WALTER AND ANDRÉE DE NOTTBECK FOUNDATION

No. 27

Sea-ice ecology in the Baltic Sea with special emphasis on bacteria

HERMANNI KAARTOKALLIO

Academic dissertation in Hydrobiology, to be presented, with the permission of the Faculty of Biosciences of the University of Helsinki, for public criticism in the Lecture Hall Aura, Finnish Institute of Marine Research, Dynamicum Building, Erik Palménin aukio 1, Helsinki, on December 2nd, 2005, at 12 noon.

HELSINKI 2005

This thesis is based on the following papers, which are referred to by their Roman numerals:

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

II Kaartokallio, H. 2001: Evidence for active microbial nitrogen transformations in sea ice (Gulf of Bothnia, Baltic Sea) in midwinter. – Polar Biol. 24: 21–28.

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

IV Kuosa, H. & Kaartokallio, H.: Experimental evidence on nutrient and substrate limitation of Baltic Sea sea-ice algae and bacteria. – Hydrobiologia (in press).

V Kaartokallio, H., Kuosa, H., Thomas, D. N., Granskog, M. A. & Kivi, K. Biomass, composition and activity of organism assemblages along a salinity gradient in sea-ice subjected to river discharge in the Baltic Sea. (manuscript).

Exeundum deinde est, ut extera Europae dicantur, transgressisque Ripaeos montes litus oceani septentrionalis in laeva, donec perveniatur Gadis, legendum. insulae complures sine nominibus eo situ traduntur, ex quibus ante Scythiam quae appellatur Baunonia unam abesse diei cursu, in quam veris tempore fluctibus electrum eiciatur, Timaeus prodidit. reliqua litora incerta. signata fama septentrionalis oceani. Amalchium eum Hecataeus appellat a Parapaniso amne, qua Scythiam adluit, quod nomen eius gentis lingua significat congelatum.

Philemon Morimarusam a Cimbris vocari, hoc est mortuum mare, inde usque ad promunturium Rusbeas, ultra deinde Cronium. Xenophon Lampsacenus a litore Scytharum tridui navigatione insulam esse inmensae magnitudinis Balciam tradit, eandem Pytheas Basiliam nominat.

We must now leave the Euxine to describe the outer portions of Europe. After passing the Riphæan mountains we have now to follow the shores of the Northern Ocean on the left, until we arrive at Gades. In this direction a great number of islands are said to exist that have no name; among which there is one which lies opposite to Scythia, mentioned under the name of Raunonia, and said to be at a distance of the day's sail from the mainland; and upon which, according to Timæus, amber is thrown up by the waves in the spring season. As to the remaining parts of these shores, they are only known from reports of doubtful authority. With reference to the Septentrional or Northern Ocean; Hecatæus calls it, after we have passed the mouth of the river Parapanisus, where it washes the Scythian shores, the Amalchian sea, the word 'Amalchian' signifying in the language of these races, frozen.

Philemon again says that it is called Morimarusa or the "Dead Sea" by the Cimbri, as far as the Promontory of Rubeas, beyond which it has the name of the Cronian Sea. Xenophon of Lampsacus tells us that at a distance of three days' sail from the shores of Scythia, there is an island of immense size called Baltia which by Pytheas is called Basilia.

PLINY THE ELDER (a.D. 23–79), NATURAL HISTORY IV(xiii): 94–95.
(Translation: Perseus Digital Library Project, http://www.perseus.tufts.edu)

CONTRIBUTIONS

	I	**II**	**III**	**IV**	**V**
Original idea	HKa	HKa	HKa	HKa, HKu	MG, HKa
Study design and methods	HKa	HKa	HKa, ML	HKu, HKa,	HKa, MG, HKu, DT
Data gathering	HKa	HKa	HKa, ML	HKa, HKu	HKa, MG, DT, HKu, KK
Responsible for manuscript preparation	HKa	HKa	HKa	HKu	HKa, HKu

DT = David Thomas, HKa = Hermanni Kaartokallio, HKu = Harri Kuosa, KK = Kai Kivi, MG = Mats Granskog, ML = Maria Laamanen

Supervised by

Prof. Harri Kuosa
Tvärminne Zoological Station
Finland

Prof. Jorma Kuparinen
University of Helsinki
Finland

Reviewed by

Doc. Pirjo Kuuppo
Finnish Environment Institute
Finland

Dr. Gerhard Dieckmann
Afred-Wegener Institute for Polar and Marine Research
Germany

Examined by

Assoc. Prof. Rolf Gradinger
University of Alaska Fairbanks
USA

Sea-ice ecology in the Baltic Sea with special emphasis on bacteria

HERMANNI KAARTOKALLIO

Kaartokallio, H. 2005: Sea-ice ecology in the Baltic Sea with special emphasis on bacteria. – W. & A. de Nottbeck Foundation Sci. Rep. 27: 1–41. ISBN 951-98521-8-2; ISBN 952-10-2796-7 PDF.

Seasonal occurrence of sea ice is an important feature in the Baltic Sea, with ice annually covering a mean of 45 % of the sea area. Sea ice affects climate processes but also provides a habitat for diverse and abundant ice organism communities that, by participating in nutrient and carbon cycles, turn sea ice into an active interface between the atmosphere and the sea. To understand wintertime ecology of polar and sub-polar seas and assess the effects of predicted and observed global change, it is necessary to understand the physical, chemical and biological properties of sea ice and their interactions. Baltic sea-ice organism communities resemble their polar counterparts; diatoms are the dominant algae, while other important algal groups include small autotrophic flagellates and dinoflagellates. The main heterotrophic organisms are the heterotrophic bacteria, diverse heterotrophic flagellates and ciliates as well as the metazoa of which rotifers are the main representatives. A systematic study of Baltic sea-ice biota has been conducted only since mid-1990s and studies including the entire ice food web of the Gulf of Finland are thus far lacking. The investigations presented in this thesis increase the level of understanding of the structure and function of sea-ice food webs in the Gulf of Finland. In addition they provide new insights into the adaptation of natural sea-ice bacterial communities to their environment and the biogeography of sea-ice bacteria, bacteria-mediated nitrogen transformations in sea ice, as well as the relationships between dissolved organic matter, algal primary productivity and bacterial productivity. Taken together these works are relevant to a more complete understanding of the ecobiogeochemistry of sea-ice systems in general.

This thesis consists of both explorative and experimental studies. The explorative work focused on the interaction between the ice biota and physicochemical properties of the ice and the internal dynamics of its food web, as well as nitrogen transformation by ice bacteria. The experimental work examined the effects of changing salinity on formation and growth of the ice bacterial community and nutrient and substrate limitation on ice algae and bacteria during various phases in winter.

The results of the explorative studies suggest that Baltic sea ice comprises a highly dynamic series of habitats in which interactions between the physical, chemical and biological properties determine the succession of ice organism assemblages and the functioning of the ice microbial food web. The ice organism assemblages in fast ice on the SW coast of Finland have specific succession sequences resembling those observed earlier in the sea ice of the Gulf of Bothnia, However, earlier timing of the ice algal bloom and the occurrence of another, minor algal biomass maximum during a low-light period in January are different from those observed in the ice from the Gulf of Bothnia. Sea-ice physical properties constrain the formation of organism assemblages. Of special significance are changes in ice porosity and brine movements, which are caused by temperature changes. Ice bacteria are the central organism group with multiple roles in the ice ecosystem. They participate in nutrient cycles not only through regeneration and uptake processes, but possibly also via active nitrogen transformation. Growth of ice bacterial assemblages is based partly on the utilization of autochthonous organic matter and thus is largely dependent on algal organic matter production in the ice. Ice bacteria contribute to carbon production in sea ice by their secondary production and act as a food source for several higher organism groups, including heterotrophic flagellates and small bactivorous ciliates.

The results of the experimental studies show that nutrient and light availability are the key factors regulating succession and biomass build-up of ice algal assemblages in Baltic sea ice. Bacterial growth is limited mainly by the availability of phosphorus, with nutrient and substrate limitation alternating along with the season. Sea-ice-derived bacteria are able to effectively adapt to changing salinity under the experimental conditions used. The variation in salinity, typical for the brine channel environment, is clearly a major factor affecting the selection of the initial bacterial community in the ice. The close association of bacterial phylotypes with Baltic sea ice from both polar areas is in accordance with findings showing a high degree of similarity between members of the Arctic and Antarctic sea-ice bacterial communities. The Baltic sea-ice bacterial community structure shows a closer resemblance to its Arctic counterpart, which is consistent with the geographical location of the Baltic Sea.

Hermanni Kaartokallio, Finnish Institute of Marine Research, Erik Palménin aukio 1, FI-00560 Helsinki, Finland and Tvärminne Zoological Station, J. A. Palménin tie 260, FI-10900, Hanko, Finland.

CONTENTS

1. INTRODUCTION

Sea ice covers up to 7 % of Earth's surface annually and is a major component in ocean circulation and global climate patterns. In addition to polar seas, seasonal sea-ice cover is formed in some temperate sea areas such as the White, Ohkotsk and Baltic seas (Dieckmann & Hellmer 2003). Seasonal occurrence of sea ice is an important feature in the Baltic Sea, with ice annually covering a mean of 45 % of the sea (Seinä & Palosuo 1996). Ice conditions vary considerably in different parts of the Baltic Sea, with ice persisting for over 6 months in the northernmost part of the Baltic Sea, the Bothnian Bay. In the southern Baltic Sea, ice appears only during severe winters. Despite the brackish nature of the parent water, sea ice in the Baltic is structurally similar and comparable to polar sea ice (Kawamura et al. 2001).

Sea-ice systems can be especially vulnerable to climate change, since the entire habitat may diminish along with a rise in temperature. The effects of global warming are predicted to affect Arctic sea ice in particular (see ACIA (Arctic Climate Impact Assessment) 2004). The present climatic scenarios also predict significant large-scale changes in the Baltic Sea region (Houghton et al. 2001). These include changes in the water balance of the entire Baltic catchment area and substantial increase in mean temperatures. The predicted changes are expected to be most extensive during the cold season. The mean winter temperatures in Northern Europe will have possibly increased by several degrees by the year 2100 (Meier 2002). The ice-covered area in the Baltic Sea would decrease by about 45 000 km^2 for each 1 °C increase in mean temperature and during mild winters only the northernmost and easternmost parts of the Gulf of Bothnia and Gulf of Finland would freeze.

Sea-ice cover is an important factor in Earth's climate system, since it alters energy flow between the atmosphere and sea. It also limits exchange of gases and influences the fate of particles contained in atmospheric deposition. In addition, sea ice provides a habitat for diverse and abundant ice organism communities that, by participating in nutrient and carbon cycles, turn sea ice to an active interface between the atmosphere and the sea. At the end of winter, organisms released from the melting ice may act as seed for the spring phytoplankton bloom (Haecky et al. 1998). Different spring bloom algal community compositions occur after ice-covered or ice-free winters in a coastal Baltic Sea site (Hajdu et al. 1996), which also illustrates the importance of sea ice in spring bloom formation. Chemical substances that have accumulated in the ice (e.g. nutrients and heavy metals) are also released into the water column during the short period of ice breakup and can contribute a significant amount to the annual input of these substances to a particular sea area (Granskog & Kaartokallio 2004). Although rapidly diluted in the water column (Thomas & Papadimitriou 2003), this short-term pulse of substances can affect the prespring bloom nutrient conditions, which largely remain to be studied in the Baltic Sea.

The study of Baltic sea-ice physical features has been well established since the 1950s but the composition and activity of ice biological assemblages have only been studied since the mid-1980s (Huttunen & Niemi 1986) and more systematically only since the mid-1990s (e.g. Norrman & Andersson 1994, Ikävalko & Thomsen 1997). Studies including several organism groups in the Baltic Sea are thus far scarce and were

mainly conducted in landfast ice in the Gulf of Bothnia (Quark area, Norrman & Andersson 1994, Haecky et al. 1998, Haecky & Andersson 1999), but occasionally also in Gulf of Bothnia pack ice (Meiners et al. 2002). During the open-water period, subbasins of the Baltic Sea differ significantly in terms of salinity and nutrient regime, total productivity, as well as structure and functioning of the planktonic food webs (Hagström et al. 2001), but currently it is unclear if analogous differences exist in sea-ice food webs during winter. The seasonal averaged biomass of ice organisms varies between the Gulf of Bothnia and Gulf of Finland (Granskog et al. 2003). Based on observations by Huttunen & Niemi (1986) and Ikävalko & Thomsen (1997), the ice algal community composition also appears to vary between different subbasins. Haecky & Andersson (1999) described the sea-ice food web in the Gulf of Bothnia; however, descriptions of the ice food webs including production estimates are still lacking from the other parts of the Baltic. Studies including bacterial production estimates or that focus solely on Baltic sea-ice bacterial assemblages are thus far scarce and confined only to the Quark area (Norrman & Andersson 1994, Haecky & Andersson 1999) and the Kiel Bight (Mock et al. 1997, Petri & Imhoff 2001).

