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Experimental evidence on nutrient and substrate limitation of Baltic Sea sea-ice algae and bacteria

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Abstract

The effect of nutrient limitation on Baltic Sea ice algae, and substrate and nutrient limitation on ice bacteria, was studied in a series of *in situ* -experiments conducted during the winter of 2002 in northern Baltic Sea. Community level changes in algal biomass (chlorophyll *a*) and productivity, and bacterial thymidine and leucine incorporation were followed for one week after the addition of nutrient and/or organic carbon rich filtered seawater to the experimental units. The results showed the major contribution of snow cover to the algal responses during the beginning of the ice-covered season. Algal communities were able to grow even in January if no snow was present. Nutrient addition did occasionally have an effect on algal biomass and productivity in the ice. Surprisingly, seeding effect from the ice to the underlying water was negatively affected by the nutrient availability in March. Bacterial limitation varied between nutrient (phosphorus) and substrate limitations. The results showed, that limitation in both algal and bacterial communities changed periodically in the northern Baltic Sea ice.

Introduction

The seasonal Baltic Sea ice is structurally similar to seasonal oceanic sea-ice, with the exception of the proximity of large rivers along the Baltic Sea coast (Palosuo, 1961). The bulk salinity of the ice shows both year-to-year and seasonal variability (Granskog et al., 2004), but it is considerable, thus enabling the existence of a well-developed brine channel system and the activity of ice biota (Norrman & Andersson, 1994; Haecky et al., 1998; Granskog et al., 2003; Granskog et al., 2004; Kaartokallio, 2004). Primary producers, bacteria and a number of protozoa are known to inhabit Baltic Sea ice. Production is based on the activity of variable groups of cold-adapted diatoms, dinoflagellates and other taxa (Ikävalko & Thomsen, 1997; Haecky et al., 1998). Algal communities may reach high biomass values in the lower layers of ice (Granskog et al., 2003), but well developed interior assemblages are also found (Haecky et al., 1998; Granskog et al., 2003). Bacteria are also active and important component of Baltic Sea ice assemblages (Norrman & Andersson, 1994; Mock et al., 1997; Kaartokallio, 2004).

Baltic Sea ice communities live under very low light conditions throughout most of the ice-covered season. It is justly proposed, based on the succession of ice algae, that light is limiting algal growth in the northern Baltic Sea (Haecky et al., 1998). The ambient light intensity above the ice and snow could enable the growth of shade-adapted algae during the first quarter of the year, but specifically even a shallow snow cover cuts away most of light reaching the ice layer (McGrath Grossi et al., 1987). In northern Baltic Sea nutrient limitation is prevalent during most of the open water growth season (Kivi et al., 1993). Thus it is

possible that nutrients limit ice productivity in clear ice conditions at least when higher light intensities are reached in late March and April. However, the question of nutrient limitation has been scarcely addressed in the studies on Baltic Sea sea-ice biota. Haecky et al. (1998) point at phosphorus limitation at the late stages of the ice-covered season, and also potential silicate limitation, in their work from the Gulf of Bothnia.

Baltic Sea bacterioplankton is known to be either carbon or nutrient (mainly phosphorus) limited during the cold-water spring period (Kuparinen & Heinänen, 1993), though the bacteria sampled from the open sea in February and March showed no responses to nutrient additions in low temperatures at all (Autio, 1998). Organic carbon is known to be abundant in the Baltic Sea sea-ice (Granskog et al., *in press*), but it may be of poor quality when bacterial metabolism is considered. Available phosphorus is most probably at least temporarily consumed to low levels during algal biomass accumulation, and the semi-closed ice system makes it difficult to substitute from the underlying water. In addition, the fresh water flowing under the ice near coastline is mostly short in phosphorus.

In order to study the effect of nutrient limitation on Baltic Sea ice algae, and substrate and nutrient limitation on bacteria, an experimental approach was applied. We conducted *in situ*-experiments with different levels of concentrations of nutrients and a carbon source. The aim of this study was to find out if sea-ice communities respond to different nutrient and substrate environments, and if succession-based differences in the responses can be found. Only community level responses are evaluated in this study, and no effort is put into separating the effects of the two main nutrients, as this is the first experimental *in situ* attempt to disentangle the basic limitation dynamics in the northern Baltic Sea sea-ice.

