameter of brine channels of around 200 µm (Weissenberger et al. 1992). We therefore assume that blocking of brine channels by EPS is of minor importance and restricted to the terminal pores of the brine-channel system in cold ice.

Bacteria

Bacterial abundance in sea ice generally follows the development of the ice-algal bloom. With the onset of algal growth in early spring, a favourable habitat for bacteria forms within the brine-channel system of the sea ice. High concentrations of DOC inside the ice (Bunch & Harland 1990, Thomas et al. 1995) support abundant and diverse bacterial communities (Junge et al. 2002, Brinkmeyer et al. 2003), which in turn are important for remineralisation of inorganic nutrients. In addition, bacterial growth also converts DOC into particulate organic carbon and therefore supports bacterivores channelling carbon through sea-ice microbial networks to higher trophic levels (Laurion et al. 1995). The bacterial abundances found in the present study are in agreement with previous Arctic fast-ice studies reporting bacterial abundances ranging between 0.2×10^5 and 1.0×10^7 cells ml^{-1} (summarised in Gradinger & Zhang 1997). TBN peaked in bottom communities which is typical for Arctic fast and pack ice (e.g. Laurion et al. 1995, Gradinger & Zhang 1997) during spring and summer. High contributions of attached bacteria are known from various marine environments (reviewed in Simon et al. 2002) and are important in particulate organic matter degradation, bacterial production (Crump et al. 1998) and for overall diversity in bacterial communities (Hodges et al. 2005). Bacteria in Arctic and Antarctic sea ice can attach to algae (McGrath Grossi et al. 1984, Smith et al. 1989, Archer et al. 1996), EP (Meiners et al. 2004) and ice surfaces (Junge et al. 2004). Our estimates of the relative contribution of bacteria attached to EP (DAPIatt) ranged from 4.6 to 28.5% of TBN (average 15.0%), which is consistent with the average value for Antarctic pack ice (14.8%, Meiners et al. 2004) and various marine habitats with values ranging between 0.5 and 25% (reviewed in Passow 2002). The ratio DAPIatt: DAPIfree was positively correlated with both EP concentration and EP area, indicating that the availability of substrate was controlling the numbers of attached bacteria.

The total number of bacteria per individual EP increased with EP size, whereas the specific number (i.e. the number of attached bacteria per aggregate volume) decreased with EP size. This phenomenon has been attributed to the increasing porosity of marine aggregates with size (Mari & Kiørboe 1996, Simon et al. 2002). The ratio of CTC-stained bacteria EP^{-1} to DAPI-stained bacteria EP^{-1} was not correlated with EP size, indicating that the quality of the particles for bacterial colonisation was similar in all size classes, i.e. no EP size class showed an increased bacterial activity.

Actively respiring bacteria

Active bacterial cells (CTC+) contributed between 3.0 and 17.2% (average 7.4%) of TBN. This result is not only consistent with other sea-ice data (Junge et al. 2002) but also with values from other aquatic habitats (Jugnia et al. 2000, Smith & del Giorgio 2003, Davidson et al. 2004, Gasol & Arístegui 2007). In the marine environment, the proportion of CTC+ cells is generally <20% of TBN and tends to correlate with other bacterial activity parameters such as cell size, production and respiration (Gasol et al. 1995, Lovejoy et al. 1996, Smith 1998, Smith & del Giorgio 2003, Davidson et al. 2004, Gasol & Arístegui 2007). In the pelagic realm, EP and larger aggregates show increased bacterial activity (Smith et al. 1992, Simon et al. 2002) including high numbers of CTC+ cells (Sherr et al. 1999, 2002). In Arctic winter sea ice (March), the proportion of CTC+ cells in melted ice samples incubated at temperatures between −2 and −20°C varied between 0.5 and 4% with almost all active cells attached to particles retained on 3 µm pore-size filters (Junge et al. 2004). In the present study, the percent contribution of actively respiring bacteria in the EP-associated bacterial fraction ranged between 7.8 and 37.6% (average 19.6%) and was 4 times higher than the relative contribution of CTC+ in the free-living bacterial fraction (range 1.1 to 11.2%, average 5.4%). Thus, sea-ice EP sustain enhanced bacterial activity throughout the spring season. The slightly elevated contribution of CTC+ (average 7.4% of TBN) compared to Junge et al.'s (2004) results can be attributed to differences in sampling methodology. The present study focused on free-living bactera and the fraction of bacteria attached to a single and relatively small class of particles (i.e. EP between 3 and 60 µm ESD), whereas Junge et al. (2004) included free-living bacteria as well as bacteria attached to a wide variety of unspecified substrates including sediment, algae and large detritus flocks retained on 3 µm pore-size filters. The small EP in the present study had high specific bacterial numbers (i.e. high numbers of attached bacteria per aggregate volume) and the combination of counts of bacteria attached to EP with size distributions of EP for calculation of the total number of attached cells might slightly overestimate the number of DAPIatt and CTC+att.

Junge et al. (2004) found a significant correlation between the number of actively respiring attached bacteria and decreasing temperatures in winter sea ice, contributing to the idea that surface attachment is important for organisms coping with extreme environmental conditions and that EPS play a cryoprotective role in sea ice (Krembs et al. 2002, Collins et al. in press). In contrast, the warmer sea ice investigated in the present study showed no significant correlations

between the proportion of DAPIatt and temperature or the proportion of CTC+att and temperature. The positive significant correlations between both TBN and CTC+ with ice temperature found in the present study can be explained with increased growth rates at higher temperatures and enhanced brine volumes as spring progresses. Modelling results by Golden et al. (1998) demonstrate that sea ice with temperatures >–5°C and ice bulk salinities >5 is permeable and allows fluid transport, i.e. exchange of brine between different depth horizons and the underlying water. With the exception of the upper horizons of the sea ice sampled in April, the sea-ice cover was well above these thresholds and allowed percolation of sea-ice brine, enhancing nutrient availability within the ice, supporting high numbers of actively respiring bacteria and high TBN.

