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ORIGINAL PAPER

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Evidence for active microbial nitrogen transformations in sea ice (Gulf of Bothnia, Baltic Sea) in midwinter

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Abstract Nutrient concentrations, chlorophyll-*a*, bacterial biomass and relative activity of denitrifying organisms were investigated from ice-core, brine and underlying water samples in February 1998 in the Gulf of Bothnia, Baltic Sea. Examined sea ice was typical for the Baltic Sea; ice bulk salinity varied from 0.1 to 1.6 psu, and in underlying water salinity was from 4.2 to 4.7 psu. In 2- to 3-months-old sea ice (thickness 0.4–0.6 m), sea-ice communities were at the winter stage; chl-*a* concentrations were generally below 1 mg m^{-3} and heterotrophic organisms composed 7–20% of organism assemblage. In 1-month-old ice (thickness 0.2–0.25 m), an ice spring bloom was already developing and chl-*a* concentrations were up to 5.6 mg m^{-3}. In relation to low salinity, high concentrations of NH_4^+, NO_2^-, PO_4^{3+} and $SiOH_4$ were found in the ice column. The results suggest that the upper part of ice accumulates atmospheric nutrient load during the ice season, and nutrients in the upper 10–20 cm of ice are mainly of atmospheric origin. The most important biological processes controlling the sea-ice nutrient status are nutrient regeneration, nutrient uptake and nitrogen transformations. Nutrient regeneration is specially active in the middle parts of the 50- to 60-cm-thick ice and subsequent accumulation of nutrients probably enhances the ice spring bloom. Nitrite accumulation and denitrifying activity were located in the same ice layers with nutrient regeneration, which together with the observed significant correlation between the concentrations of nitrogenous nutrients points to active nitrogen transformations occurring in the interior layers of sea ice in the Baltic Sea.

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Introduction

The Gulf of Bothnia, a temperate brackish-water sea area in the northern Baltic Sea, is ice covered annually for 4–6 months. Ice formation starts in December and ice is thickest in February/March, when mean ice thickness is 50–80 cm in the Bothnian Bay and 25–40 cm in the Bothnian Sea (Mälkki and Tamsalu 1985). Despite its low salinity, sea ice in the Baltic Sea is structurally quite similar to polar sea ice (Leppäranta et al. 1998) and ice-associated organism assemblages closely resemble those found in the Arctic and Antarctic (e.g. Norrman and Andersson 1994; Ikävalko 1997; Mock et al. 1997). The organism assemblages in Baltic sea ice consist of diatoms, photo- and heterotrophic flagellates, cyanobacteria, heterotrophic bacteria and metazoa (Huttunen and Niemi 1986; Norrman and Andersson 1994; Laamanen 1996; Ikävalko 1997; Mock et al. 1997). Diatoms and various autotrophic flagellates are the most important algal groups; hetero- and mixotrophic organisms (flagellates and ciliates) compose 2–10% of the total carbon biomass (Haecky et al. 1998). The structure and functioning of the Baltic sea-ice ecosystem have not yet been satisfactorily described, but the presence of primary producers, consumers and bacteria indicates the existence of an active microbial food web.

In the Gulf of Bothnia, the amounts of algal carbon and chlorophyll-*a* in sea ice are relatively low (< 1 mg m^{-3} of chl-*a*) until a dense interior algal bloom with chlorophyll-*a* concentration of 5–20 mg m^{-3} occurs in March and April. Concentrations of silicate, phosphate and ammonium tend to rise during the winter months, and are subsequently lowered or even exhausted as the ice spring bloom develops (Norrman and Andersson 1994; Haecky et al. 1998). The accumulation of inorganic nutrients and abundant heterotrophic organisms found in seasonal Baltic sea ice indicates active wintertime nutrient regeneration inside the sea-ice matrix. Heterotrophic flagellates and bacteria are most abundant in the middle parts of the ice column

(Norrman and Andersson 1994; Ikävalko and Thomsen 1997) and, as in Arctic sea ice (Gradinger et al. 1992), heterotrophic communities seem to be interior in Baltic sea ice. Nutrient remineralisation is associated with heterotrophic carbon mineralisation and thus the interior parts of ice are the most obvious places for nutrient regeneration. Earlier studies on Baltic sea ice have included nutrient measurements (Norrman and Andersson 1994; Rahm et al. 1995; Mock et al. 1997; Haecky et al. 1998) but detailed studies on the sea-ice nutrient status in the Baltic Sea have not been conducted. The aim of this study was to investigate wintertime nutrient status of seasonal sea ice in the Gulf of Bothnia and reveal the different processes affecting nutrient dynamics. Special attention was paid to nitrogen cycle processes. In order to investigate the existence of active nitrogen reduction in the ice environment, the relative activity of denitrifying organisms was measured.