Materials and methods

Study area

The experiments were made at Santala Bay, about 20 km from Tvärminne Zoological Station, SW Finland. Santala Bay is a sheltered brackish water bay without any major inflows of fresh water, but still it is affected by runoff from the land. The sheltered character of the bay enables maximum ice season length and ice thickness found in the area. The water salinity is Santala Bay is, during open water season, from 4 to 5 PSU, which makes Santala Bay ice standard Baltic sea ice. In 2002 the ice-covered period was only 3 months due to the uncharacteristically mild winter.

Experimental set-up and sampling

Four identical successive experiments were run in January, mid-February, early and late March, the duration of each experiment being one week. The experimental set-up consisted of nine transparent 1-m long Plexiglas tubes (∅ 10 cm) with closed bottom equipped with an outlet valve (Fig. 1). In the beginning each experiment twelve ice cores were obtained using a CRREL-type power auger (MARK II, Kovacs Enterprises, Lebanon NH). Under-ice water samples were obtained from immediately under the ice using a 1-l Ruttner-type water sampler. Ice temperature was immediately recorded using a Testo 720 electrical thermometer. Three cores were immediately cut into 5–10 cm sections with a handsaw, placed in clean plastic containers and transported to the laboratory for melting and subsequent processing. Nine cores

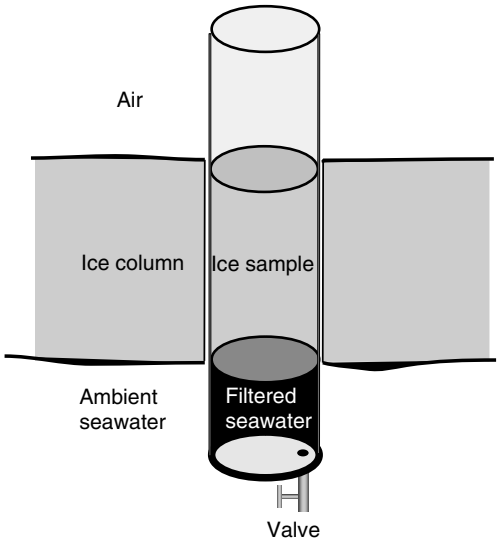

Figure 1. A diagram of the experimental setup with a Plexiglas tube.

were put into the Plexiglas tubes containing 2 l of sterile filtered seawater (Sartobran 300 capsule 0.45 + 0.2 μm, Sartorius GmbH, Göttingen, Germany), originating from a much more open sea area near Tvärminne, amended with nutrient and carbon additions. Three tubes served as a manipulation control with no additions, two received carbon (sucrose, final conc. 1 mg C l^{-1}), two nutrients (NO_3-N 160 μg N l^{-1}, PO_4-P 40 μg l^{-1}) and two both carbon and nutrients. Tubes were placed to their original boreholes, forming a 3 × 3 array with 30–40 cm spacing between the tubes, and adjusted to the right level so, that ice cores inside the tubes retained their original vertical position. Tubes were fastened with nylon cord and screws to the ice and incubated for 6–7 days. Plexiglas tubes were wrapped with 1 mm thick plastic foam foil up to the ice surface level to enhance recovery after incubation period. At the end of the experiment, the Plexiglas tubes were recovered from the boreholes, under-ice water inside the tubes sampled *via* bottom outlet valve and cores handled as described above. In addition to the cores recovered from Plexiglas tubes, one ice core and under-ice water sample reflecting the ambient situation was obtained within 30 cm of the experimental field. In the laboratory ice sections were immediately sampled for bacterial and primary production measurement and then thawed in a cold room at +5 °C overnight. Direct melting was used, as it has been shown to be suitable for the study area (Kaartokallio, 2004). After thawing the samples were mixed carefully and sampled for further analyses.