1.1 The internal sea-ice environment

In contrast to polar seas with multiyear sea ice, only first-year ice exists in the Baltic Sea. Near the coastline landfast ice extends to areas with water depths of 5–15 m; further offshore the ice cover is highly dynamic, moving along with the currents and wind (Leppäranta 1981). Ice formation begins with a 'dynamic' phase during which ice crystals form in the water, float to the surface and freeze together, forming a closed ice cover. This initial ice cover consists of ice crystals of a few millimetres in diameter with random crystal orientation and forms a 'granular ice' layer (Fig. 1) that is structurally different from the columnar ice produced by subsequent thermodynamic ice growth (Eicken 2003). The granular ice layer also contains meteoric ice, i.e. snow-ice and superimposed ice that are formed from incorporation of snow through melting and refreezing into the ice. The meteoric ice fraction can form up to 40 % of the entire ice thickness in the main study area of this thesis, Santala Bay (for details see below; Granskog 2004). The dynamic ice formation phase is followed by static or thermodynamic growth. As heat is extracted from the sea surface by the cold atmosphere, the ice becomes

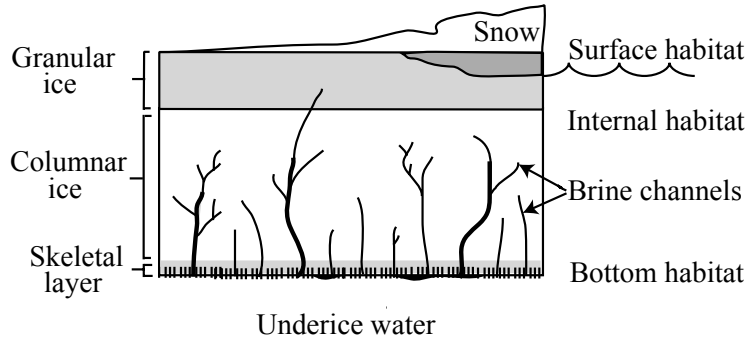

Figure 1. Schematic illustration of Baltic Sea landfast ice structure and different ice habitats. Redrawn after Arrigo (2003).

thicker as individual ice crystals extend downwards, forming columnar ice (Fig. 1). A thin lamellar layer called the skeletal layer exists at the ice-water interface. As ice growth proceeds this lamellar layer is consolidated into ice and the majority of dissolved constituents present in the parent water are rejected from the ice. Part of the water, including dissolved constituents, is trapped between the growing ice crystals, forming small channels and pockets at the ice crystal junctions. These liquid-filled spaces contain a concentrated solution referred to as brine. Columnar ice is composed of vertically elongated ice crystals that can grow to several centimetres in diameter and tens of centimetres in length (Eicken 2003). Depending on ice growth conditions, an intermediate ice layer forms between the granular and columnar ice layers (Granskog et al. 2004). In landfast ice, increase in ice thickness is mostly governed by static, i.e. thermodynamic growth, whereas dynamic thickness growth processes (e.g. drifting and rafting) are more important in offshore areas (Granskog 2004). The studies presented in this thesis were conducted on landfast ice near the coastline.

When saline water freezes, part of the material dissolved in the water is trapped within the ice, the resultant ice being a matrix of ice crystals, gas bubbles and a brine-filled channel system (Eicken 2003). The channel system consists of thin tubes and pockets, which in the Baltic Sea are usually less than 200 μm in diameter. In Antarctic sea ice, about 80 % of the channels are interconnected, with the remaining 20 % forming isolated pockets (Weissenberger et al. 1992). When the temperature of the ice rises, the pores enlarge and coalesce in the vertical direction (Eicken et al. 2000). The concentration and volume of the brine are directly proportional to ice temperature, and therefore also the volume as well as the morphology of the brine channel system are dependent on changes in the ice temperature. Since the ice temperature follows changes in the air temperature in a matter of hours (Granskog et al. 2003), especially in the relatively thin ice cover of the Baltic Sea, the brine salinity and volume also change rapidly. In addition to changes in temperature and salinity, the brine moves inside the ice sheet and across the ice-water interface due to gravity drainage and thermodynamic processes (Eicken 2003). These brine movements across the ice-water interface are important for ice biota because they transport nutrients and dissolved organic matter (DOM; Gradinger et al. 1992).

Within the ice sheet, the brine channel system is the actual habitat of the ice biota (Fig. 1). It is a semienclosed system, in which changes in physical and chemical factors can be extreme. The habitat is characterized by the presence of highly variable salinity, pH, dissolved gas concentrations and dissolved inorganic nutrients (Thomas & Papadimitriou 2003). The light field in the ice interior can also change as a result of variable snow cover and changes in incoming solar radiation (e.g. McGrath Grossi et al. 1987). Physicochemical processes primarily govern these ice properties but the organism assemblages inhabiting the brine channel system can themselves alter their physical and chemical environment. The brine channel system is a very concentrated habitat, all organism biomass being confined to the brine that occupies less than 10 % of the ice volume. The concentrated nature of the environment can lead to very high biomasses of ice organisms. For example, in the Baltic Sea the calculated maximum chlorophyll concentrations can be 800-2000 μg chl-*a* l^{-1} in the brine fraction

(Granskog et al. 2005b). The brine channel surfaces are important for the colonization of ice organisms (Krembs et al. 2000) and it is likely that many of the biological and chemical interactions in the ice system resemble those found in aquatic biofilms rather than open-water ecosystems (Krembs et al. 2000, Mock & Thomas 2005). Other ice habitats include the surface habitat at the snow-ice interface and the bottom habitat in the skeletal layer at the ice-water interface (Fig. 1). The surface habitat is less common in the Baltic Sea, but can be important during ice melting or flooding events, when the snow layer on top of the ice submerges the entire ice sheet. Bottom habitats are ubiquitous, but adequate sampling of this fragile habitat requires the use of divers. Therefore I have focused on the ice interior habitat within the framework of this thesis.

Previous studies from polar areas (e.g. Gradinger et al. 1992, Fritsen et al. 1994, Hudier & Ingram 1994) and using experimental systems (Krembs et al. 2000, 2001) suggest a close coupling between physical and chemical properties of and biological processes occurring in sea ice. The temperature in thin ice changes rapidly with air temperature (Krembs et al. 2001, Granskog et al. 2003), leading to fluctuations in variables that are dependent on ice temperature, such as brine salinity, concentrations of dissolved constituents and surface areas inside the brine channel system. In addition, temperature-dependent changes in ice porosity, brine channel morphology and brine transport processes may be crucial factors shaping ice organism community structure and functioning (Gradinger et al. 1992, Krembs et al. 2000, Granskog et al. 2005b). Brine salinity in Baltic sea ice ranges between 6 and 30 psu (Ikävalko & Thomsen 1997, Mock et al. 1997; II). The salinity is lower than in polar seas; the reported median values for Baltic sea-ice brine salinity (calculated from sea-ice bulk salinity and temperature) are 10.1 psu (Meiners et al. 2002) and measured brine salinity 7.9 psu (recalculated from the data presented in II).

1.2 Composition and succession of ice organism assemblages

In polar sea ice, the most important organism group with regard to biomass and production are the ice algae, most often dominated by pennate diatoms (Brierley & Thomas 2002). The main heterotrophic organism groups in the polar sea ice are the bacteria, diverse heterotrophic flagellates and ciliates, which are the most diverse groups among the nonalgal protists in sea ice (Lizotte 2003). Pennate diatoms are also the biomass-wise dominant algae in the sea-ice of the various Baltic Sea subbasins (Huttunen & Niemi 1986, Ikävalko & Thomsen 1997, Haecky et al. 1998, Haecky & Andersson 1999). Other important algal groups in the Baltic sea ice are small autotrophic flagellates (Crypto-, Hapto-, Chryso- and Prasinophytes; Ikävalko & Thomsen 1997) and dinoflagellates, the latter occasionally even being the dominant algal group (IV). The main heterotrophic organisms in Baltic sea ice are the heterotrophic bacteria (Mock et al. 1997, Meiners et al. 2002), diverse heterotrophic flagellates of various sizes (Ikävalko & Thomsen 1997, Haecky & Andersson 1999, Meiners et al. 2002; I), ciliates and metazoa, of which rotifers are the main representatives (Meiners et al. 2002, Werner & Auel 2004; I, V).

Microbial communities in first-year sea ice probably fit into many of the theoretical frameworks of sequential succession, including pioneer community and primary

succession. The initial incorporation of organisms into new ice, i.e. the pioneer community, reflects the community composition available in the water column during freezing, whereas subsequent changes in species composition reflect selection, succession and latter colonization. Primary succession beyond the pioneer stage is feasible within only a few months because this is sufficient time for major shifts to occur in community structure. The changes occurring in community structure over time are primarily defined by the species present and secondarily by the response to changing environmental conditions. This primary succession ends due to loss of the habitat at icemelt (Lizotte 2003).

The initial colonization during sea-ice formation is typically followed by a low-productive winter stage, blooming of the ice algae and finally a heterotrophy-dominated stage late in the season (Grossmann & Gleitz 1993, Günther & Dieckmann 1999). Biomass accumulation of the sea-ice algae generally follows the seasonal increase in solar radiation beginning at the transition of winter and spring and lasts until the onset of icemelt (Cota et al. 1991, Norrman & Andersson 1994, Haecky & Andersson 1999). Heterotrophic processes increase in significance in late bloom and postbloom situations late in the sea-ice season (e.g. Stoecker et al. 1993, Vezina et al. 1997). Such a successional sequence, beginning with a low-productive winter stage followed by an algal bloom and a heterotrophy-dominated postbloom situation, was documented in the Gulf of Bothnia, the northernmost part of the Baltic Sea (Haecky & Andersson 1999).

1.3 Dissolved organic matter and nutrients in sea ice

The concentrations of dissolved organic carbon (DOC) in the sea-ice environment are in general significantly higher than in surface waters (Thomas & Papadimitriou 2003). In the Baltic Sea, however, the first measurements of ice DOC (Granskog et al. 2005a, b) showed that DOC concentrations in ice are lower than in the underlying waters due to the generally high concentrations of terrestrially derived DOC in the Baltic Sea water (e.g. Hagström et al. 2001). In the ice, the DOC is believed to originate mainly from material incorporated into the ice during its formation (Giannelli et al. 2001, Thomas et al. 2001), as well as autochthonous matter produced by organisms inhabiting the ice, the latter being largely comprised of carbohydrate-rich polysaccharides (reviewed by Brierley & Thomas 2002, Thomas & Papadimitriou 2003). Granskog et al. (2005b) reported an accumulation of DOM derived from algal biomass in the lower ice layers in the Gulf of Finland. The accumulation of refractory DOM observed in sea ice is an exception in aquatic systems, it being usually consumed rapidly by bacteria (Pomeroy & Wiebe 2001).

The main nutrient source in the sea-ice internal habitat is the initial nutrient entrapment during ice formation and, in older sea ice, nutrient transport with brine movement (Dieckmann et al. 1991, Golden et al. 1998). Brine movement can transport nutrients across the ice-water interface from the underlying water, in which dissolved nutrient concentrations typically exceed those within the ice (Gradinger et al. 1992). Snow on ice accumulates nutrients from atmospheric deposition, these nutrients can be incorporated into the ice sheet via snow-

ice formation and even transported deeper into the ice sheet when melting events occur (Granskog et al. 2003, Granskog & Kaartokallio 2004). Changes in ice temperature and porosity are important factors determining nutrient transport within the ice (Fritsen et al. 1994, Golden et al. 1998, Granskog et al. 2003). Recycling of nutrients from allochthonous and autochthonous biomass in the ice through decomposition and nutrient regeneration by ice heterotrophs also constitutes an important nutrient source inside the ice (Cota et al. 1991). Ice bacteria degrade particulate organic matter (POM) and regenerate nutrients (Helmke & Weyland 1995), as do phagotrophic protists in the sea-ice environment (Stoecker et al. 1993).

1.4 Sea-ice food web

Due to space limitation in the brine channels, internal sea-ice food webs are often considered to be severely truncated, meaning that organisms larger than the upper size limits of the channels are lacking (Krembs et al. 2000). This simplifies the ecosystem by lowering the diversity of ecological relationships. Although sea-ice food webs should thus be fairly simple to describe conceptually, sampling difficulties, the ephemeral nature of the sea-ice system and significant interactions with the underlying water column severely limit the ability to quantify the sea-ice microbial food webs (Lizotte 2003). Different 'short circuits' in the flow of energy and organic matter are typical of microbial food webs inside the sea ice. These include herbivory by ciliates and flagellates, ciliate bacterivory and direct utilization of DOM by heterotrophic flagellates (Sherr 1988, Gradinger et al. 1992, Stoecker et al. 1993, Laurion et al. 1995, Sime-Ngando et al. 1997, Vezina et al. 1997, Haecky & Andersson 1999). Although DOM accumulates in sea ice, recycling of DOM via the microbial loop is still considered to be a major link between primary and secondary producers (Gradinger et al. 1992). The significance of the microbial loop and micrograzer herbivory is assumed to increase with decreasing algal productivity (Laurion et al. 1995, Sime-Ngando et al. 1997). Sea-ice bacteria are often larger than bacteria in the underlying water, both in polar oceans and the Baltic Sea, and their average cell size increases along with the age of the ice (e.g. Grossmann & Dieckmann 1994, Gradinger & Zhang 1997, Mock et al. 1997; I, II). The larger cell size is usually assumed to be a result of enhanced substrate availability, due to high DOM concentration, or lowered grazing pressure in the ice. Although several studies of Arctic sea ice suggest tight coupling between bacterial biomass and DOM (Gradinger et al. 1992, Thomas et al. 1995 and references therein), the coupling between primary production (supplying autochthonous DOM) and bacterial production (consuming DOM) in the ice is not straightforward, and there are seasonal differences between winter and spring (Stewart & Fritsen 2004). Estimates of bacterial production vary from 10 % of primary production to net heterotrophy (Mock & Thomas 2005).