Materials and methods

Sampling

Ice, brine and water samples were collected in February 1998 at eight different coastal locations in the vicinity of 63°33′N, 19°49′E in the Gulf of Bothnia, Baltic Sea. All sampling locations were situated within 1 km distance from land and each other. Ice cores were taken from 1- to 2.5-month-old land-fast ice. The ice thickness at the sampling area was 21–62 cm; snow cover varied between 0 and 18 cm and water depth was 6–14 m. A motor-driven stainless steel ice corer, 13 cm in diameter, was used for ice coring. The ice temperature was measured immediately after coring from small holes drilled to the core at 5-cm intervals. Ice cores were cut with a hand-saw into 10-cm sections and each section was placed in a 2.5-l acid-washed plastic container. The brine samples were obtained by drilling shallow ''dead-end'' holes into the ice and collecting the brine with 100-ml syringes and silicone tubing. Brine samples could not be recovered from sampling stations 6 and 7 because of meltwater accumulation on the ice. At the end of the sampling period the air temperature decreased and brine sampling was conducted at stations 9 and 10, situated close to stations 6 and 7. Different sample types obtained from sampling stations are shown in Table 1. Samples from the water immediately beneath the ice were taken from a drill hole with a 5-l water sampler. Water and brine samples were stored in 200-ml dark, acid-washed glass bottles. Samples for determination of dissolved nutrients were taken in 50-ml polypropylene centrifuge tubes. All samples were kept dark and cold after the collection and transported to the laboratory immediately. Ice-core samples were thawed at +5 °C within 24–38 h and sub-sampled for further analyses.

Physico-chemical parameters and chlorophyll-*a*

Salinity was measured from all samples (thawed ice-core sections, brine and water) with a WTW LF 196 salinometer. The brine content of ice was calculated following the equations given by Leppäranta and Manninen (1988). Dissolved inorganic nutrients (ammonium, nitrate, nitrite, phosphate, silicate) were determined immediately after ice melting at the Umeå Marine Sciences Center (UMSC) using a Technicon TRAACS 800 autoanalyser. The subsamples for total nitrogen and phosphorus determination were frozen in 40-ml acid-washed glass bottles and analysed later in the Finnish Institute of Marine Research using a Skalar 5100 autoanalyser. Standard seawater procedures (Grasshoff et al. 1983) were used for all nutrient determinations. In order to compare nutrient concentrations between ice and water column and reveal effects of biological processes on them, dissolved nutrients were normalised to surface seawater salinity (Dieckmann et al. 1991; Gleitz et al. 1995). Organic nitrogen was defined as total nitrogen (TN) − dissolved inorganic nitrogen (DIN), and therefore contains both dissolved organic nitrogen (DON) and particulate organic nitrogen (PON).

For chlorophyll-*a* determination, duplicate 50 ml of sample water (thawed ice, brine, water) was filtered onto 25-mm Whatman GF/F filters. The filters were placed in 10 ml 96% ethanol and chlorophyll-*a* was extracted at room temperature in the dark for 24 h. The extract was filtered through 0.2-μm pore-size Sartorius Minisart filters and fluorescence was measured with a Perkin-Elmer LS-30 fluorometer calibrated with pure chlorophyll-*a*. Chlorophyll-*a* concentrations were calculated according to HELCOM (1988).

Total bacterial number and bacterial biovolume

Sub-samples of 10 ml were taken from thawed ice cores, brine and water samples and fixed with 37% formalin (final concentration 1% formaldehyde). The ice cores were thawed without osmotic buffering (Garrison and Buck 1986) since ice bacteria are able to survive short-term salinity reductions (Helmke and Weyland 1995). One millilitre of each formaldehyde-fixed sample was filtered onto black 0.2-μm pore-size polycarbonate filter (Poretics) and stained for 5 min with 0.015% acridine orange solution. The air-dried filters were stored in the dark at room temperature. Total bacterial numbers were counted 5 months after sampling using epifluorescence microscopy Bacterial biovolume was determined by image analysis (Massana et al. 1997).

Activity of denitrifying organisms

For estimating the relative activity of denitrifying organisms in sea ice, brine and underlying water, the acetylene inhibition method was used (e.g. Gerdhardt 1981). Glass vials (20 ml) containing 5 ml of semi-solid nitrate-nutrient agar (Brettar and Höfle 1993) were inoculated with 200 μl of sample water (thawed ice, brine, water). The vials were sealed gas-tight with rubber stoppers and aluminium seal rings and 0.15 ml acetylene was injected to gas phase. All samples were incubated for 14 days in the dark at 0 °C. After

Table 1 Sampling stations, sample types and parameters analysed. Abbreviations *i*, *b* and *w* refer to ice, brine and water samples, respectively. Physical parameters include salinity and temperature measurement; biological parameters include chlorophyll-*a*, total bacterial number and bacterial biovolume