Primary and bacterial production

For primary and bacterial production measurement samples containing known amount of ice crush and concentrated seawater (Kaartokallio, 2004) were prepared as follows. Each intact 5–10 cm ice core section was crushed using a spike and electrical ice cube crusher approximately 10 ml of crushed ice were placed in a scintillation vial and weighed with a Sartorius 1413 laboratory balance. To better simulate brine pocket salinity and ensure even distribution of labelled substrate, 2–4 ml of 2 × concentrated (by evaporation) filtered (through 0.2 μm) seawater from sampling area were added to the scintillation vials. Production was measured immediately after sample collection and all work was done in a cold room at + 5 °C.

Bacterial production was measured using a ^{14}C-leucine (Kirchman, 1985) and ^{3}H-thymidine (Fuhrman & Azam, 1980, 1982) incorporation methods and dual labelling. Two aliquots and a formaldehyde-killed absorption blank were amended with L-[U-^{14}C] leucine (Amersham; UK, sp. act. 307 mCi $mmol^{-1}$) diluted with carrier leucine in a proportion of 1:5 and [methyl–^{3}H] thymidine (NEN; MA, USA, sp. act. 84.3 Ci $mmol^{-1}$). The concentrations used, 14 nM for thymidine and 1100 nM (ice samples) and 400 nM (water samples) for leucine, were tested to be above the saturating concentration. Samples were incubated in the dark at −0.2 °C in a cooled incubator (LMS 205, LMS UK) for 5–14 h, incubation stopped with addition of formaldehyde and samples processed using standard cold-TCA extraction procedure. A Wallac WinSpectral 1414 counter and InstaGel (Perkin–Elmer) cocktail were used in scintillation counting.

Primary production was measured using an apparent net method (Steemann Nielsen, 1952; Niemi et al., 1983). Samples (two replicates and a dark control) were amended with 50 μl of $NaH^{14}CO_3$ containing 1 μCi (DHI, Denmark) and incubated in constant light of 80 μE at −0.2 °C in a cooled incubator (LMS 250, LMS, UK) for 4 h. The incubations were stopped by taking 4 ml aliquot from the sample and acidifying it with 100 μl of 1 N hydrochloric acid. After 24 h of keeping the sample cap open, 7 ml of InstaGel (Perkin–Elmer) scintillation cocktail was added to the sample and it was counted with a Wallac WinSpectral 1414 liquid scintillation counter. The light level was chosen to represent typical optimum light level of ice algae of the area, with no light inhibition (Kuosa, unpublished). When the primary production was calculated, the values were corrected for different volumes (see above, the density of ice was assumed to be 0.91). Total inorganic carbon content (TIC) in the Baltic Sea water is mainly related to salinity and temperature (and pH). The concentrations may be measured directly or calculated according to specific formulas. In the melted ice samples the variability of TIC may be high according to the bulk salinity and changes caused by the activity of the ice organisms (Gleitz et al., 1995). In this case the TIC values were calculated

with the aid of the formulas given by Buch (1945), which, however, may deviate from measured values in Baltic Sea ice (Kuosa, unpublished). Direct measurements of DIC would be preferred, but in this case that does not have an effect on the analysis as the bulk salinities among the experimental units were similar, and the correlation between bulk salinity and DIC in the small data set ($n = 50$) was linear. That was also tested with the corrected values based on the linear correlation, which produced the same statistical results.

Physicochemical parameters and chrorophyll-a

Salinity was measured from all samples (thawed ice core sections and water) using a YSI 63 temperature-conductivity-pH meter, calibrated with YSI standard solutions. The concentrations of dissolved inorganic nutrients (PO_4-P, NH_4-N, NO_2-N and NO_3-N) were determined manually immediately after ice thawing using standard seawater procedures (Grasshoff et al., 1983). For determination of chlorophyll-*a*, 50-ml aliquots of sample water (thawed ice or water) were filtered through 25-mm Whatman GF/F filters. The filters were placed in 10 ml 94% v/v ethanol and chlorophyll-*a* was extracted at room temperature in the dark for 24 h. The extract was filtered through a GF/F filter and fluorescence was measured with a Shimadzu RFPC 5001 fluorometer, calibrated with pure chlorophyll-*a*. The chlorophyll-*a* concentrations were calculated according to HELCOM (1988).