1.5 Sea-ice bacteria

Heterotrophic bacteria are the most abundant group of prokaryotic organisms in polar sea ice and are also important with regard to biomass (Mock & Thomas 2005, Lizotte 2003). Together with unicellular algae they represent the two major

organism groups within the sea ice, and have thus been the most intensively studied groups to date (Mock & Thomas 2005). The abundance and biomass of sea-ice heterotrophic bacteria vary widely between < 0.01 and 28 × 10^6 cells ml $^{-1}$ and 2–270 µg C l^{-1}, respectively (Mock et al. 1997 and references therein), the highest biomass usually being found in association with high algal biomass. Association with particles is common among the ice bacteria, as over 50 % of bacteria are attached to particles or surfaces (e.g. sediment grains, detritus, ice crystal boundaries and other organisms (Sullivan & Palmisano 1984, Junge et al. 2004). This may form an important mechanism for survival and growth in subzero temperatures. A rich diversity of bacteria was found in both Antarctic and Arctic sea ice (Brinkmeyer et al. 2003), of which species from the α- and γ-subclasses of the phylum Proteobacteria and the *Cytophaga-Flavobacteria-Bacteroides* group (phylum Bacteroidetes) were mainly represented. In addition, phylotypes (phylotype meaning a group of identical polymerase chain reaction (PCR) products obtained from environmental samples, Kemp & Aller 2004) from the β-subclass of the phylum Proteobacteria, characteristic for freshwater environments, predominate in Arctic ice melt ponds in summer. In general, sea-ice bacterial phylotypes are very similar in both polar areas, which implies the occurrence of similar selection mechanisms in these two geographically separated environments (Brinkmeyer et al. 2003, Mock & Thomas 2005). In contrast to other marine environments, the majority of sea-ice bacteria appear to be cultivable (Brown & Bowman 2001, Brinkmeyer et al. 2003). The culturability of sea-ice bacteria can attain level as high as 62 % (Junge et al. 2002), whereas that of seawater bacteria is can be as low as 0.01 % (Amann et al. 1995). The high culturability of sea-ice bacteria may be related to the highly concentrated biological assemblages and high concentrations of DOM frequently found in polar sea ice and also in the Baltic Sea (Granskog et al. 2004, 2005b; V), leading to a prevalence of species that are able to grow under rich culturing conditions. However, in the only published study from the Baltic Sea (Kiel Bight), sea-ice bacterial communities showed no resemblance to their polar sea-ice counterparts (Petri & Imhoff 2001). Interestingly, the authors reported the occurrence of 16S ribosomal DNA (rDNA) sequences related to those of fermenting bacteria and anoxygenic phototrophic purple sulphur bacteria, which implies the occurrence of anoxic microzones in the sea-ice environment (Petri & Imhoff 2001). Results indicating active denitrification in Baltic sea ice (II) and in Arctic sea-ice (Rysgaard & Glud 2004) further support the hypothesis for the existence of such microzones.

1.6 Role of bacteria in ice food webs

Sea-ice bacteria are the key organisms with regard to diversity and biomass and consequently play multiple roles in sea-ice food webs. They decompose POM, producing DOM, and recycle autochthonous and allochthonous DOM via the microbial loop. Bacterial secondary production supplies particulate carbon, which subsequently serves as an important food source for several groups of higher organisms in the ice food webs.

1.7 Regulation of ice bacterial communities

The overall factors that regulate growth and production of sea-ice bacteria are generally common for all sea-ice-dwelling organisms and can be divided into three categories:

1) The physical ice properties, of which the most important are temperature, brine salinity and ice porosity (the latter affecting the space available for colonization) and brine movement.

2) Availability of nutrients and substrate, defined by multiple factors in the ice internal environment, including properties of the parent water, ice growth processes and (physical) interactions between the ice and water column during winter. Biological factors also contribute significantly to nutrient and substrate availability of which the most important are uptake and regeneration of nutrients, as well as production and consumption of DOM. Dissolved inorganic nutrients, especially phosphorus, may be more important than DOC, which accumulates in the sea-ice environment.

3) Food web interactions, mainly flagellate and ciliate grazing on bacteria. Reduced grazing pressure on sea-ice algae and bacteria resulting from restricted access of grazers to the brine channels has been a commonly suggested reason for the observed buildup of high bacterial and algal biomass in ice (Brierley & Thomas 2002). However, the abundance of well-adapted heterotrophic protists capable of bacterivory in the internal environments of sea ice indicate the existence of functional microbial food webs in ice and effective protozoan grazing on bacteria (Stoecker et al. 1993, Sime-Ngando et al. 1999, Haecky & Andersson 1999; I).

2. OUTLINE OF THE THESIS

Here I attempt to better understand the ecology of sea-ice organisms, especially sea-ice bacteria in the Baltic Sea. The thesis consists of five studies, including both explorative (I, II, V) and experimental work (III, IV). The main emphasis in I and V is on the interaction between the ice biota and physicochemical properties of the ice as well as the internal dynamics of its food web. They provide an overview of the overall structure and dynamics of the sea-ice food web, as well as the role of bacteria. I also focus (II) on the interaction between ice bacteria and nitrogen transformation and on the regulation of formation and growth of the ice bacterial community in III and IV, which are based on experimental work. The effect of changing salinity on the structure and physiology of the sea-ice and open-water natural bacterial communities is studied (III). Finally, an experimental study was conducted on the effects of nutrient and substrate limitation on ice algae and bacteria during various phases in winter (IV).

The main objectives of the investigations presented here were to examine: 1) temporal and spatial scales in the structure and functioning of the sea-ice food web in landfast sea ice on the SW coast of Finland (I, IV, V) and 2) the role of bacteria in the Baltic sea-ice food web and interactions between bacteria and the physical and chemical environments, especially factors that regulate the formation and growth of ice bacterial communities (II–IV).

These investigations increase the level of understanding of the structure and function of sea-ice food webs in the Gulf of Finland (I, V, IV). In addition they provide

new insights into the adaptation of natural sea-ice bacterial communities to their environment and the biogeography of sea-ice bacteria (III), bacteria-mediated nitrogen transformations in sea ice (II), as well as relationships between DOM, algal primary productivity and bacterial productivity (V). Taken together these works are relevant to a more complete understanding of the ecobiogeochemistry of sea-ice systems in general.

3. STUDY AREA

3.1 Characteristics of the northern Baltic Sea

The Baltic Sea is one of the world's largest brackish water basins with a surface area of 422 000 km^2 and volume of 21 000 km^3. The mean depth of the Baltic Sea is only 55 m, and in the Gulf of Finland and the Bothnian Bay less than 40 m (Voipio 1981). The surface salinity varies from 9 psu in the southern Baltic Proper to <1 psu in the innermost parts of the Gulf of Finland and the Bothnian Bay. On the SW coast of Finland, the surface salinity in winter is usually between 5 and 6 psu and in the Quark area between 4 and 5 psu. The Baltic Sea is heavily influenced by river discharge, and the sea has a positive water balance, meaning that river runoff and precipitation exceed evaporation. The brackish nature of the sea is maintained by intermittent inflows of saline North Sea water through the Danish Straits (Stigebrandt 2001). Due to a strong choking effect of the shallow Danish Straits at the entrance of the Baltic Sea, tidal sea-level variation is generally only 1-10 cm (Stigebrandt 2001). The Baltic Sea extends over a large geographical area in the north-south dimension, which leads to regional differences in the annual primary production dynamics caused by variations in solar radiation. The characteristics of ice winter also vary considerably in the subbasins of the Baltic Sea.

3.2 Ice winter in the Baltic Sea

The Baltic Sea belongs to the seasonal sea-ice zone and freezes over annually. The ice season in the Baltic Sea normally extends from October-November to May-June, with an annual areal maximum usually in late February to early March. The interannual variation in the ice cover is large, ranging from 10 % to 100 % of the Baltic Sea (Haapala & Leppäranta 1997). Ice covers an average of approximately 200 000 km^2, which equals almost half the entire Baltic Sea. During mild winters the maximum extent of the ice is well below 100 000 km^2. Landfast ice is typically found close to the coastlines and extends to the outer skerries, where the water depth is about 5–15 m. In offshore areas the ice cover is more dynamic.

Ice formation begins at the northernmost Bothnian Bay and the easternmost Gulf of Finland in October-November. Next to freeze are the Quark, the entire Bothnian Bay and the coastal areas of the Bothnian Sea. In average winters, the ice also covers the Bothnian Sea, Archipelago Sea, Gulf of Finland as well as the northern part of the Baltic Proper. In severe winters the Danish Straits and the southern Baltic Proper are also covered with ice.

In spring, along with increasing solar radiation, the ice begins to melt northwards. The northern Baltic Proper is normally open by early April. In early May the ice prevails only in the Bothnian Bay and has completely melted by early June. The mean

duration of ice winter varies from 20 days in the northern Baltic Proper to more than 6 months in the northern Bothnian Bay (Seinä & Peltonen 1991).

3.3 Description of study sites

All work presented in this thesis was carried out on coastal fast ice of the Baltic Sea. Sampling and experiments (I, IV) were conducted in Santala Bay, a small semienclosed body of water north of the Hanko Peninsula in SW Finland (Fig. 2). The bay is located between the peninsula and islands but has strong water exchange with the adjacent open sea. Due to the sheltered location it also freezes over in mild winters. The ice season usually starts during mid-January and lasts to mid-April (Granskog et al. 2004). The mean water depth is approximately 6 m. The bay has no significant freshwater input and no under-ice freshwater lenses have been observed. Sampling (III, V) was conducted east of the Hanko Peninsula. The experimental laboratory work presented in III was carried out at the Tvärminne Zoological Station. The samples (II) were collected in the Quark area, Gulf of Bothnia, in the vicinity of Umeå Marine Sciences Centre, Umeå University (Fig. 2). Santala Bay was chosen for the study due to its sheltered character, with a long ice season and maximum ice thickness found in the area. The ice studied originates from water with a salinity range of 4–6 psu, which are typical values for the majority of fast ice-covered coastal areas of the Baltic Sea, except the Bothnian Bay and easternmost Gulf of Finland and is therefore considered to represent well the Baltic Sea landfast sea ice in these areas.

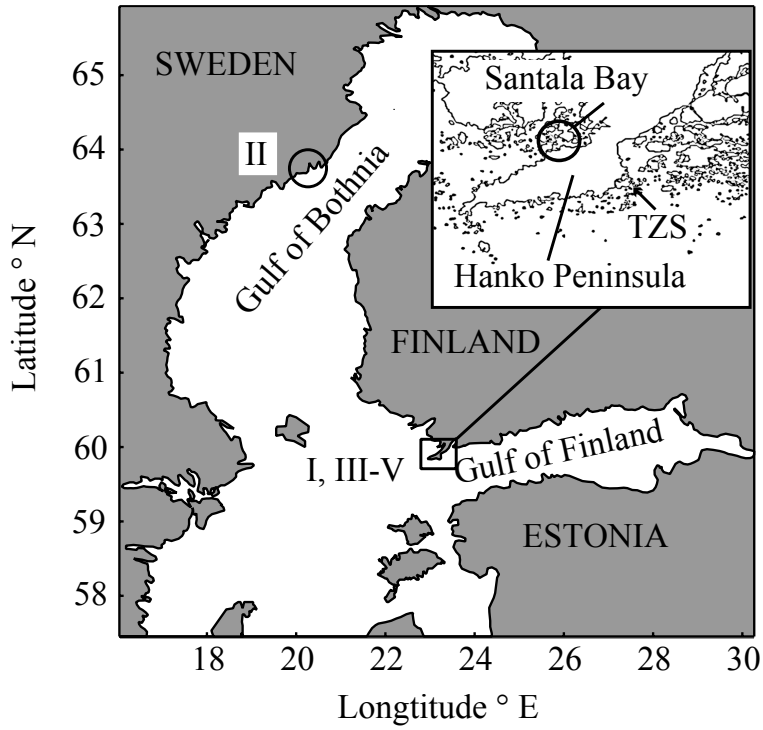

Figure 2. Map of the northern Baltic Sea, showing the study areas at the entrance of the Gulf of Finland (rectangle and map insert) and in the Quark area in the Gulf of Bothnia (circle). Roman numerals next to the study areas refer to articles I-V. TZS refers to Tvärminne Zoological Station.

4. MATERIALS AND METHODS

4.1. Sampling

Coastal landfast ice was chosen for sampling, due to its easy accessibility and the feasibility of sampling the same location over the entire ice winter. Three different sampling schemes were applied to obtain explorative results (I, II, V). A time-series sampling (I) was featured from a single location extending over an entire ice winter (from January to April). A 'snapshot' study presented intensive sampling from a limited area over a period of one week (II). Sampling was designed to cover an inshore-offshore salinity gradient from a freshwater source to the open sea (V). All three sampling schemes covered different aspects and gave differing views of the structure and functioning of the sea-ice ecosystem. They also included ice of different types and ages, due to the spatially and temporally complex ice formation patterns at the study sites.

Ice samples were obtained with a CRREL-type power auger (internal diameter 9 cm, except in II where a 13-cm-diameter corer was employed); these produce vertical ice cores from the entire ice sheet. Ice and snow thickness and ice temperature (by drilling small holes at 5-cm intervals in the fresh ice core and measuring the temperature using an electrical thermometer) were measured immediately in the field. The ice cores were divided into 5-10-cm sections with a handsaw and placed in plastic containers. Water samples from below the ice were usually obtained using a one liter Ruttner-type water sampler employed through a borehole.

Since the horizontal variation in chemical and biological properties of sea ice is often high, especially in pack ice (e.g. Eicken et al. 1991), the reliability of the point sampling used in I was assessed by conducting a separate test to study horizontal variability within the sampling plot used. According to the results nearly all parameters (except particulate organic carbon (POC) and dissolved organic nitrogen (DON)) revealed a significantly smaller spatial rather than temporal variability over the sampling period (see I for details). This is in accordance with the results of a recent study assessing horizontal variation in the Baltic Sea ice (Granskog et al. 2005b), where variability in algal biomass was insignificant at < 20-m scales. The observed relatively small variation was probably due to the homogenous nature of fast ice governed by thermodynamic ice growth, in comparison to pack ice.