Station	Ice	Brine	Water	Physical parameters	Nutrients	N_2O production	Biological parameters
1	+	+	+	i,b,w	i,b,w	i,b,w	i,b,w
2	+	+	+	i,b,w	i,b,w	i,b,w	i,b,w
3	+	+	+	i,b,w	i,b,w	i,b,w	i,b,w
6	+		+	i,w	i,w	i,w	i,w
7	+		+	i,w	i,w	i,w	i,w
8	+	+	+	i,b,w	i,b,w	i,b,w	i,b,w
9		+	+	b	b	b	b,w
10		+	+	b	b	b	b,w

incubation, N_2O was measured by gas chromatography from the gas phase of the vials. Measurement was done by using a Micromat HRGC 412 gas chromatograph furnished with an electron capture detector (10 mCi ^{63}Ni), operated at 310 °C. A stainless steel Hewlett Packard Hayesep Q column (10 ft, 1/8″, mesh 80/100) at 50 °C with carrier gas flow of 20 ml/min (90/10 vol/vol Ar/CH_4, AGA, Finland) was used for sample separation. Measurement was calibrated against N_2O-air mixtures (AGA) prepared each day. Standard deviation of N_2O measurement was less than 8% and detection limit 20 ppm(v). Average standard deviation between parallel samples in a separate sample series was 20% ($n = 6$, data not shown).

Results

Air temperature varied remarkably in the course of the sampling period; during the first sampling period, temperature rose rapidly from −4 to +8 °C, stayed above zero for several days and fell again (Fig. 1). Variation in the air temperature influenced sea-ice temperature within hours and its increase caused ice surface melting. Brine salinity decreased from 20–30 psu to approximately 6 psu during the warm period as a consequence of internal melting and increased brine volume. Calculated brine volume varied between 1 and 25%. The ice structure was investigated from the cores by eye and was similar at all sampling stations: the uppermost 10–20 cm of ice was opaque (granular ice or frozen snow) and the rest clear congelation ice. In the lowermost 5 cm, brine channels were enlarged and ice was visibly porous.

The studied sea ice was divided into two categories according to age, thickness and salinity. Salinity was higher in approximately 1-month-old "thin ice" (stations 3 and 8, thickness < 0.25 m) than in 2- to 3-month-old "thick ice" (stations 1, 2, 6, 7, 9 and 10, thickness > 0.4 m). All sampling stations, sample types and parameters measured are shown in Table 1. The salinity range of brine and underlying seawater is shown in Table 2. Snow cover was present only on the first sampling day: at stations 1 and 2, snow thickness was 13 and 18 cm, respectively, while at station 3 only 6 cm.

Except the uppermost ice layer, chlorophyll-*a* concentration in sea ice exceeded concentration of the underlying water (Fig. 2). In thick ice, concentrations were generally below 1 mg m^{-3} whilst concentrations of up to 5.6 mg m^{-3} were measured in thin ice. In thick ice, an interior chlorophyll maximum was typical while in thin ice, a pronounced bottom maximum occurred (Fig. 2). Chlorophyll-*a* concentration in thick ice-brine samples was lower than the expected values based on total chlorophyll-*a* content of the melted ice and calculated brine volume. Only 0.3–1.6% (thick ice) and 1.8–68% (thin ice) of the excepted chlorophyll-*a* content was found in the brine samples.

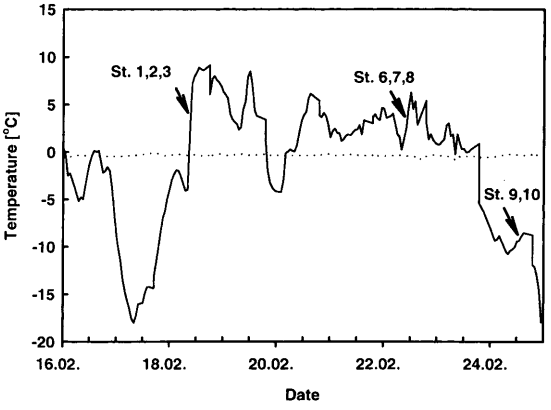

Fig. 1 Time course of air (*solid line*) and water (*dashed line*) temperature at study area during sampling period in February 1998. *Arrows* denote sampling occasions; sampling station numbers are shown (data: Umeå Marine Sciences Center)

In the ice column, highest concentrations of ammonium, nitrite, nitrate and phosphate were found in the uppermost part, whilst silicate concentration was highest in the middle part. Nitrate and ammonium were the dominant inorganic nitrogen compounds (Table 2). Phosphate concentrations in brine were remarkably low, which led to extremely high N:P ratios. N:P ratios were 273–2487 in brine, 25–39 in underlying water and 3–238 in sea ice. Values near the Redfield ratio 16:1 were observed in the lowermost 20 cm of ice and the ratio increased towards the upper parts of the ice column.