Data analysis and statistical methods

The data dealing with algal parameters (chlorophyll-*a* and primary productivity) was divided only to two different categories: the units with no nutrient addition (5) and the units with nutrient additions. This was made because there is no reason to separate carbon additions in algal parameters. The preliminary analysis of data also showed that carbon did not have an effect on algal parameters. The difference between start values and control values at the end of experiment and the difference between nutrient manipulations at the end of the experiment was analysed with the parametric *t*-test. The data on bacterial productivity between the start values and controls was also analysed with the parametric t-test. The bacterial data at the end of the experiment was analysed with ANOVA taking into account also substrate additions, and if there was a statistically significant effect the groups were tested with an *a posteriori*-test (Tukey) to find similar groups.

Results

Ice formation was quick in early January 2002, and ice devoid of snow cover was already 22 cm thick at the beginning of the 1st experiment (Table 1). Simultaneously, a very well developed algal community had arisen throughout the whole ice column with chlorophyll values up to 20 $\mu g\ l^{-1}$ in the lower layer (Fig. 2). After the 1st experiment some snow fell, which, together with the low ambient light levels, inhibited algal growth and led to lowered chlorophyll-*a* values (Fig. 2). Between the 2nd and 3rd experiments snow melted away, and another growth burst took place in the lower ice layer with chlorophyll-*a* values up to over 30 $\mu g\ l^{-1}$. Already during the 3rd experiment, and after that air temperatures were continuously high leading the apparent flushing of the algae from the ice, significantly diminishing chlorophyll-*a* values (Fig. 2). Primary productivity results confirm the high activity of the algal community at the start of the 3rd experiment, and the flushing effect starting at the 3rd experiment at the lower ice layer (Fig. 3). In the beginning of the 1st and 3rd experiments, algal community in the lower ice was dominated by the dinoflagellate *Scrippsiella hangoei*, which formed 70–80% of the algal biomass. In 2nd experiment the initial lower ice algal biomass consisted mainly of the *S. hangoei* and small unidentified flagellates, and in the 4th experiment the chlorophyte *Dictyosphaerium* sp. and small unidentified flagellates.

Algae were obviously nutrient limited already in January (Figs. 2 and 3). The sudden growth had probably used up available phosphorus from the ice layer and with very low phosphorus values in the underlying water, the P-demand had no possibilities to be met (Table 1). Another period of nutrient limitation was seen during the 3rd experiment in the upper ice layer. During that time the quite even vertical biomass distribution during the beginning of the season had changed to one with

Table 1. Basic parameters for the four experiments. Both the start and end values of air temperature and snow depth are given. Upper and lower denotes the start values for the upper and lower part of the ice, respectively, water the values for under-ice water at the start of the experiment and FSW the values of the added filtered seawater

	Air temp (°C)	Ice (cm)	Snow (cm)	Salinity (PSU)	NO_3-N ($\mu g\ l^{-1}$)	NH_4-N ($\mu g\ l^{-1}$)	PO_4-P ($\mu g\ l^{-1}$)
1st	$+0.3 \rightarrow 0$	22	$0 \rightarrow 0$				
Upper				0.6	32.9	22.8	1.4
Lower				0.6	60.1	25.1	1.2
Water				1.8	2837	138.3	3.0
FSW				5.4	132.6	16.5	26.9
2nd	$+0.9 \rightarrow -4.8$	23	$3 \rightarrow 0$				
Upper				0.3	98.7	61.9	2.1
Lower				0.3	58.3	24.9	2.1
Water				2.2	202.5	11.7	4.0
FSW				5.2	106.3	7.4	26.7
3rd	$+1.1 \rightarrow +4.2$	25	$5 \rightarrow 2$				
Upper				0.3	65.5	32.7	2.5
Lower				0.3	19.7	7.4	1.7
Water				3.2	164.0	5.6	7.0
FSW				5.9	212.8	6.1	28.2
4th	$+5.0 \rightarrow +2.0$	23	$0 \rightarrow 0$				
Upper				0.1	46.7	37.0	2.5
Lower				0.2	24.4	17.2	1.5
Water				1.3	125.2	74.1	2.5
FSW				5.6	87.4	6.8	21.1

prominent algal biomass in the lower ice layer (Fig. 2). The threefold difference in phosphorus concentrations between the plain added filtered seawater and the nutrient manipulations most probably led to higher penetration of nutrients to sea ice due to much steeper concentration gradient. During the most intensive flushing period in March the development of algal communities in the underlying water appeared to be negatively related to added nutrients (Figs. 2 and 3).