4.1.1 Ice sample processing. The actual habitat of sea-ice organisms, the brine channel system, is found between the ice crystals and is very difficult to separate from ice as such. Several techniques have been applied to observe ice organisms in an environment that has been disturbed as little as possible. The most widely applied method is to collect brine from dead-end holes drilled in the ice. This brine-collecting method was also used in II. The other methods used for observation of ice organisms in their natural environment include centrifugation of ice cores to collect brine (e.g. Weissenberger et al. 1992) or observation of organisms in experimental ice systems (Krembs et al. 2000). However, in most sea-ice studies measurements are carried out on melted sea-ice samples and by applying appropriately modified methods commonly used in open-water studies. For measurements of bacterial production and enzyme activity, crushed ice or small ice cube samples were used (e.g. Helmke & Weyland 1995, Guglielmo et al.

2000). A bacterial production measurement modification using mixed crushed ice/concentrated seawater samples for sea ice was applied (I, IV, V). Other parameters were measured in thawed ice samples (see below for details).

In biological sea-ice studies salinity-buffered melting of ice samples is usually applied (Garrison & Buck 1986). Buffering is considered necessary for salinity reductions greater than 7 psu to avoid osmotic shock fatal to ice organisms during sample melting (Garrison & Buck 1986). It is typically done by adding large amounts of filtered seawater to the thawing ice sample. The measured values are then corrected to the actual ice volume placed in containers. In studies included in this thesis, direct melting without buffering was used, since methodological tests done in the main study area on the SW coast of Finland showed no differences in primary productivity and (chl-*a*) recovery between seawater-buffered and direct melting (see I for details). The suitability of direct melting for Baltic sea-ice samples is most likely due to the generally low salinity of the ice compared with that of polar sea ice. The direct melting method was also used in several sea-ice studies in the Baltic Sea (Norrman & Andersson 1994, Haecky et al. 1998, Haecky & Andersson 1999).

4.2 Experiments

The experimental studies described in this thesis (III, IV) include *in situ* experiments using intact ice cores, as well as experiments using sea-ice and open-water bacterial communities in batch cultures. All experimental work was carried out using ice and water from Santala Bay or the vicinity of Tvärminne Zoological Station.

4.2.1 Experimental setups. Batch culture experiments were designed to test the salinity responses of sea-ice and open-water bacterial communities (III). The water samples were taken from 10-m depths at the ice edge of an open-water area. The ice samples for the experiments were obtained 2 or 3 days earlier from 40-cm-thick level ice of Santala Bay (Fig. 2) and melted. Two separate experimental series were prepared, one containing only the open-water bacterial community and the other containing the open-water community plus an additional 10 % (of the total number of bacteria in the open-water series) of bacteria from melted ice. The salinity of the experimental units was adjusted with synthetic sea salt, resulting in four salinity levels (5, 12, 19 and 26 psu), each in duplicate in both series. To ensure sufficient bacterial growth, all units were amended with carbon (sucrose) and phosprorus (PO_4-P). All units were incubated at 0 °C in darkness for 17 or 18 days, and sampling was done at 1-4-day intervals.

A novel experimental method for *in situ* studies in the sea-ice environment was developed within the framework of this thesis (presented in detail in IV). The experimental setup consisted of nine transparent 1-m-long Plexiglas tubes with a closed bottom equipped with an outlet valve, that enabled sampling from the water phase in the tube (Fig. 3). At the beginning of each experiment, 12 ice cores were obtained using a power auger. Three cores were immediately used for measurements at the start of the experiment. Nine cores were put into the Plexiglas tubes containing 2 l of sterile filtered seawater, originating from a nearby open sea area, amended with nutrient (nitrate as NO_3-N and phosphorus as PO_4-P) and carbon (sucrose) additions. Seawater containing nutrient and carbon additions surrounded the ice

core in the Plexiglas tube. The purpose of the setup was to introduce nutrients and carbon into the ice cores so that it would be available for ice organism communities (Fig. 3) Three tubes served as manipulation controls with no additions, two tubes received carbon, two nutrients and two both carbon and nutrients. The tubes were placed into their original boreholes, forming a 3×3 array with 30–40-cm spacing between the tubes, and adjusted to the correct level so that the ice cores inside the tubes retained their original vertical position. The tubes were incubated for 6–7-d *in situ*.

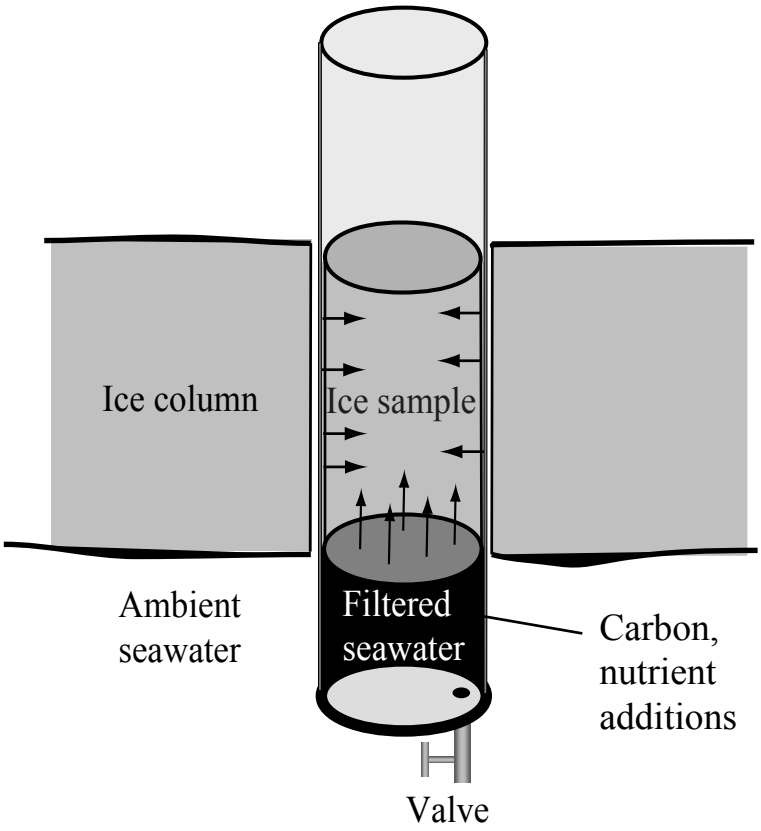

Figure 3. A diagram of the experimental setup with a Plexiglas tube. Arrows denote the vertical and lateral transport of nutrient and carbon additions into the ice cores.

4.3. Measurements

The measurements obtained (I–V) are summarized in Table 1 with references to the methods used. Description of the most important methods used in this thesis and their modifications from current standard procedures are given in the following.

4.3.1 Bacterial production measurements. Bacterial activity was measured using the leucine incorporation method (Kirchman et al. 1985) or simultaneous measurements of thymidine (Fuhrman & Azam 1980, 1982) and leucine incorporation. Generally, several methods for ice sample preparation were used to measure sea-ice bacterial

Table 1. Summary of the main methods used for ice and water samples in (I–V). Underlined bold initials refer to abbreviations used in the respective columns. In addition to parameters given in the table, salinity of ice and water samples was measured (I–V).

Parameter	Bacteria				Algae, Protozoa			Nutrients, POC & DOM	
	Abun-dance	Cell size	Secondary production	Denitrif. potential, community structure	Abundance, Species composition	Primary production	Biomass	Nutrients	Organic carbon, nutrients
Method	AODC	Image analysis	**L**eucine, **T**hymidine incorp.	**A**cetylene method PCR-**D**GGE	**A**lgae **P**rotozoa Microscopy, Lugol fix	^{14}C-incorp.	Chl-a Fluoro-metry	**D**issolved, **T**otal Standard methods	HTCO (DOC, POC) Persulphate wet oxidation (DON/P)
Reference	Hobbie et al. 1977	Massana et al. 1997	Furhman & Azam 1980, 1982, Kirchman et al. 1985	e.g. Gerhardt 1981 (Ac) Muyzer et al. 1993 (D)	Utermöhl 1958	Steemann Nielsen 1952	HELCOM 1988	Grasshoff et al. 1983	Qian & Mopper 1996, Kattner & Becker 1991 Salonen 1979
I	×	×	L		A, P		×	D, T	POC, PON
II	×	×		Ac			×	D, T	
III	×	×	L, T	D					
IV			L, T			×	×	D, T	
V	×	×	L, T		A, P	×	×	D, T	DOC, DON/P, POC

production. These include the use of melted ice samples (e.g. Grossmann & Dieckmann 1994) or of the brine fraction only (Haecky & Andersson 1999). The drawbacks of the melting method are 1) delay in sample processing caused by sample melting (usually > 24 h after sampling) and 2) demolition of the actual microhabitats of ice bacteria in the brine channel system. The problem in using the brine fraction only is the remaining high proportion of surface-associated bacteria in the brine channel system and that the cells do not necessarily move along with the brine and may have different relative activity in comparison to the free-living bacteria in the brine (shown in II). To overcome these problems a modification for rapid processing of ice samples was developed within the framework of this thesis. Similar methods were also used by Guglielmo et al. (2000) in Antarctic sea ice. However, they used a 100-fold lower leucine concentration than that found to be saturating in our studies with crushed ice samples.

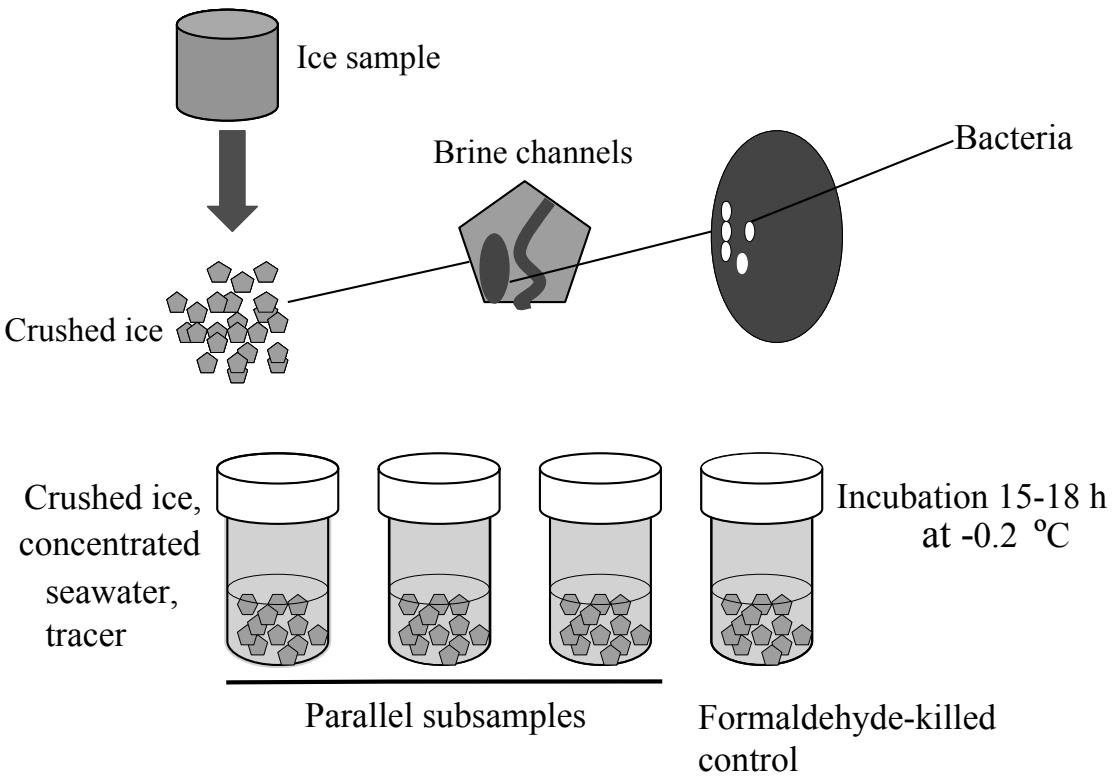

Figure 4. Illustration of "crushed ice" bacterial secondary production measurement method developed within the framework of this thesis.

Bacterial production (I, IV, V) was measured as follows (Fig. 4): immediately after sample collection, each intact 5-cm ice core section was crushed using a spike and electrical ice cube crusher in a cold room at +5 ºC. The density of the ice was determined from each ice core section by immersing a weighed ice piece in a measuring glass. Approximately 10 ml of crushed ice was placed in a scintillation vial and weighed with a laboratory balance. To better simulate salinity in the brine channels and ensure even distribution of labelled substrate, 2–4 ml of 2× concentrated (by evaporation) and filtered (through 0.2 µm) autoclaved seawater from the sampling area was added to the scintillation vials. The samples were subsequently spiked with radioactively labelled ^{14}C-leucine or both ^{14}C-leucine and ^{3}H-thymidine (Table 1). The final leucine concentrations were 200-500 nmol l^{-1} in the water samples and 900-1100 nmol l^{-1} in the ice samples. The thymidine concentrations used were usually 14 nmol l^{-1} in both ice and water samples. The leucine and thymidine concentrations were tested before the actual measurements and were above the saturating level. The samples were incubated in the dark at –0.2 ºC for 16–20 h, and incubations were stopped by adding formaldehyde. To ensure that the leucine incorporation was linear over a 20-h period an incorporation kinetics experiment was conducted prior to the actual measurements. The samples were processed using the standard cold-trichloroacetic acid (TCA) extraction procedure. Radioactivity in the samples was measured with a Wallac WinSpectral 1414 liquid scintillation counter (Wallac, Turku, Finland) using InstaGel (PerkinElmer, Wellesley, MA, USA) cocktail as the solvent. The leucine and thymidine incorporation rates were corrected for the actual ice volume using the measured ice density and sample weight.