While the DOC concentrations in the upper parts of the sea ice were within the range previously observed (Thomas et al. 1995, Krembs et al. 2002), the values in the bottom communities were high and significantly exceeded concentrations reported by others. We calculated brine DOC concentrations based on bulk sea-ice DOC concentrations and brine volume fractions. These values reflect the concentrations that bacteria are exposed to *in situ*, and ranged from 0.22 to 8.34 mmol C l^{-1}, indicating favourable conditions for bacteria suspended in the brine. Despite these high DOC concentrations, the attached bacterial fraction showed a higher contribution of actively respiring cells, suggesting that attachment to particles provides significant advantages. There is little information on DOC composition in sea-ice brine, and a large fraction of the DOC may be refractory material that cannot be utilised by free-living bacteria. We therefore assume that the number of active bacteria living suspended in the brine remained relatively low due to a large proportion of undegradable DOC, while bacteria attached to potentially nutrient-rich particulate substrates showed a high percentage of active cells. Other factors enhancing the activity of the EP-attached bacterial fraction are freezing-point depression and enzyme stabilisation by EPS, as suggested by Junge et al. (2006), who studied the marine psychrophilic bacterium *Colwellia psychrerythraea* at sub-freezing temperatures in laboratory cultures.

Besides bottom-up control, top-down processes including virus-mediated mortality and protozoan grazing are impacting on bacterial populations and their activity (Yager et al. 2001, Sherr et al. 2002). High virus concentrations affecting both eukaryote and prokaryote populations have been reported from the sea-ice habitat (Maranger et al. 1994, Borriss et al. 2003, Gowing 2003, Gowing et al. 2004) and are suggested to significantly affect sea-ice bacterial populations in winter and early spring (Wells & Deming 2006). Bacterial ingestion rates by ice-associated heterotrophic flagellates can be high (Laurion et al. 1995, Riedel et al. 2007b), and selective grazing of active cells, including CTC+ cells, has been reported for marine habitats (Sherr et al. 1992, del Giorgio et al. 1996). Ikävalko & Gradinger (1997) report on the occurrence of surface-associated protozoans in Greenland Sea pack ice; it appears reasonable to assume that they are able to graze bacteria from the surface of larger particles and ice-associated biofilms, which together with viral activity probably influenced the numbers of attached/free-living and actively respiring/inactive bacteria in the present study.

Riedel et al. (2007a) hypothesise that EPS may be directly utilised as a carbon source by bacteria and other heterotrophs fostering nutrient regeneration in the sea-ice habitat. Their study showed a significant relationship between EPS concentrations and NH_4 regeneration rates, and the authors postulate that EPS also faciliates grazing by heterotrophic protists, thereby enhancing nutrient regeneration. The high numbers of respiratory active bacteria attached to EP found in the present study may serve directly in NH_4 production and may also be more easily grazed by sea-ice protists. Grazing rates of sea-ice protists feeding on suspended bacteria are similar to those in temperate habitats (Laurion et al. 1995), and sea-ice protists feed on particles over a wide size range, including the smallest size fraction of EP investigated in the present study (Scott et al. 2001). EP colonised by bacteria may therefore serve as an important food source for heterotrophic sea-ice protists fostering nitrogen remineralisation. In contrast, Riedel et al. (2007b) found negative correlations between EPS concentration and experimentally derived ingestion rates of bacterivores in Arctic fast ice. They suggest that EPS at times may also interfere with the mobility and feeding of heterotrophic protists by coating and clogging feeding appendages. The high number of small EP found in the present study supports this hypothesis, although EP concentrations were generally 1 to 2 orders of magnitude lower than bacterial abundances.

Enrichment factors (Simon et al. 2002) were calculated to compare bacterial abundances associated with individual EP versus the abundances of free-living bacteria suspended in the sea-ice brine. The average enrichment factors for DAPI-stainable cells and actively respiring bacteria were 14 500 and 53 900, respectively, illustrating the importance of EP serving as substrate for bacteria and also as microbial hotspots in Arctic sea ice. The increased bacterial activity associated with the particles suggest that EP may serve as hotspots for respiration, nutrient remineralisation and grazing and thus may strongly influence biogeochemical cycling and food web structure in Arctic sea ice.

Densely colonised EP may provide a highly enriched food source, especially during the sea-ice melt season when concentrations of free-living bacteria are diluted in increasing brine volumes, while bacterial concentrations on particles remain constant.

Large numbers of EP that are colonised by bacterial assemblages showing a large fraction of actively respiring cells may be released into the water column during ice-melt season, seeding the microbial food web in the pelagic realm during Arctic spring and early summer. We hypothesise that the fate of attached and suspended bacteria released from sea ice will be different due to different settling speeds and pelagic grazing activity. EP observed in the present study did not harbour any particles except bacteria and, similar to pelagic TEP (Azetsu-Scott & Passow 2004), can be assumed to be neutrally buoyant, serving as ideal vectors for horizontal distribution of bacteria.

Further studies are needed to elucidate the importance of bottom-up versus top-down control, e.g. the effect of substrate availability, grazing and viral mortality, of both free-living and particle-associated bacteria in sea ice. Bacteria attached to EP are assumed to respond differently to environmental processes, e.g. changes in brine solute concentrations and grazing pressure, than the free-living bacterial fraction and should be considered as a decoupled subpopulation.

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