All normalised nutrient concentrations (Fig. 3) in brine and sea ice exceeded underlying water concentrations; only in thin ice was silicate concentration lower than in underlying water. In thick ice, internal maxima of ammonium, nitrite, phosphate and especially silicate

Table 2 Nutrient concentration range and mean values (*in parentheses*) in different sample types. Upper ice layer means 10–20 cm below the ice-air interface; lower part means lowermost 10 cm of ice [*n* number of samples, *S* salinity (psu), *T* temperature (°C), *TN* total nitrogen, *TP* total phosphorus, *n.d.* no data]; nutrient concentrations (μM)

Sample	*n*	*S*	*T*	NO_3^-	NO_2^-	NH_4^+	$SiOH_4$	PO_4^{3+}	TN	TP
Ice	9	0.1–1.6	−2.7–0.3	5.5–12.2	0.07–0.24	1.7–7.6	0.9–8.8	0.07–0.22	15.4–38.6	0.13–0.36
Upper part		(0.7)	(−0.82)	(8.3)	(0.15)	(5.61)	(5.5)	(0.14)	(27.2)	(0.23)
Middle part	1	0.2–1.4	−1.6–0.2	0.2–3.6	0.05–0.11	0.4–2.0	1.5–10.6	0.05–0.25	9.0–29.6	0.13–0.59
Lower part	7	(0.6)	(−0.56)	(1.5)	(0.08)	(1.23)	(6.7)	(0.14)	(17.6)	(0.31)
Brine	6	6.0–31.5	n.d.	22.7–302.1	0.3–4.7	1.7–46.6	30.4–288.6	0.04–0.67	47.6–430	0.27–1.14
		(15.3)	(132.2)	(1.8)	(18.00)	(114.6)	(0.22)	(190)	(0.63)	
Water	6	4.2–4.7	−0.2–0.1	6.0–6.3	0.10–0.17	0.1–0.2	23.1–25.9	0.16–0.26	18.0–24.4	0.27–0.43
		(4.5)	(−0.2)	(6.1)	(0.13)	(0.15)	(24.6)	(0.21)	(20.6)	(0.38)

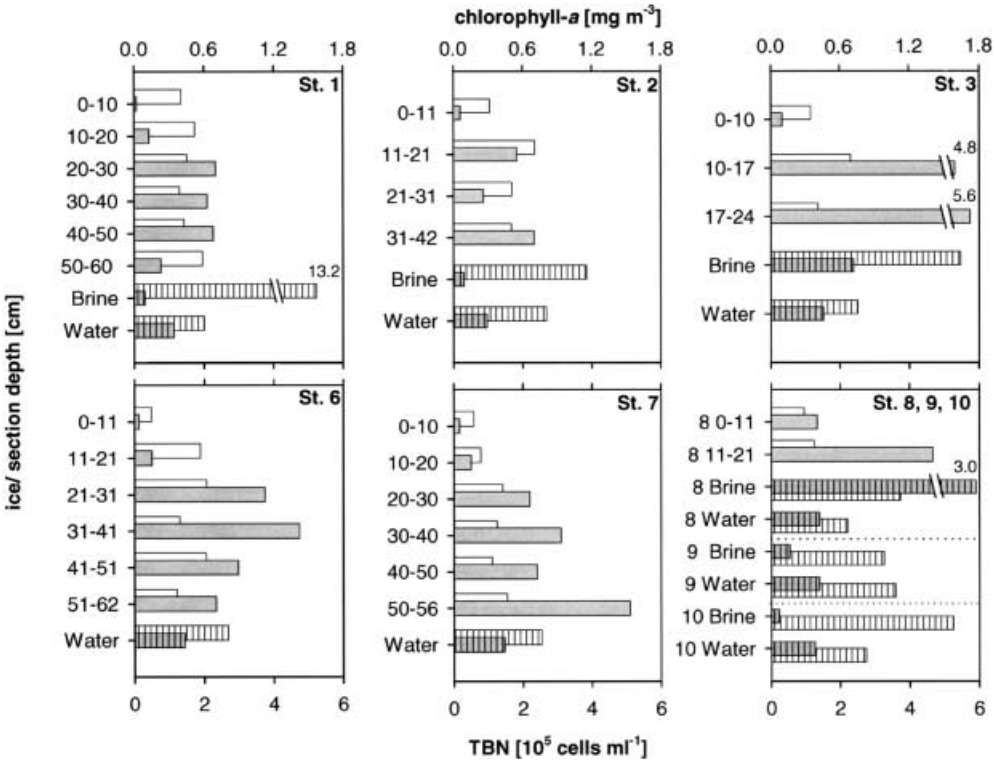

Fig. 2 Vertical profiles of chlorophyll-*a* (chl-*a grey shaded bars*) and total bacterial number (TBN *unshaded bars*) in melted ice cores, brine and underlying water. The *dashed bars* denote underlying water samples

were found. Nitrate concentration decreased from top to the bottom of ice and showed no maxima in the interior part of the ice. In the bottom 10 cm of thick ice, nutrient concentrations were close to the underlying water concentrations. In thin ice, no clear vertical gradients were found, probably due to coarse sectioning of the ice cores. There was a significant positive correlation between all nitrogenous nutrients in pooled ice-core data, while phosphate and silicate showed no significant correlation with any other nutrient (Table 3).