Dual labelling with thymidine and leucine was used (III, V), the incubation time being shorter in III (at 0 ºC for 2 h). A shorter incubation time was used because of higher bacterial activity in batch cultures compared with natural samples. Bacterial production presented as carbon values (I, V) was calculated from the leucine incorporation data according to Bjørnsen & Kuparinen (1991). The thymidine incorporation (I, V) was not used in the carbon production estimates, because by using the crushed ice method it is very difficult to estimate the conversion factor between the thymidine incorporation and cell production needed in the calculations. Possible problems in the conversion factor experiment include maintaining the liquid and ice phases in the crushed ice samples for extended periods of time as well as difficulties in reliably subsampling the experimental vessel containing the crushed ice.

4.3.2 Bacterial abundance and cell volume. Bacterial abundance was measured using the acridine orange direct count (AODC, Hobbie et al. 1977) method (Table 2). Subsamples of 20 ml were taken from the thawed ice and water and fixed with 25 % glutaraldehyde (electron microscopy grade, final concentration 1 %). Prior to counting, 5–10 ml of each sample were filtered onto a black 0.2-µm pore-sized polycarbonate filter and stained for 5 min with 0.015 % acridine orange solution. The number of bacteria was counted using a Leitz Aristoplan epifluorescence (Leitz, Oberkochen, Germany) microscope equipped with an I3 filter and PL Fluotar 100× 12.5/20 oil immersion objective (Leica Microsystems, Wetzlar, Germany). At least 200 cells from ≥ 20 fields were counted with the aid of a New Porton E11 counting grid (Canemco & Marivac, Canton de Gore

(Lakefield), Quebec, Canada). The bacterial cell volume was determined using image analysis (Massana et al. 1997). At least 200 bacteria were recorded from each filter using a Photometrics CH250/A charged-couple device camera (Photometrics, Tucson, AZ, USA) connected to a Leitz Aristoplan epifluorescence microscope and PMIS image acquisition software. The digital images were analysed with National Instruments LabView-based (National Instruments, Austin TX, USA) LabMicrobe software (DiMedia, Kvistgård, Denmark). The bacterial biomass was calculated using these empirically derived average cell volumes for each sample, and a carbon conversion factor of 0.125 pg C μm^{-3} (Pelegri et al. 1999).

Table 2. Abundance, cell volume, biomass and secondary production of sea-ice and underice water bacteria in the Baltic Sea. Values are means and (range) except for Meiners et al. (2002), where median (range) for Gulf of Bothnia sampling stations are given. GoF = Gulf of Finland, GoB = Gulf of Bothnia. UIW = Underice water.

	Abundance ($\times 10^6$ cells ml^{-1})		**Cell volume (μm^3)**		**Biomass (μg C l^{-1})**		**Production (μg C $l^{-1}h^{-1}$)**	
	Ice	**UIW**	**Ice**	**UIW**	**Ice**	**UIW**	**Ice**	**UIW**
I (GoF)	0.5 (0.1–1.5)	1.8 (1.2–2.2)	0.21 (0.11–0.32)	0.15 (0.1–0.22)	13.6 (2.1–48.2)	33.6 (25.0–50.3)	0.3 (0.0–0.7)	0.2 (0.0–1.1)
II (GoB)	0.8 (0.2–5.0)	1.2 (0.8–2.0)	0.18 (0.13–0.27)	0.12 (0.1–0.13)	12.2 (0.0–29.6)	14.3 (11.4–18.3)		
IV (GoF)							0.5 (0.1–1.7)	0.7 (0.2–1.0)
V (GoF)	0.4 (0.01–4.4)	2.2 (1.0–3.0)	0.08 (0.03–0.14)	0.04 (0.04–0.06)	4.6 (0.6–56.3)	12.4 (6.1–22.8)	0.3 (0.0–2.4)	0.9 (0.07–4.0)
Meiners et al. 2002 (GoB)	0.2 (0.01–1.7)	0.4 (0.1–0.8)			3.6 (0.7–22.7)	3.6 (0.9–5.7)		
Mock et al. 1997 (Kiel Bight)	(0.7–7)	(0.6–5.5)	0.2 (0.1–0.4)	0.08 (0.03–0.13)	(15–270)	(6.9–96.5)		(0.01–0.91)
Haecky & Andersson 1999 (GoB)	(0.01–0.3)	(0.8–1.3)			1.2 (0.5–2.2.)		0.2 (0.0–1.1)	
Norrman & Andersson 1994 (GoB)	(0.1–1.5)						0.2 (0.0-0.4)	

4.3.3 Activity of denitrifying bacteria. To estimate the relative activity of denitrifying bacteria in sea ice, brine and underlying water (II) the acetylene inhibition method was used (e.g. Gerdhardt 1981). A series of 20-ml glass vials containing 5 ml of semisolid nitrate-nutrient agar were inoculated with 200 µl of sample water (thawed ice, brine, water). The vials were sealed gastight and acetylene was injected into the headspace of the vials. The samples were incubated for 14 days in the darkness at 0 °C. After incubation, N_2O was measured with gas chromatography from the gas phase of the vials. The measurement was calibrated against mixtures of N_2O and air prepared fresh each day.

4.3.4 Structure of ice and open-water bacterial communities. The structure of bacterial communities (III) was assessed using DGGE (denaturing gradient gel electrophoresis) of PCR-amplified partial 16S ribosomal RNA (rRNA) genes by sequencing of DGGE bands at the start and end of the experiments. Community DNA was isolated at the start of the experiment from the original melted ice samples and from the sample water used for preparation of the experimental units for the bacterial community structure analysis. The bacterial cells were collected by filtering onto 0.2-µm pore-sized polyethersulphone filters, and stored in a lysis buffer. DNA was extracted using the hot phenol method following Giovannoni et al. (1990). The DNA extracts obtained were subsequently purified using a purification kit.

The partial 16S rRNA genes were amplified using PCR from the community DNA with the general eubacterial primers F984GC and R1378 (Heuer et al. 1997). The PCR products were loaded onto DGGE gels with a vertical urea-formamide denaturing gradient. The gels were stained with a nucleic acid stain and photographed under ultraviolet (UV) light. Some of the most prominent bands with interesting positions in the gels were excised for sequencing.

For the sequencing, the partial 16S rRNA genes from the DGGE bands were amplified with PCR as described above, using 5 µl of the DGGE band sample as a template and sequenced using an ABI Prism 310 automated sequencer (Applied Biosystems, Foster City, CA, USA). The above-mentioned primers were used for the sequencing reactions, and the amplicons were resolved in both directions. The sequences were deposited in the GenBank under accession numbers AY271857 to AY271864. All lanes of the gel images were scanned and the bands were detected from intensity histograms. To reveal similarities between different communities, the presence or absence of the bands was subsequently used for cluster analysis using the hierachical clustering method (ward linkage, percent distance).

5. RESULTS AND DISCUSSION

5.1 Seasonal succession of algae and bacteria

The seasonal development of ice algal communities differed from those described in earlier studies of Baltic sea ice (Haecky et al. 1998, Haecky & Andersson 1999). The first ice algal bloom already appeared in mid-January in thin ice without snow cover (I, IV). This, however, was most probably due to the unusual absence of snow cover, which allows enhanced light penetration into the ice. These early blooms in January have not been reported in the Gulf of Bothnia, where algal biomass

remains low during the winter months and the only bloom begins in late March as a response to increasing irradiation (Norrman & Andersson 1994, Haecky & Andersson 1999, I). The main bloom of ice algae, with predominance of diatoms (Meiners et al. 2002; I, V) or dinoflagellates (IV) developed in March. After the termination of the bloom in late March a more heterotrophic post-bloom situation with elevated total leucine incorporation (TLI) and total thymidine incorporation (TTI) values was established (I, IV). In comparison to the ice algal bloom, the total organism biomass decreased by 81 % and the proportion of heterotrophic organisms increased in the post-bloom conditions (Fig. 5). Such a heterotrophic phase late in the ice winter was also reported by Haecky & Andersson (1999) from the Gulf of Bothnia. In polar sea ice, the spring-summer period is also accompanied by maximal heterotrophic activity (Kottmeier et al. 1987, Bunch & Harland 1990).

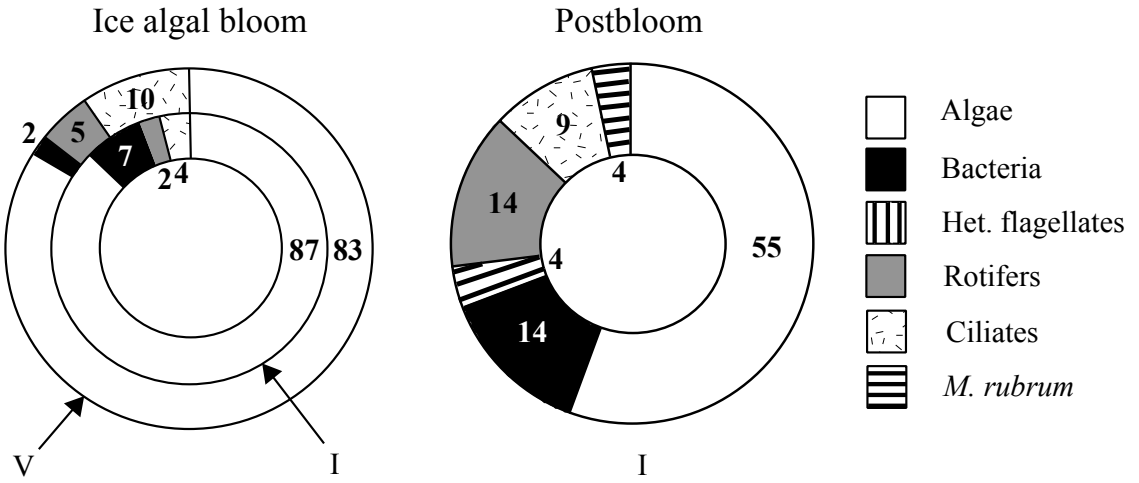

Figure 5. Biomass percentages of ice organism groups during an ice algal bloom and in a postbloom situation. Numbers denote percentages of total organism biomass. Data from I and V. I: Bloom, March 8, Postbloom March 28. V: Stations 5–8. Mean values for the entire ice column at a given date are presented.

Based on chl-*a*, the ice algal biomass in general was similar to that observed in earlier studies conducted with Baltic sea ice (Norrman & Andersson 1994, Mock et al. 1997, Haecky et al. 1998, Haecky & Andersson 1999, Meiners et al. 2002). The ice organism assemblages were autotrophy-dominated, with algal biomass comprising in average over 80 % of the total organism biomass in ice (I, V). Bacteria formed a remarkable proportion of organism biomass (with a mean ± standard deviation of 17 ± 9.4 %) and were a central heterotrophic organism group in a monitoring study (I).

In V, where sampling occurred during the main ice algal bloom in March, the bacterial biomass proportion was lower at only 2 %, the difference probably being due to the timing of sampling. This is consistent with the observed lower biomass proportion of bacteria (7 %) during the ice algal bloom event also observed in Paper I. When the biomass proportions during the ice algal bloom (I, V) were compared, the proportions of heterotrophic and autotrophic biomass were similar, the biomass of bacteria being higher in I and ciliates and rotifers in V (Fig. 5).

5.2 Formation and composition of bacterial communities

During ice formation, bacteria incorporated in the developing ice cover must withstand severe physicochemical stress caused by extreme salinity and ice nucleation (Nichols et al. 1995). Immediately after incorporation into forming ice, bacterial metabolic activity is strongly reduced as the initial community originating from parent water declines (Grossmann & Gleitz 1993, Grossmann & Dieckmann 1994, Deming 2002). The reduced metabolic activity is later reversed when another ice-adapted psychrophilic community becomes established. The initial suppression and subsequent increase in metabolic activity, indicated as increase in TLI, was also evidenced in the Baltic sea-ice (I). The TLI values in the ice were initially low and increased one week after ice formation. A simultaneous decrease in the initially high bacterial abundance and increase in bacterial cell size was also observed in ice (I), while in the underlying water the bacterial abundance and cell size remained more constant. The increase in bacterial cell size during the first stages of ice growth also reported from Antarctic sea ice (Grossmann & Dieckmann 1994).

The occurrence of specific ice-adapted bacterial communities in sea ice of the Baltic Sea, resembling those found in polar sea ice, is shown in III; all eight bacterial 16S rRNA gene sequences obtained from the sea ice studied were closely related to sequences from polar sea ice (4/8), other polar environments (1/8) or Baltic Sea open water (3/8). However, no obvious resemblance with the only published report on Baltic sea-ice bacterial community structure from the Kiel Bight (Petri & Imhoff 2001) was found. The close association between phylotypes from the Baltic Sea (III) and from both polar areas corresponds with the recent findings of Brinkmeyer et al. (2003) on phylogenetic convergence between Arctic and Antarctic sea-ice bacterial communities. Moreover, this study is also the first observation of the occurrence of true ice-related psychrophiles from landfast ice in a temperate sea area. In our study, the majority of sequences obtained belonged to the γ- and α- subclasses of Proteobacteria and the *Cytophaga-Flavobacteria-Bacteroides* group, with two exceptions that were associated with the β-proteobacteria and Actinobacteria (III). Previous studies showed that these are also the predominant bacterial groups in polar sea ice (Bowman et al. 1997, Brown & Bowman 2001, Junge et al. 2002, Brinkmeyer et al. 2003). The predominance of freshwater-associated β-proteobacteria was also reported from Arctic sea-ice melt pools in summer but not in the Antarctic (Brinkmeyer et al. 2003). The bacterial community in the ice studied thus appears to resemble more its Arctic counterpart, which is consistent with the geographical location of the Baltic Sea as well as the high freshwater influence prevalent in both areas.