Bacterial productivity (leucine and thymidine incorporation) increased clearly during the 1st experiment (Fig. 4). The incorporation rates appeared to reach substrate limitation at least in the lower ice layer and added water. During the 2nd experiment ice bacteria were clearly nutrient limited. During that time phosphorus concentrations were very low both in the ice and the ambient seawater underlying ice (Table 1). Bacteria either transferred or grown in the filtered seawater seemed to be more carbon limited, most probably resulting from the relatively high phosphorus concentration in the added filtered water.

Discussion

The first two weeks of January 2002 were exceptionally cold. This led to the rapid formation of a relatively thick ice cover. The formation of high algal biomass already during the darkest time of the year together with the responses in the 1st experiment show that even during low ambient irradiation ice algal growth is possible. This is not in contradiction with the work by Haecky & Andersson (1999) as the early ice season in 2002 must be considered an aberration, normal situation being featured by a well-developed snow cover. However, temporarily clear areas (leads etc.) at any time of winter may well be areas of active algal growth. After the 1st experiment even a shallow snow cover resulted in declining biomass and apparently light limited communities with no responses to manipulations. The biomass maximum after the disappearance of the snow cover later in March represents normal dynamics, and the effect of nutrients during that period is not surprising (Haecky et al., 1998). Snow cover is of utmost