Total nitrogen concentrations in the sea ice were highest in the upper and lowest in the middle part (Fig. 4). In the upper part of thick ice, TN was dominated (30–65%) by the inorganic nitrogen compounds, nitrate and ammonium concentrations being roughly equal. In the middle and lower parts of the ice, inorganic nitrogen constituted only 10–25% of TN, ammonium being usually the dominant form. Nitrate was found to occur in excess in brine compared to ice values and most of the brine TN was nitrate. In the underlying water, 20–35% of TN was inorganic, mainly nitrate.

Total bacterial number (TBN) in underlying water was of the same order of magnitude as in melted ice samples but lower compared to the brine values (Fig. 2). As for chlorophyll-*a*, only a fraction of TBN could be recovered in brine samples: TBN values in the brine fraction were only 10–13% and 18–66% in thick and thin ice, respectively, of expected values based on melted ice TBN and calculated brine volume. Vertical distribution of TBN in the ice column was even and no clear maxima occurred. The average biovolume of sea-ice bacteria was 0.178 μm^3, reaching maximum values in the middle parts of the ice column (Fig. 5). In brine and water, the average bacterial biovolumes were 0.143 and 0.117 μm^3, respectively. The median value of cell biovolume was lower in brine than in melted ice cores (*U*-test, $n = 16$, $P = 0.000$ in 13 cases). Large bacterial cells (biovolume > 0.2 μm^3) were most abundant in the interior part of the ice column (Fig. 5).

Visible bacterial growth was observed in all culture vials after 14 days incubation. Colonies were generally small and often had yellow or orange coloration. Measured N_2O production was highest in culture vials inoculated with brine and thawed ice from the middle parts of thick ice (Fig. 3). In the upper and lower parts of thick ice, thin ice and water, N_2O production was low or not detectable.

Discussion

In thick, 2- to 3-month-old sea ice, communities were clearly at the winter stage during the study period. Chlorophyll-*a* concentrations in melted ice cores (Fig. 2) were in the same range as earlier measured winter values (Haecky et al. 1998), and heterotrophic organisms, especially *Cryothecomonas armigera* (Thomsen et al. 1991) composed a substantial part of the organism assemblage (Table 4). In thin ice and thin-ice brine higher chlorophyll-*a* concentrations and algal cell numbers suggest that the ice spring bloom was already developing. Increase in snow cover and ice thickness greatly reduce the amount of downwelling irradiance in the ice column