5.3 Effect of salinity variation on ice bacterial communities

Sea-ice bacterial assemblages must be able to cope with continual, irregular salinity fluctuations, since brine salinity and chemical composition change over short time scales along with ice temperature (Eicken 2003, Granskog et al. 2003). These rapid changes are demonstrated by results showing that the brine salinity decreased from 20–30 psu to approx. 6 psu within days during the warm period as a consequence of internal melting and increased brine volume (II). Frequency, magnitude, and rate in the variation of salinity in the brine channel system are believed to be the major selective factors shaping the ice bacterial community (Nichols et al. 1995, Junge et al. 2002). The results in III, the first study of the responses of sea-ice natural bacterial communities to variations in salinity, showed that bacterial communities derived from Baltic sea ice were able to adapt to increasing salinity without major changes in their growth rates, maximal cell numbers or community structure. This is consistent with the results from experimental work on a single sea-ice bacterial species (Nichols et al. 1999, 2000). The open-water bacterial assemblages, on the other hand, appeared to suffer from osmotic stress along with the increase in salinity and responded with greater changes in community structure and physiology. As seen during changes in physiological and community structure responses to increasing salinity in our experimental system, the results clearly support the assumptions on the role of salinity fluctuation as the major selective factor shaping the sea-ice bacterial community structure (III).

5.4 Abundance, biomass and secondary production of sea-ice bacteria

5.4.1 Bacterial abundance and biomass. Bacterial abundance, cell volume and biomass were all in the same range as reported earlier from the Baltic sea ice (Table 2). The abundance values presented in II were erroneously too low due to miscalculation, and corrected values appear in Table 2. The bacterial biomass in ice was generally three times lower than in underice water. Temporal variation in mean bacterial biomass in the ice was low, and the biomass was relatively stable throughout the season (I). The results (I, II, V) show that bacterial cells were significantly larger in the fast ice than in the underlying water both in the Gulf of Bothnia and Gulf of Finland, which is concordant with earlier observations from polar and Baltic sea ice (Table 2). Various explanations for the large bacterial cell sizes in sea ice have been suggested, including enhanced substrate availability due to high nutrient and DOM concentrations, as well as physiological effects of low temperature (see e.g. Grossmann & Dieckmann 1994, Gradinger & Zhang 1997, Pomeroy & Wiebe 2001). Decreased grazing pressure and subsequent longer life of individual bacterial cells (Krembs et al. 2000, Brierley & Thomas 2002) could also explain the larger cell size. The results (I) support the latter explanation, since increased grazing pressure was accompanied by a decrease in bacterial abundance and cell volume, followed by an increase in biomass-specific bacterial production. In experimental studies conducted in the same area during summer, heterotrophic nanoflagellates exerted size-selective grazing, favouring large rod-shaped bacteria (Kuuppo-Leinikki 1990).

5.4.2 Bacterial secondary production. The 'crushed ice method' developed for the work covered in this thesis for bacterial production measurement (I, IV, V) gave reliable results, as indicated by the high correlation between independently measured primary and bacterial productivity (V), as well as overall agreement of bacterial production values with earlier measurements from Baltic sea ice (Table 2). The significantly higher production-to-biomass (P:B) ratio indicated that the ice bacterial communities were more active than those in the underlying water (I, V). Furthermore, the results (III) show that the ice-derived bacterial community was metabolically more active and capable of faster growth compared with its open-water counterpart.

A high TLI:TTI ratio was found in the ice compared with the underice water, with mean values of 32 and 110 in ambient ice and 18 and 16 in underice water (IV and V, respectively). The overall higher TLI:TTI ratios (reported also by Mock et al. 1997) in Baltic sea ice, compared with the commonly measured values of 5-10 in water and Antarctic sea ice (Chin-Leo & Kirchman 1988, Grossmann & Dieckmann 1994), may arise from specific physiological properties of sea-ice bacterial communities in the brackish water of the Baltic Sea. The observed changes in TLI:TTI ratio along the salinity gradient (V) also indicate that the physiology of bacterial communities may explain high ratios (see also Shiah & Ducklow 1997). Mock et al. (1997) discussed the potential compensatory use of the amino acid flux as a substrate rather than in biosynthesis as an explanation for the high TLI values. The actual reason for the high TLI:TTI ratios is outside the scope of this thesis, but we speculated (IV) that they may be related to the enhanced requirement for proteins, e.g. for UV protection or osmoregulation in low-salinity Baltic sea ice (compared with polar sea ice).

5.5 Physical properties of the sea ice affecting organism assemblages

5.5.1 Brine movements, meltwater flushing. Sea-ice brines move within the ice sheet and across the ice-water interface mainly due to gravity drainage and thermodynamic processes (Eicken 2003). These brine movements across the ice-water interface can be important to ice biota because they transport nutrients and DOM (Gradinger et al. 1992).

The results of this thesis suggest that brine transport is also important for the Baltic Sea ice organism assemblages. Brine motion does not necessarily cause movement of all organisms inhabiting the brine channel system, since flagellated cells and small bacteria move more easily along with the brine movements than diatoms and larger bacterial cells (II). Enrichment of smaller bacteria into the brine fraction was observed (II), possibly caused by the attachment of large bacterial cells on diatoms or organic particles (Sullivan & Palmisano 1984).

Prolonged warm weather periods (with air temperature ≥ 0 °C) caused warming of the ice sheet, changes in the permeability of the ice and subsequent flushing of meltwater (I, IV). The physical aspects that affected the chemical and biological properties of the ice including changes in nutrient and chl-*a* concentrations were discussed in detail in Granskog et al. (2003). Similar flushing events were also reported by Sime-Ngando et al. (1999) from sea ice of a cold temperate lagoon.

High flagellate and metazoan abundance in the lower portion of the ice (I) coincided with periods of warm weather, probably

due to active migration from the underlying water. Space limitation is considered to be the greatest restriction preventing protozoan and metazoan grazers from effectively grazing standing stocks of bacteria and algae in sea ice (Krembs et al. 2000, Brierley & Thomas 2002). Ice warming and increase in brine channel diameter could allow metazoans to partially overcome the size limitation and reach otherwise isolated channels and pockets inside the ice matrix (I).

The results (I, II) indicate that temperature and permeability evolution of ice controlled algal biomass in the studied ice, analogously to the results from an experimental ice system (Krembs et al. 2001). Through taxon-specific drainage rates of ice algae (Haecky et al. 1999, II) changes in ice permeability may also affect the community composition of ice organisms.

The chemical composition of brine differed from that of melted whole ice, the most important differences being lower phosphate values and higher nitrate than expected from salinity distribution alone (II). These findings corroborate the view of Krembs et al (2000) and Mock & Thomas (2005) that the sea-ice internal habitat resembles that of a biofilm located on the walls of brine channels with an overlying flowing liquid phase, i.e. the brine fraction.

5.5.2 Salinity of underlying water. The salinity of the underlying water is the main factor controlling growth of algal assemblages both in the ice and underice water. In sea-ice-covered coastal systems it is affected by river water flowing under the ice (Gosselin et al. 1986, Legendre et al. 1992). In the ice, this control functions through changes in the thickness and porosity of the ice and in the underice water through direct physiological effects of salinity on individual species. Correlation between the ice biota and physicochemical variables in a river-sea salinity gradient was examined, but significant correlations between salinity and chl-*a* or protozoan biomass in ice could not be detected (V); however, the salinity of the underlying water affects the ice porosity and thereby the available space for colonization of the ice organisms. Granskog et al. (2005b) reported extremely high algal biomass values (800–2000 µg chl-*a* l^{-1} in the brine, based on chl-*a* and calculated brine volumes) which increased with porosity in the same natural salinity gradient created by an underice river plume as described in V. This would suggest that ice porosity, in addition to the nutrient regime, may be a crucial factor in limiting ice algal biomass accumulation (Granskog et al. 2005b).

5.6 Interactions between ice bacteria and the chemical environment

5.6.1 Nutrients and DOM in ice and growth limitation by their availability. The main nutrient source for sea ice organisms is the initial nutrient entrapment during the freeze-up and vertical growth of the ice sheet and, in older sea ice, nutrient transport from the underlying water occurring with brine movement (Dieckmann et al. 1991, Gradinger et al. 1992, Golden et al. 1998). Other important nutrient sources are the regeneration processes inside the ice (Cota et al. 1991). In the Gulf of Bothnia, the accumulation of ice algal biomass is dependent on the amount of nutrients, particularly phosphorus, trapped inside the ice and available during the ice algal bloom period (Haecky & Andersson 1999). The main ice algal bloom in the Gulf of Bothnia can occur in the intermediate layers of ice rather than ice layers near the ice-water interface (Haecky et al. 1998). The

highest dissolved nutrient concentrations were measured in these intermediate ice layers, which underlines the importance of initial entrapment of nutrients and subsequent recycling of this nutrient pool in the Gulf of Bothnia (II). In the Gulf of Finland, conditions may be different because nutrient supply into the ice driven by physical forcing (i.e. meltwater flushing or brine exchange processes) can be the determining factor in nutrient dynamics and also be important for primary productivity (Granskog et al. 2003, I). However, the observed correlations between PO_4-P and bacterial secondary production (as TTI; V), elevated PO_4-P and ammonium (NH_4-N) concentrations in lower ice as well as high PO_4-P concentrations observed during the heterotrophic post-bloom situation (I), indicate active nutrient remineralization by ice heterotrophic assemblages, specifically bacteria, in the sea-ice of the Gulf of Finland.

The first attempt to study growth limitation of Baltic sea-ice algae and bacteria experimentally is presented in IV. The experiments show that light, nutrient and substrate limitation of ice algae and bacteria changes with progress of the ice winter and ice layer (IV). Algal growth appeared to be sequentially light- or nutrient-limited along with the winter progression, as shown in studies from Antarctica (Robinson et al. 1998), the Canadian Arctic (Gosselin et al. 1990), and the Baltic Sea (Haecky et al. 1998). The light conditions may even have an indirect effect on bacterial productivity because light increases primary production, which in turn enhances the exudation of DOM from algae, which is assumed to be the primary substrate for sea-ice bacteria. The results (II) point to continuous nutrient limitation in the upper portion of the ice, which is consistent with results in I and V and point to the fact that phosphorus is the main growth-limiting nutrient. In addition to regenerated phosphorus, the main phosphorus supply available to ice organisms during the ice-covered period is in the underice water and it is thus probable that the upper ice layers farthest from water are the most nutrient-limited. Organism communities in the lower ice are able to benefit from this storage, since phosphorus accumulates in the lower ice in the study area (I, Granskog et al. 2005b).

The mechanisms that transport nutrients across the ice-water interface are not well understood, but active movement of organisms across the ice-water interface cannot be ruled out next to brine exchange processes (IV). During the open-water period, coastal waters in the main study area are limited by nitrogen or co-limited by both nitrogen and phosphorus, and the role of nitrogen transported by river water is unquestionably crucial for the productivity of the ice organism assemblages studied (Kivi et al. 1993; V). In offshore areas, a potential source of nitrogen would be nitrogen precipitated on the upper surface of the ice from the atmosphere and subsequently introduced into the ice e.g. by meltwater flushing (Granskog et al. 2003) or a propagating freezing front (Fritsen et al. 1994).

In the ice studied, the bacterial communities were either phosphate- or substrate-limited, as was found experimentally in the Baltic Sea during the open-water period (Lignell et al. 1992, Kuparinen & Heinänen 1993). Periods of bacterial substrate limitation occurred when the algae were nutrient-limited (IV), which together with the observed significant correlations between bacterial biomass and production and algal biomass and potential productivity in the explorative studies (I, V) implied tight coupling between the DOM derived from algae

and thus the supply of carbon for the microbial loop. Haecky & Andersson (1999) and Mock et al. (1997) reported a strong linkage between primary and bacterial production from Baltic sea ice in the Gulf of Bothnia and southern Baltic Sea, respectively. Several reports on Arctic sea ice indicate tight coupling between bacterial biomass and DOM (Gradinger et al. 1992, Thomas et al. 1995 and references therein).

The first report of actual simultaneous measurements of bacterial production and DOM in a sea-ice system was presented (V). Linear correlations between ice DOC and bacterial parameters were not observed; however, coupling between DON and bacterial parameters and also PO_4-P was observed (I, V). It is therefore suggested that the DON concentration may reflect the readily utilizable fraction of DOM in the ice studied (see also Thomas et al. 1995, Guglielmo et al. 2000). DOC and DON were not interrelated (V; discussed in detail by Granskog et al. 2005b), which is thought to illustrate the complex nature of the ice DOM pool, originating partially from allochthonous (parent water, terrestrial sources) and autochthonous (ice algal production, degradation of fresh POM) sources.