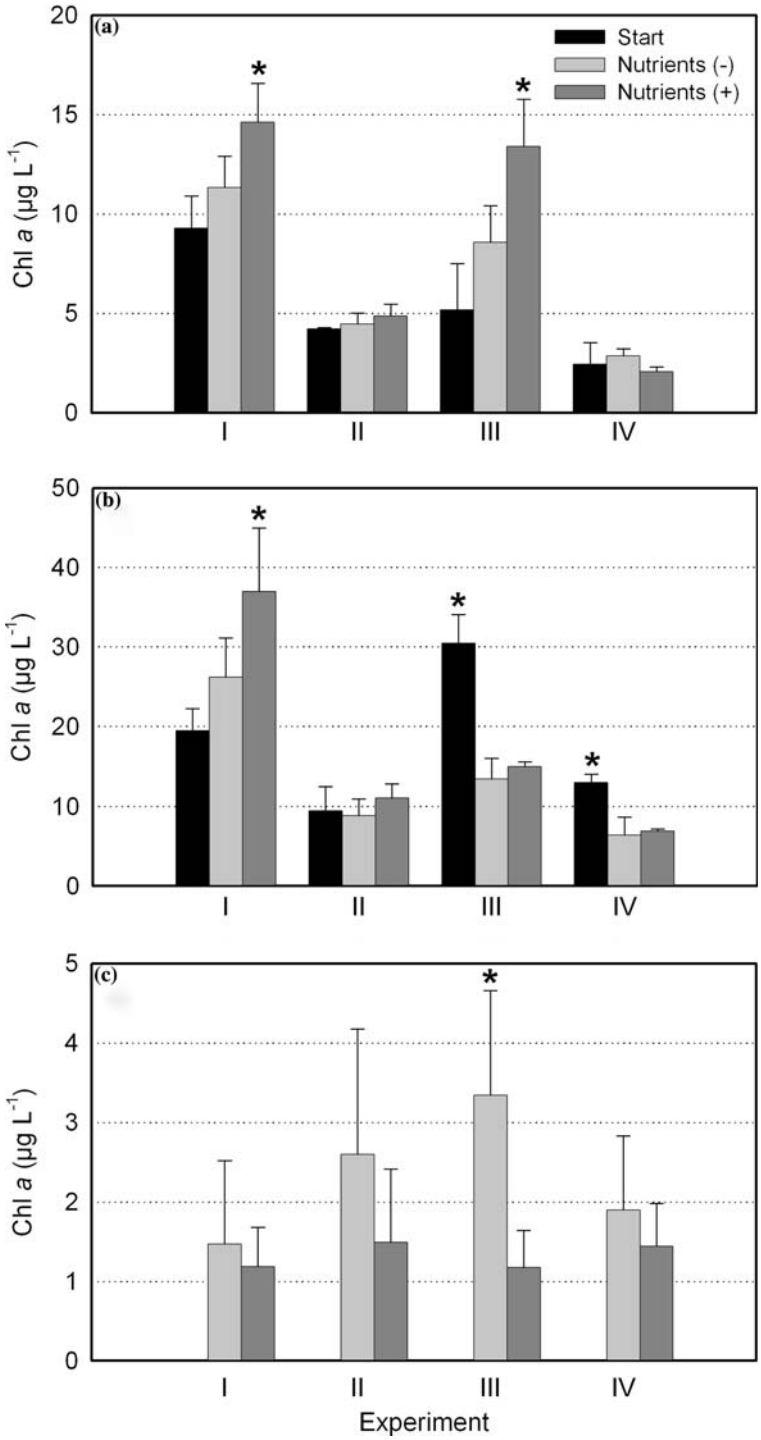

Figure 2. Chlorophyll-*a* concentrations in the upper (a) and lower (b) ice layers and added filtered sea water (c). The mean start value and the average values (± intervals) without N(−) and with N(+) nutrient additions at the end of the experiments are given. * above the start column denotes statistically significant difference between the start and control values, otherwise * denotes statistically significant ($p < 0.05$) difference between the units without and with nutrient additions.