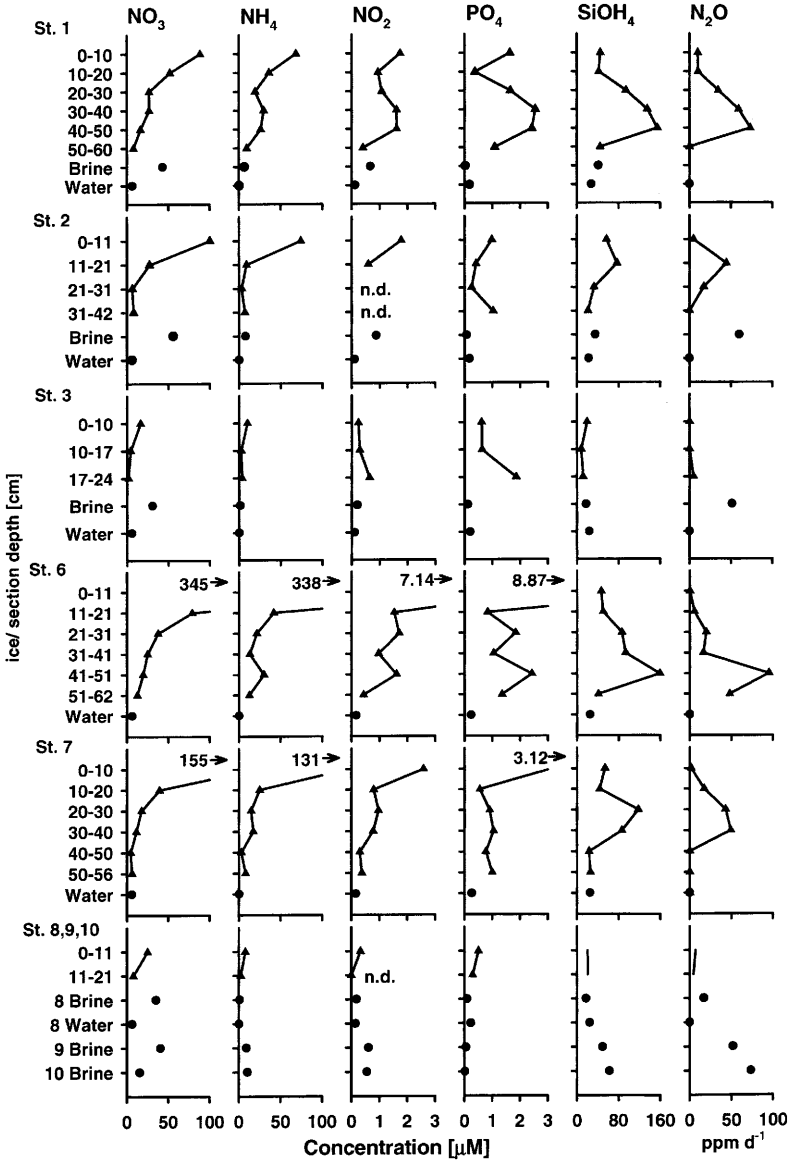

Fig. 3 Vertical profiles of salinity-corrected dissolved inorganic nutrient concentrations. N_2O production in culture vials (*n.d.* no data)

(Maykut 1985). Better light conditions due to the thinner snow and ice cover in thin ice may explain observed differences as increase in available light initiates the sea-ice spring bloom in the study area (Norrman and Andersson 1994; Haecky and Andersson 1999).

Brine motion inside the brine channel system does not necessarily cause movement of ice organisms inhabiting channels and pockets. Investigations from Antarctic pack ice show that only a small fraction of organisms is recovered in brine samples while most stay within the brine channel system (Gradinger et al. 1992 and references therein). This is also the case for Baltic sea ice, since the measured concentrations of chlorophyll-*a* and organic nitrogen in thick ice brine were noticeably lower than expected (Figs. 2, 4). Cell counts revealed that an especially large fraction of diatoms and other non-flagellated algal cells stayed inside the ice matrix while flagellated cells were more easily recovered in the brine fraction (Table 4). Heterotrophic organisms and bacteria also showed different quantitative and qualitative distributions in thawed ice and brine samples. Attachment of large bacterial cells on diatoms or organic particles (Sullivan and Palmisano 1984) possibly caused the observed relative enrichment of smaller bacterial cells into the brine fraction. The chemical composition of brine differed also from the expected values. Phosphate did not concentrate in brine in relation to salinity or other dissolved nutrients, which can be seen in the extremely high N:P ratios in brine. Similar low brine phosphate concentrations were seen in western Baltic sea ice (Mock et al. 1997) and Antarctic pack ice (Garrison et al. 1990). Phosphate is possibly adsorbed onto surfaces, e.g. on diatoms and sediment (Leppäranta et al. 1998) remaining inside the brine channel system.

Most of the brine TN was dissolved inorganic nitrogen, mainly nitrate, while in melted ice samples organic nitrogen was dominant. The reason for the observed nitrate excess in relation to other DIN species

Table 3 Pearson correlation matrix of biological and chemical parameters of the pooled ice-core data. Samples from the upper 10 cm of ice are excluded to remove the effect of atmospheric precipitation on correlations [V_{bact} median value of bacterial cell volume; N_2O nitrogen dioxide production in culture vials ($n = 21$)]; correlation coefficients ≥0.54 are significant, $P \leq 0.01$

	S	Chl *a*	N_2O	V_{bact}	NO_2	NO_3	NH_4	$SiOH_4$
Chl *a*	0.11	–						
N_2O	−0.69	−0.20	–					
V_{bact}	−0.31	−0.28	0.43	–				
NO_2	−0.07	−0.13	−0.02	0.10	–			
NO_3	0.26	−0.37	−0.28	0.08	0.83	–		
NH_4	0.14	−0.38	−0.04	−0.01	0.87	0.92	–	
$SiOH_4$	−0.41	−0.61	0.62	0.60	0.29	0.16	0.30	–
PO_4	0.28	0.45	−0.18	−0.51	−0.07	−0.22	−0.10	−0.43

in brine remains unclear. Different quotas of organic and inorganic N in brine and melted ice are obviously due to entrapment of organic particles inside the ice matrix. Unfortunately, methods used in this study cannot distinguish DON and PON. Information on DON distribution in the ice column would be helpful in understanding nitrogen dynamics and will be included in further studies.

Nutrient concentrations varied greatly within and between sample types, but were generally in good agreement with those of Rahm et al. (1995), measured from sea ice in the same area, and also in the same range as western Baltic Sea ice values (Mock et al. 1997). Concentrations of all nitrogenous nutrients and phosphate were elevated in the upper part of thick ice. High concentrations of dissolved inorganic nitrogen, as well as the conservative behaviour of silicate in the uppermost 20 cm of thick ice (Fig. 3), suggest that atmospheric precipitation is controlling the nutrient status of the upper 20 cm of ice. This is consistent with earlier observations of atmospheric nutrient load accumulation on top of the ice in the Gulf of Bothnia (Rahm et al. 1995). Salinity of the uppermost 10 cm of ice was low and this layer is at least partly frozen snow.