5.6.2 Nitrogen transformations. Accumulation of temporary intermediate compounds of the nitrogen cycle was frequently observed in the sea ice of both polar areas (Oradiovskiy 1974, Clarke & Ackley 1984, Meese 1989, Garrison et al. 1990, Thomas et al. 1995). High concentrations of nitrate and organic carbon, as well as abundance of actively respiring heterotrophic organisms create suboxic microsites in the brine channels and pockets, a potential site for nitrogen reduction analogous to water-filled soil pore spaces (Tiedje 1988). Thomas et al. (1995) observed a high correlation between nitrite (NO_2-N) and DOC in Arctic multiyear ice cores and suggested that nitrite accumulation is associated with decomposition of organic carbon, which indicates active nitrate reduction. Antarctic sea ice hosts a high diversity of psychrophilic bacteria, and the bacterial strains closely related to denitrifying species are abundant (Gosink & Staley 1995, Bowman et al. 1997, Zumft 1997). The first focused attempt to study nitrate reduction or denitrification in a sea-ice environment was presented (II). An indirect culture method (that does not measure actual denitrification, but instead the denitrification potential of the bacterial assemblage present in the sample) was used in the study. Rysgaard & Glud (2004) provided direct evidence of anoxia and active denitrification in Arctic sea ice. Denitrifying activity in the Gulf of Bothnia was present in ice layers with high heterotrophic biomass, nutrient regeneration and accumulation of nitrite (II). These interior layers of 2–3-month-old ice were probably the sites where nitrogen transformation occurs in sea ice in the Gulf of Bothnia.

Even if the culture method used to estimate the activity of denitrifying organisms in this study does not provide information on the actual denitrification rates, it reveals the enrichment of denitrifying organisms in the intermediate layers of thick ice and together with elevated nitrite concentrations indicates active nitrate reduction and also possible denitrification. Although phosphorus probably plays a more significant role in limiting primary and bacterial production in the Baltic sea ice, the removal of nitrogen from the ice during winter may affect the amount of nitrogen released from melting sea ice prior the annual phytoplankton spring bloom in the water column. Other possible nitrogen transformations mediated by ice bacteria, e.g. nitrification, were not

examined in this thesis, although high correlations between nitrogenous nutrients in the ice data (II) suggest that they may be active in the area.

5.7 Bacteria as a trophic link in the sea-ice food web

5.7.1 Grazers of bacteria. Food webs found within sea ice are usually severely truncated, due to the size limitation in brine channels (Krembs et al. 2000). Exceptions to the size-structured food webs, such as ciliate and flagellate herbivory, ciliate grazing on bacteria and direct utilization of DOM by heterotrophic flagellates are common. Bacteria are the major heterotrophic organism group in ice with regard to both abundance and biomass and are thus of great importance in sea-ice food webs. Small heterotrophic flagellates are generally considered to be important consumers of bacterial biomass, but small bacterivorous ciliates are known to be efficient grazers in sea-ice interior habitats (e.g. Delille et al. 2002, Sime-Ngando et al. 1999) and most probably also in Baltic sea ice (I). Sea-ice bacteria are also affected by other mortality factors, e.g. viruses (Maranger et al. 1994), but these could not be addressed within the framework of this thesis.

5.7.2 Grazing interactions. Grazing interactions and interactions between the ice biota and DOM pool in the ice studied, (I,V),

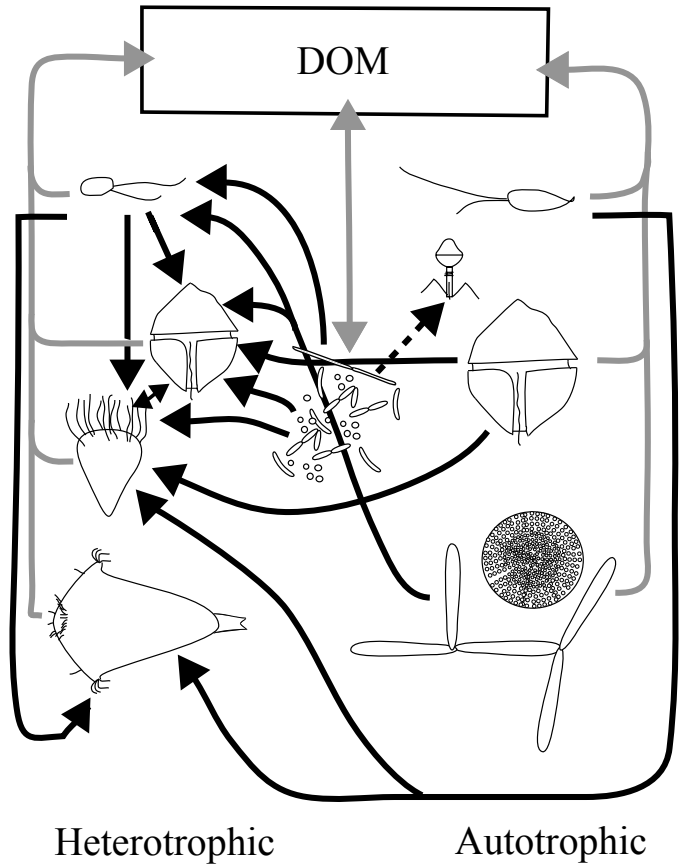

Figure 6. Schematic representation of possible grazing and organism-DOM interactions in sea ice in the Gulf of Finland based on I and V.

are schematically represented in Figure 6. The high P:B ratios of ice bacteria and their relatively low biomass proportion, compared with those of their potential grazers, point to high grazing pressure on ice bacteria (I, V). The flagellate biomass observed in the ice was very low, whereas small ciliates belonging mainly to the genus *Strombidium* with equivalent spherical diameters (ESDs) of 10–30 µm were ubiquitous throughout the ice column in early March (I). Ciliates are diverse and clearly the central organism group in terms of grazing interactions in the ice studied. For example, large ciliates of the genus *Strombidium* (ESD 50–80 µm) found in ice (V) select for flagellate-sized particles of 10–15 µm (H. Kuosa, K. Kivi, unpublished data) and ciliates of the genus *Bursaria*, also found in ice (I; V), can harbour several autotrophic dinoflagellate cells (visible under the light microscope) at the same time.

The low flagellate biomass is assumed to result from effective metazoan grazing, primarily by the rotifer *Synchaeta littoralis*, which is known to feed on small algae and was abundant in the lower ice. This is supported by the observed high variation in flagellate abundance (I, IV), which was significantly connected with variations in rotifer abundance (I). Haecky & Andersson (1999) also attributed the relatively high rotifer biomass in the lowermost ice to loss of part of the algal biomass. In contrast to Arctic and Antarctic sea ice (Werner & Auel 2004 and references therein) rotifers play an important role in the Baltic sea-ice ecosystem (V). In the study by Haecky & Andersson (1999) in the Gulf of Bothnia, very high heterotrophic microflagellate biomass was found which, based on their calculated carbon demand, used food sources other than bacteria, e.g. small primary producers or direct uptake of DOM. The results of this thesis suggest that grazing interactions and ice food web structure vary in different subbasins of the Baltic Sea. However, additional studies including all organism groups are needed to assess the relative importance of regional and interannual variations.

6. CONCLUDING REMARKS

The results presented in this thesis suggest that Baltic sea ice comprises a highly dynamic series of habitats in which interactions between physical, chemical and biological properties determine the succession of ice organism assemblages and the functioning of the ice microbial food web. The ice organism assemblages in fast ice on the SW coast of Finland have specific succession sequences resembling those observed earlier in the sea ice of the Gulf of Bothnia. However, earlier timing of the ice algal bloom and the occurrence of another, minor algal biomass maximum during a low-light period in January are different from those in the ice from the Gulf of Bothnia.

Sea-ice physical properties constrain the formation of organism assemblages. Of special significance are changes in ice porosity and brine movements, which are caused by temperature changes. The variation in salinity in the brine channel environment is clearly another important factor affecting the selection of the initial bacterial community in the ice. Nutrient and light availability are the key factors regulating succession and biomass build-up of ice algal assemblages in Baltic sea ice. Bacterial growth is mainly limited by the availability of phosphorus. Nutrient and substrate limitation progresses along with season. Growth of ice bacterial assemblages is based partly on the utilization of autochthonous organic

matter and thus appears to be largely dependent on algal organic matter production in the ice. Formation and dynamics of pack ice differ from the fast ice studied in this thesis, with probable consequences for ice biogeochemistry and organism communities. Thus the studies concerning the eco-biogeochemistry of Baltic Sea pack ice are clearly needed.

The close association of bacterial phylotypes with Baltic sea ice from both polar areas is in accordance with findings showing a high similarity between members of the Arctic and Antarctic sea-ice bacterial communities. However, based on the limited data produced within the framework of this thesis, the Baltic sea-ice bacterial community structure shows a closer resemblance to its Arctic counterpart, which is consistent with the geographical location of the Baltic Sea as well as the prevalent high freshwater influence in both areas.

Ice bacteria are the central organism group with multiple roles in the ice ecosystem. They participate in nutrient cycles by regeneration and uptake processes, but also possibly via active nitrogen transformation. Bacteria decompose POM and recycle DOM. By their secondary production they contribute to the production of autochthonous carbon in the sea ice and act as a food source for several higher organisms, including heterotrophic flagellates and small bactivorous ciliates.

7. ACKNOWLEDGMENTS

This thesis is based on studies carried out at the Finnish Institute of Marine Research (FIMR) and the Tvärminne Zoological Station during 1999-2003. This work was financed by the Walter and Andrée de Nottbeck Foundation, FIMR and the Academy of Finland, Research Programme BIREME.

I want to express my gratitude to people who supported me during my early steps in science as well as in the various phases of this thesis. I feel myself privileged having had the possibility to work with exceptional people who have offered not only a very challenging and stimulating environment, but also true friendship and lots of fun. Without this 'greenhouse' to grow in, my way would have been far more difficult.

First of all I am grateful to Riitta Autio, who introduced me to the fascinating world of marine bacteria and has continually been my solid supporter and forbearing interlocutor in diverse professional and practical questions. Harri Kuosa has been my supervisor and mentor for years. Our collaboration began when he promised to supervise my MSc thesis with the quite unusual (in other words desperate) topic of denitrification potential in sea ice. I think most senior scientists would have strictly avoided this kind of obscure endeavour, but he gladly took me in. I am grateful to Harri for support and collaboration in my scientific attempts as well as lengthy discussions on truly various topics. Jorma Kuparinen, my other supervisor, supported me throughout the various phases of this thesis, beginning in 1999 when he, as head of the Department of Biological Oceanography at FIMR, hired me for a couple of months to begin my sea-ice studies there. After leaving FIMR, he has continued sponsorizing me in research projects funded by the Academy of Finland. David Thomas, although not my official supervisor, has helped me in various ways in my work. I am especially grateful to David for providing me a larger view into the world of sea-ice research. Field expeditions with him and other colleagues to Tvärminne and Umeå have been a lot of fun but also a lot of hard work together. Mats Granskog has been my principal co-worker in many investigations concerning the vital links between sea-ice physics, chemistry and biology. I want to thank him for fruitful and inspiring collaboration within and outside the scope of this thesis. Janne-Markus Rintala and Jonna Piiparinen share the same enthusiasm for the sea-ice-dwelling creatures and it has been important to me to have had them around during these years. Field expeditions beginning from the 1998 trip with Janne to Umeå, have also always been really memo-

rable. Johanna Ikävalko introduced me to sea-ice research during the Finnish-German ice research course at Tvärminne as far back as 1997 and has been my project leader for years. I want to thank her for trusting me to work independently, which has been really valuable. The other WANS Ice project members, Jens, Kimmo, Kristian and Jari are also thanked for collaboration.

I have been working at FIMR since I started my PhD project and still feel at home there. I want to thank the people at the Department of Biological Oceanography for an inspiring and relaxed working environment and also for lots of support in form of practical help and discussions. Among others, I want to thank Markku, Susanna, Laura, Maima, Emil, Pipsa, Siru, Vilma, Jukka, and Mika. Most of the field and laboratory work of this thesis was done at the Tvärminne Zoological Station. I want to express my deep gratitude to Jouko Pokki, Lallu Keynäs, Raija Myllymäki, Bebbe Åström, Elina Salminen, Mervi Sjöblom, Ulla Sjölund and Totti Sjölund for all their help over the years. The Umeå Marine Sciences Centre, especially Agneta Andersson and Ulf Båmstedt there, is also acknowledged for providing excellent conditions for field work. Pirjo Kuuppo and Gerhard Dieckmann, as reviewers, significantly improved the final version of the thesis, which is gratefully acknowledged. The English of this thesis was corrected by James Thompson.

My dearly beloved family, Satu, Nuutti and Ohto, have been my firm ground to return to after a day's work in marine science. It has been really invaluable to be able to forget everything at work in a matter seconds after getting home.

8. REFERENCES

ACIA, 2004: Impacts of a Warming Arctic: Arctic Climate Impact Assessment. – Cambridge University Press.

Amann, R. I., Ludwig, K. & Schleifer, K. H. 1995: Phylogenetic identification and in situ detection of individual microbial cells without cultivation. – Microb. Rev. 59: 143–169.

Arrigo, K. R. 2003: Primary production in sea ice. – In: Thomas, D. N. & Dieckmann, G. S. (eds.), Sea Ice: An Introduction to its Physics, Chemistry, Biology and Geology, pp. 143–183. Blackwell Science, Oxford, UK.

Bjørnsen, K. B. & Kuparinen, J. 1991: Determination of bacterioplankton biomass, net production and growth efficiency in the Southern Ocean. – Mar. Ecol. Prog. Ser. 71: 185–194.

Bowman, J. P., McCammon, S. A., Brown, M. V., Nichols, D. S. & McMeekin, T. A. 1997: Diversity and association of psychrophilic bacteria in Antarctic sea ice. – Appl. Environ. Microbiol. 63: 3068–3078.

Brierley, A. S. & Thomas, D. N. 2002: Ecology of southern ocean pack ice. – Adv. Mar. Biol. 43: 171–276.