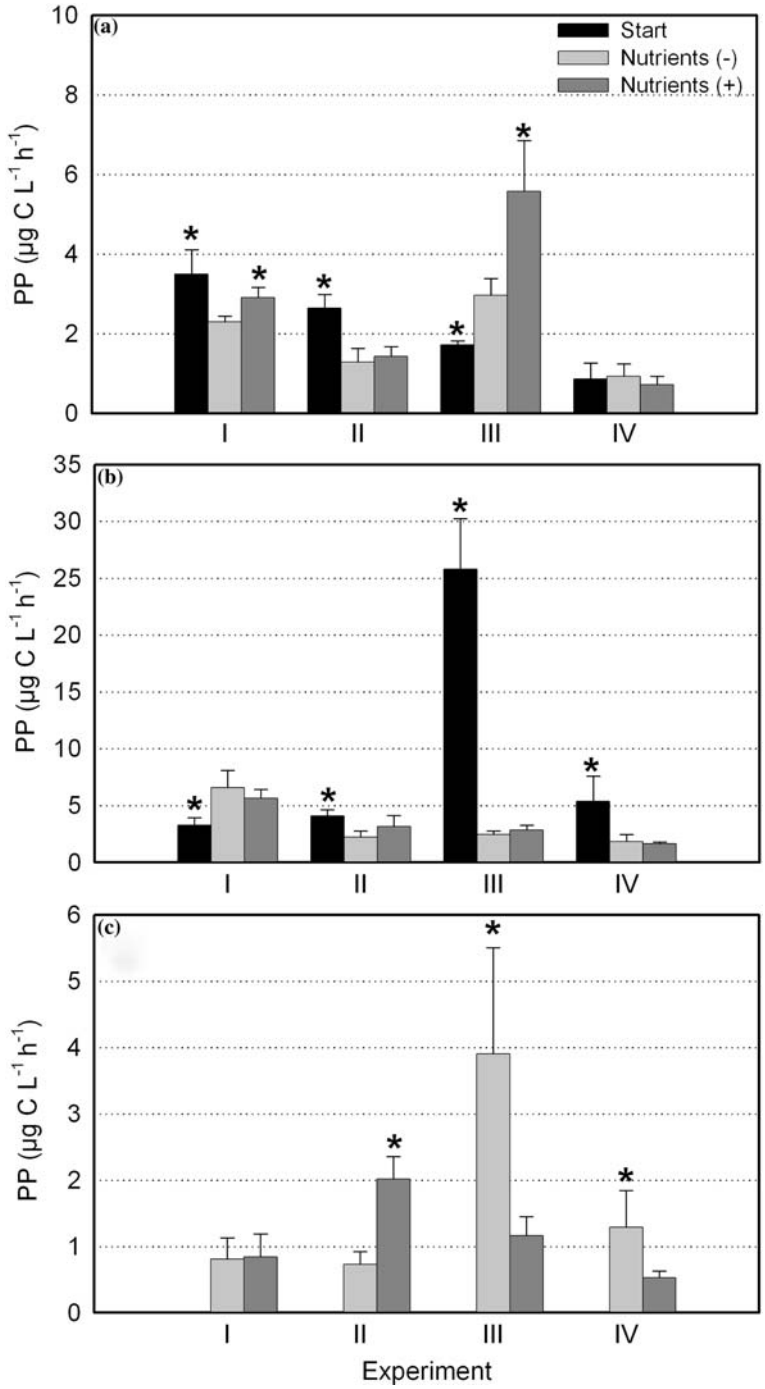

Figure 3. Primary productivity values in the upper (a) and lower (b) ice layers and added filtered sea water (c). The mean start value and the average values (± intervals) without N(−) and with N(+) nutrient additions at the end of the experiments are given. * above the start column denotes statistically significant difference between the start and control values, otherwise * denotes statistically significant ($p < 0.05$) difference between the units without and with nutrient additions.

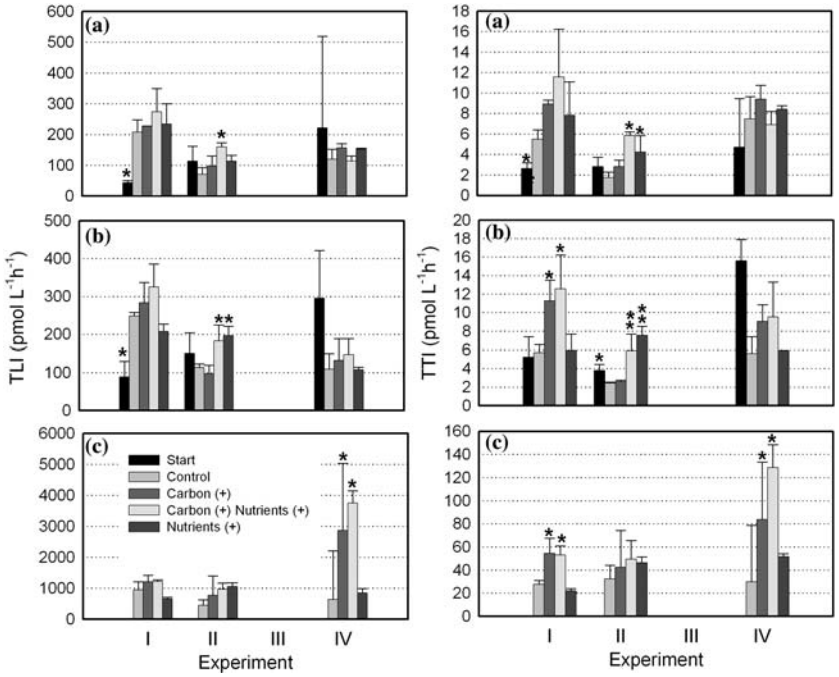

Figure 4. The incorporation of radiolabelled leucine (TLI) and thymidine (TTI) in the upper (a) and lower (b) ice layers and added filtered sea water (c). The mean start value and the average values (± intervals) without additions (control) and with either single nutrient (N) or organic carbon (C) additions or both (NC), at the end of the experiments are given. * above the start column denotes statistically significant difference between the start and control values, otherwise * or ** denotes statistically significant difference ($p < 0.05$ or $p < 0.01$, respectively) shown in ANOVA. The stars are above those groups belonging to a different entity from the control based on an *a posteriori*-test.

importance in regulating light environment in the ice layer (Palmisano et al., 1987), which has a direct relationship to ice algal community development (McGrath Grossi et al., 1987; Gosselin et al., 1990).