Weak correlation between various nutrients and salinity in pooled ice-core data (Table 3) suggests that different biological processes are the main factors controlling the nutrient status of middle and lower parts of sea ice in the Gulf of Bothnia. Middle layers of thick ice especially seem to be sites for active heterotrophic processes, as heterotrophic organisms are abundant and average bacterial cell volume is enlarged (Table 4, Fig. 5). Gradinger and Zhang (1997) observed a similar bacterial size distribution pattern in Arctic multiyear ice floes as reported in this study and they assumed that high DOM concentration in the ice environment is one of the factors responsible for the increased bacterial cell sizes. Accumulation of ammonium and phosphate (Fig. 3), abundance of heterotrophic organisms and enlarged bacterial cell size in the middle layers of thick ice point to active carbon mineralisation and adjacent regeneration of N and P. Norrman and Andersson (1994) measured the highest bacterial production and heterotrophic flagellate numbers in the middle layers of approximately 50-cm-thick ice during the ice spring bloom. Heterotrophic activity seems to be located in the same layers also under pre-bloom conditions in February. The accumulation of phosphate, silicate and ammonium in the middle part of ice probably enhances the interior algal bloom during March/April (Norrman and Andersson 1994; Haecky et al. 1998).

The nitrogen cycle in the sea-ice environment is an interesting, although so far, understudied subject. Accumulation of temporary intermediate compounds of the nitrogen cycle is frequently observed in sea ice of both polar areas (Oradiovskiy 1974; Clarke and Ackley

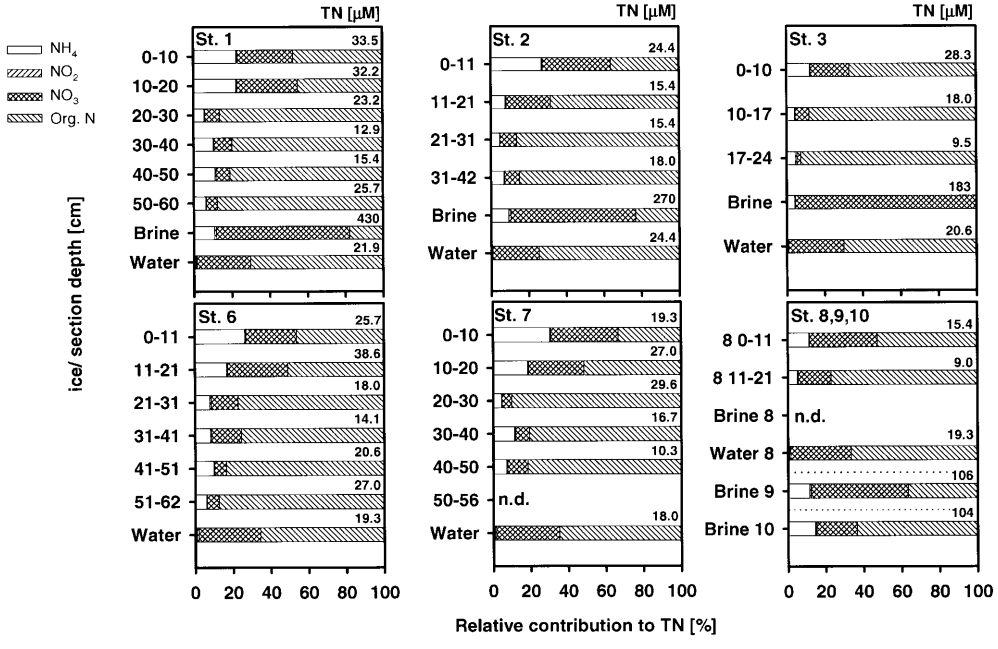

Fig. 4 Amount of total nitrogen (*TN*) and proportion of different nitrogen species in total nitrogen (*n.d.* no data)