The algal dynamics together with the positive nutrient limitation results point at a continuous nutrient limitation in the upper layer of the ice, resulting in low algal biomasses. Considering the apparent phosphorus limitation the main storage of nutrients is water underlying the ice. It is logical that the ice layers farthest away from the water nutrient stores are most nutrient-limited. It is difficult to deduce nutrient limitation from bulk nutrient values (Robinson et al., 1998), but the threefold phosphorus concentration in the added FSW clearly had periodically positive effects compared to the normal, actually rather high, concentrations in the underlying seawater.

It is interesting that during the most intensive flushing period, the amount of biomass flushing form the ice appeared to be negatively related to added nutrients. Again, primary productivity results confirmed the effects; upper ice layers benefited from the nutrient additions from time to time, and during the flushing period good nutrient status appeared to keep active algal cells in the ice matrix. However, the amount of chlorophyll-*a* and primary productivity in the water under the ice core was surprisingly stable at the end of all four experiments, most probably showing that much of the material flushed during the most intensive period (3rd experiment) was already dying. Thus, in concordance with the results of Haecky et al. (1998), prominent seeding effect is hardly observed. However, this aspect requires much more attention as our results point at an active mechanism, and the effect of nutrient availability, on seeding.

The effect of substrate and nutrients on bacterial activity appeared to change during the season. Available labile substrate was clearly limiting bacterial productivity during part of the season, mainly during the 1st experiment. Considering that specific experiment, the limiting role of carbon

is not surprising as algal community was clearly nutrient limited, and probably not provided substantial amounts of labile organic carbon to bacteria. This suggests a similar strict relationship between DOC derived from primary production and bacterial activity in the Baltic Sea like Bunch & Harland (1990) found in the Arctic sea ice. DOC is known to be generally related to algal biomass in a variety of ice communities (Smith et al., 1997), although uncoupling between DOM production and consumption in sea ice environment is also reported (reviewed in Pomeroy & Wiebe, 2001; Brierley & Thomas, 2002). However, without deeper knowledge on the DOC dynamics in the studied sea ice it remains to be seen how close the relationship really is. Long before the 2nd experiment, all available phosphorus had already been used by ice communities and, probably consequently (as substrate is derived from dying algae), bacteria were nutrient limited. The 4th experiment proved to be different. Limitation was present only considering bacteria either transferred or grown in the filtered seawater under the ice core. Nutrients (phosphorus) were apparently limiting, while substrate availability was of no importance. This may be related to the fast decline of algae during the late stages of the ice season. It is feasible, that the developing phytoplankton bloom used most available nutrients as evidenced by nutrient limitation of phytoplankton biomass and productivity in the upper layer, but at the same time produced a wealth of labile substrates or that dying algae leaked DOC.

The existence of bacterial limitation in cold environment appears to differ from the previous work by Autio (1998), in which cold temperature was found to prevent growth. However, based on the activity of bacteria in the ice (Kaartokallio, 2004) and the present study, it is clear that Baltic Sea ice bacterial communities are capable in responding to suitable growth conditions. The basic difference of these works is that Autio (1998) took the samples in February and March from open water. Obviously, well cold-adapted bacterial communities do exist in Baltic Sea ice, but not necessarily at the same time in the water mass. Thus periodic limitation of bacterial activity by substrate availability and nutrients may exist in Baltic Sea ice as is found experimentally in open water (Kuparinen & Heinänen, 1993).

The interplay between light and different nutrient limitations has been shown from the Antarctica (Robinson et al., 1998), Canadian arctic (Gosselin et al., 1990), and the Baltic Sea (Haecky et al., 1998). Robinson et al. (1998) stress the fact that nutrient limitation is very difficult to show from nutrient concentration data. Bacterial dependence from DOC has also been shown, but similarly it is difficult to pinpoint the periods of different limitation. Experimental approach may prove to be useful to study certain aspects of limitation, and if combined with other types of studies may complement our view on the effect of environmental changes to the ice biota.

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