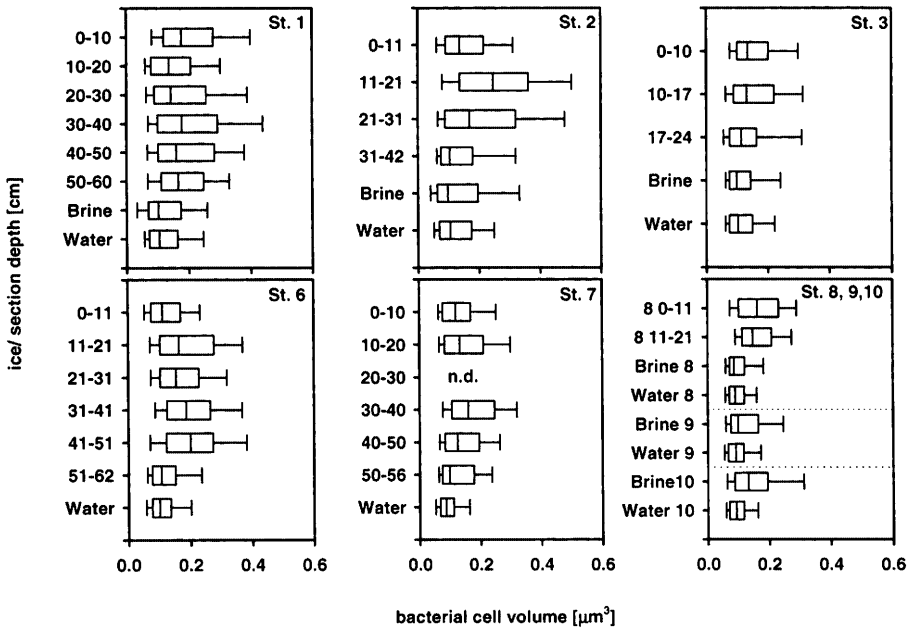

Fig. 5 Box and Whisker plots of the bacterial cell volume in sea ice, brine and underlying water. Whisker gaps denote 10th and 90th percentiles (*n.d.* no data)

Table 4 Cell counts and proportion of different organisms in ice, water and brine samples. Ice values are means of pooled ice samples (*Hnano* small heterotrophic flagellates, *C.a. Cryothecomonas armigera*). The *last column* gives the proportion of heterotrophic cells to all counted cells (data: J.-M. Rintala)

Station	Sample type	Autotrophic				Heterotrophic				
		Cells l^{-1} ($\times 10^4$)	Diatoms (%)	Flagellated (%)	Others (%)	Cells l^{-1} ($\times 10^4$)	Hnano (%)	*C.a.* (%)	Others (%)	Heterotrophic cells (%)
1	Ice	12.2	53	30	17	1.07	13	63	24	7
	Brine	12.4	7	82	11	7.16	20	75	5	37
	Water	16.8	49	25	26	1.02	31	0	69	6
2	Ice	20.9	17	36	46	3.53	15	64	21	19
	Brine	28.4	16	75	9	50.8	4	79	16	64
	Water	16.8	26	25	49	1.02	31	0	69	6
3	Ice	59.2	18	40	54	2.21	29	44	27	4
	Brine	34.1	27	24	49	0.53	0	60	40	2
	Water	6.3	1	68	31	0.13	0	0	100	2

1984; Meese 1989; Garrison et al. 1990; Thomas et al. 1995). Sea-ice nutrient data from the Baltic Sea also show elevated concentrations of reduced nitrogen compounds (Norrman and Andersson 1994; Rahm et al. 1995; Mock et al. 1997; Haecky et al. 1998). Although correlation between the concentrations of different nutrients in sea ice is usually low, nitrogenous nutrients are often significantly correlated with each other, which points to the occurrence of active nitrogen transformation in the sea-ice environment (Meese 1989; Thomas et al. 1995). Furthermore, N_2O, an intermediate compound of denitrification and nitrification, has been shown to be emitted to the atmosphere from sea ice in both polar areas (Gosink 1980).

Denitrification is known to occur in microsites surrounded by fully aerobic environments, e.g. water-filled soil pore spaces, although lowered oxygen concentration in the environment is the major controlling factor (Tiedje 1988). The respiratory activity of organisms is the major mechanism that removes oxygen from the microsites and it is driven by available organic carbon (Tiedje 1988). High concentrations of nitrate and organic carbon and abundance of heterotrophic organisms make suboxic microsites in brine channels and pockets a potential site for nitrogen reduction. Thomas et al. (1995) observed a high correlation between nitrite and DOC in Arctic multiyear ice cores and suggested that nitrite accumulation is associated with organic carbon decomposition, which points to active nitrate reduction. Denitrification has been also offered as a possible explanation for observed high nitrite concentrations in 1st-year (Gradinger and Ikävalko 1998) and multiyear Arctic sea ice (Meese 1989). Antarctic sea ice hosts high psychrophilic bacterial diversity, and bacterial strains closely related to denitrifying species are abundant (Gosink and Staley 1995; Bowman et al. 1997; Zumft 1997). However, nitrate

reduction or denitrification in the sea-ice environment have not yet been directly studied.

Significant positive correlation between all nitrogenous nutrients in pooled ice core data indicates the occurrence of active nitrogen transformations in Baltic sea ice. Denitrifying activity, i.e. the highest N_2O production in cultures, was located in the same ice layers with high heterotrophic activity, nutrient regeneration and accumulation of nitrite (Fig. 3). These interior layers of 2- to 3-months-old ice are probably the sites for the nitrogen transformations occurring in Baltic sea ice. The culture method used to estimate activity of denitrifying organisms in this study does not give information about actual denitrification rates, but the enrichment of denitrifying organisms in the middle layers of thick ice, together with elevated nitrite concentrations, indicate active nitrate reduction and also possible denitrification. High correlation between ammonium and nitrate, as well as ammonium and nitrite, in pooled ice-core data suggests that nitrification is also active in the ice environment. However, any nitrate accumulation was not observed.

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