Brinkmeyer, R. K., Knittel, J., Juergens, H., Weyland, H., Amann, R. & Helmke, E. 2003: Diversity and structure of bacterial communities in Arctic versus Antarctic sea ice: a comparison. – Appl. Environ. Microbiol. 69: 6610–6619.

Brown, M. V. & Bowman, J. P. 2001: A molecular phylogenetic survey of sea-ice microbial communities (SIMCO). – FEMS Microbiol. Ecol. 35: 267–275.

Bunch, J. N. & Harland, R. C. 1990: Bacterial production in the bottom surface of sea-ice in the Canadian Subarctic. – Can. J. Fish. Aquat. Sci. 47: 1986–1995.

Chin-Leo, G. & Kirchman, D. L. 1988: Estimating bacterial production in marine waters from the simultaneous incorporation of thymidine and leucine. – Appl. Environ. Microbiol. 54: 1934–1939.

Clarke, D. B. & Ackley, S. F. 1984: Sea ice structure and biological activity in the Antarctic marginal ice zone. – J. Geophys. Res. 89: 2087–2095.

Cota, G. F., Legendre, L., Gosselin, M. & Ingram, R. G. 1991: Ecology of bottom ice algae. I. Environmental controls and variability. – J. Mar. Syst. 2: 257–277.

Delille, D., Fiala, M., Kuparinen, J., Kuosa, H. & Plessis, C. 2002: Seasonal changes in microbial biomass in the first-year ice of the Terre Adelié area (Antarctica). – Aquat. Microb. Ecol. 28: 257–256.

Deming, J. W. 2002: Psychrophiles and polar regions. – Curr. Op. Microbiol. 5: 301–309.

Dieckmann, G. S. & Hellmer, H. H. 2003: The importance of sea ice: an overview. – In: Thomas, D. N. & Dieckmann, G. S. (eds.), Sea Ice: An Introduction to its Physics, Chemistry, Biology and Geology, pp. 1–21. Blackwell Science, Oxford, UK.

Dieckmann, G. S., Lange, M. A., Ackley, S. F. & Jennings, J. C. 1991: The nutrient status in sea ice of the Weddell Sea during winter: effects of sea ice texture and algae. – Polar Biol. 11: 449–456.

Eicken, H. 2003: From the microscopic to the macroscopic, to the regional scale: growth, microstructure and properties of sea ice. – In: Thomas, D. N. & Dieckmann, G. S. (eds.), Sea Ice: An Introduction to its Physics, Chemistry, Biology and Geology, pp. 22–81. Blackwell Science, Oxford, UK.

Eicken, H., Bock, C., Wittig, R., Miller, H. & Poertner, H.-O. 2000: Magnetic resonance imaging of sea-ice pore microstructure. – Cold Regions Science and Technology 31: 207–225.

Eicken, H., Lange, M. A. & Dieckmann, G. S. 1991: Spatial variability of sea-ice properties in the northwestern Weddell Sea. – J. Geophys. Res. 96: 10603–10615.

Fritsen, C. H., Lytle, V. I., Ackley, S. F. & Sullivan, C. W. 1994: Autumn bloom of Antarctic pack-ice algae. – Science 266: 782–784.

Fuhrman, J. A. & Azam, F. 1980: Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica and California. – Appl. Environ. Microbiol. 39: 1085–1095.

Fuhrman, J. A. & Azam, F. 1982: Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. – Mar. Biol. 66: 109–120.

Garrison, D. L. & Buck, K. R. 1986: Organism losses during ice melting: a serious bias in sea ice community studies. – Polar Biol. 6: 237–239.

Garrison, D. L., Close, A. R. & Gordon, L. I. 1990: Nutrient concentrations in Antarctic pack ice during the austral winter. – CRREL monogr. 90–91: 35-40.

Gerdhardt, D. 1981: Manual of Methods for General Bacteriology. – Am. Soc. Microbiol. Washington DC.

Giannelli, V., Thomas, D. N., Haas, C., Kattner, G., Kennedy, H. A. & Dieckmann, G. S. 2001: Behaviour of dissolved organic matter and inorganic nutrients during experimental sea ice formation. – Ann. Glaciol. 33: 317–321.

Giovannoni, S. J., DeLong, E. F., Schmidt, T. M. & Pace, N. R. 1990: Tangential flow filtration and preliminary phylogenetic analysis of marine picoplankton. – Appl. Environ. Microbiol. 56: 2572–2575.

Golden, K. M., Ackley, S. F. & Lytle, V. I. 1998: The percolation phase transition in sea ice. – Science 282: 2238–2241.

Gosink, J. J. & Staley, J. T. 1995: Biodiversity of gas vacuolate bacteria from Antarctic sea ice and water. – Appl. Environ. Microbiol. 61: 3486–3489.

Gosselin, M., Legendre, L., Therriault, J.-C. & Demers, S. 1990: Light and nutrient limitation of sea-ice microalgae (Hudson Bay, Canadian Arctic). – J. Phycol. 26: 220–232.

Gosselin, M., Legendre, L., Therriault, J.-C., Demers, S. & Rochet, M. 1986: Physical control of the horizontal patchiness of sea-ice microalgae. – Mar. Ecol. Prog. Ser. 29: 289–298.

Gradinger, R., Spindler, M. & Weissenberger, J. 1992: On the structure and development of Arctic pack ice communities in Fram Strait: a multivariate approach. – Polar. Biol. 12: 727–733.

Gradinger, R. & Zhang, Q. 1997: Vertical distribution of bacteria in Arctic sea ice from the Barents and Laptev Seas. – Polar. Biol. 17: 448–454.

Granskog, M. A. 2004: Investigations into the physical and chemical properties of Baltic Sea ice. – Dissertation, University of Helsinki. 35 pp.

Granskog, M. A., Ehn, J. & Niemelä, M. 2005a: Characteristics and potential impacts of under-ice river plumes in the seasonally ice-covered Bothnian Bay (Baltic Sea). – J. Mar. Syst. 53: 187–196.

Granskog, M. A. & Kaartokallio, H. 2004: An estimation of the potential fluxes of nitrogen, phosphorus, cadmium and lead from sea ice and snow in the northern Baltic Sea. – Water Air Soil Poll. 154: 331–347.

Granskog, M. A., Kaartokallio, H. & Shirasawa, K. 2003: Nutrient status of Baltic Sea ice: Evidence for control by snow-ice formation, ice permeability, and ice algae. – J. Geophys. Res. 108, C8, 3253, Doi:10.1029/2002JC001386.

Granskog, M. A., Kaartokallio, H., Thomas, D. N. & Kuosa, H. 2005b: The influence of freshwater inflow on the inorganic nutrient and dissolved organic matter within coastal sea ice and underlying waters in the Gulf of Finland (Baltic Sea). – Est. Coast. Shelf Sci. 65: 109–122.

Granskog, M. A., Leppäranta, M., Kawamura, T., Ehn, J. & Shirasawa, K. 2004: Seasonal development of the properties and composition of landfast sea ice in the Gulf of Finland, the Baltic Sea. – J. Geophys. Res. 109(C2), C02020, doi:10.1029/2003JC001874.

Grasshoff, K., Ehrhardt, M. & Kremling, K. 1983: Methods of Seawater Analysis. 2nd ed. – Verlag Chemie, Weinheim.

Grossmann, S. & Dieckmann, G. S. 1994: Bacterial standing stock, activity and carbon production during formation and growth of sea ice in the Weddell Sea, Antarctica. – Appl. Environ. Microbiol. 60: 2746–2753.

Grossmann, S. & Gleitz, M. 1993: Microbial responses to experimental sea-ice formation: implications for the establishment of Antarctic sea-ice communities. – J. Exp. Mar. Biol. Ecol. 173: 273–289.

Guglielmo, L., Carrada, G. C., Catalano, G., Dell'Anno, A., Fabiano, M., Lazzara, L., Mangoni, O., Pusceddu, A. & Saggiomo, V. 2000: Structural and functional properties of sympagic communities in the annual sea ice at Terra Nova Bay (Ross Sea, Antarctica). – Polar Biol. 23: 137–146.

Günther, S. & Dieckmann, G. 1999: Seasonal development of algal biomass in snow-covered fast ice and the underlying platelet layer in the Weddell Sea, Antarctica. – Ant. Sci. 11: 305–315.

Haapala, J. & Leppäranta, M. 1997: The Baltic Sea ice season in changing climate. – Bor. Environ. Res. 2: 93–108.

Haecky, P. & Andersson, A. 1999: Primary and bacterial production in sea ice in the northern Baltic Sea. – Aquat. Microb. Ecol. 20: 107–118.

Haecky, P., Jonsson, S. & Andersson, A. 1998: Influence of sea ice on the composition of the spring phytoplankton bloom in the northern Baltic Sea. – Polar Biol. 20: 1–8.

Hagström, Å., Azam, F., Kuparinen, J. & Zweifel, U.-L. 2001: Pelagic plankton growth and resource limitations in the Baltic Sea. – In: Wulff, F. V., Rahm, L. A. & Larsson, P. (eds.), A Systems Analysis of the Baltic Sea, pp. 177–210. Springer-Verlag.

Hajdu, S., Larsson, U. & Skärlund, K. 1996: Phytoplankton in the Himmelfjärden: changes in a nutrient-enriched coastal ecosystem in the Baltic Sea. – Swed. Environ. Protection Agency 4565: 63–79.

HELCOM 1988: Guidelines for the Baltic monitoring programme for the third stage. Part D. Biological determinants. – Baltic Sea Environmental Proceedings no. 27D.

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

Heuer, H., Krsek, M., Baker, P., Smalla, K., & Wellington, E. M. H. 1997: Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. – Appl. Environ. Microbiol. 63: 3233–3241.

Hobbie, J. E., Daley, R. J., & Jasper, S. 1977: Use of Nuclepore filters for counting bacteria by fluorescence microscopy. – Appl. Environ. Microbiol. 33: 1225–1228.

Houghton, J. T., Ding, Y., Griggs, D. J., Noguer, M., van der Linden, P. J., Dai, X., Maskell, J. & Johnson, C. A. 2001: Climate Change 2001: The Scientific Basis. – Cambridge University Press, United Kingdom.

Hudier, E. & Ingram, G. 1994: Small scale melt processes governing the flushing of nutrients from a first-year sea ice, Hudson Bay, Canada. – Oceanol. Acta 17: 397–403.

Huttunen, M. & Niemi, Å. 1986: Sea-ice algae in the Northern Baltic Sea. – Memoranda Soc. Fauna Flora Fennica 62: 58–62.

Ikävalko, J. & Thomsen, H. A. 1997: The Baltic Sea ice biota (March 1994): a study of the protistan community. – Europ. J. Protistol. 33: 229–243.

Junge K. F., Eicken H & Deming J. W. 2004: Bacterial Activity at -2 to -20 ºC in Arctic Wintertime Sea Ice. – Appl. Environ. Microbiol. 70: 550–557.

Junge, K. F., Imhoff, J., Staley, J. & Deming, J. W. 2002: Phylogenetic diversity of numerically important Arctic sea-ice bacteria cultured at subzero temperature. – Microb. Ecol. 43: 315–328.

Kattner, G. & Becker, H. 1991: Nutrients and organic nitrogenous compounds in the marginal ice zone of Fram Strait. – J. Mar. Syst. 2: 385–394.

Kawamura, T., Shirasawa, K., Ishikawa, N., Lindfors, A., Rasmus, K., Granskog, M. A., Leppäranta, M., Martma, T. & Vaikmäe, R. 2001: Time series observations of the structure and properties of brackish ice in the Gulf of Finland, the Baltic Sea. – Ann. Glaciol. 33: 1–4.

Kemp, P. & Aller, J. Y. 2004: Estimating prokaryotic diversity: When are 16S rDNA libraries large enough? – Limnol. Oceanogr.: Methods 2: 114–125.

Kirchman, D., K'Nees, E. & Hodson, R. 1985: Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. – Appl. Environ. Microbiol. 49: 599–607.

Kivi, K., Kaitala, S., Kuosa, H., Kuparinen, J., Leskinen, E., Lignell, R., Marcussen, B. & Tamminen, T. 1993: Nutrient limitation and grazing control of the Baltic plankton community during annual succession. – Limnol. Oceanogr. 38: 893–905.

Kottmeier, S. T., Grossi, S. M. & Sullivan, C. W. 1987: Sea ice microbial communities. VIII. Bacterial production in annual sea ice of McMurdo Sound, Antarctica. – Mar. Ecol. Prog. Ser. 35: 175–186.

Krembs, C., Gradinger, R. & Spindler, M. 2000: Implications of brine channel geometry and surface area for the interaction of sympagic organisms in Arctic sea ice. – J. Exp. Mar. Biol. Ecol. 243: 55–80.

Krembs, C., Mock, T. & Gradinger, R. 2001: A mesocosm study of physical-biological interactions in artificial sea ice: effects of brine channel surface evolution and brine movement on algal biomass. – Polar Biol. 24: 356–364.

Kuparinen. J. & Heinänen, A. 1993: Inorganic nutrient and carbon controlled bacterioplankton growth in the Baltic Sea. – Est. Coast. Shelf Sci. 37: 271–285.

Kuuppo-Leinikki, P. 1990: Protozoan grazing on planktonic bacteria and its impact on bacterial population. – Mar. Ecol. Prog. Ser. 63: 227–238.

Laurion, I., Demers, S. & Vézina, A. F. 1995: The microbial food web associated with the ice algal assemblage: biomass and bacterivory of nanoflagellate protozoans in Resolute Passage (High Canadian Arctic). – Mar. Ecol. Prog. Ser. 120: 77–87.

Legendre, L., Martineau, M. J., Therriault, J. C. & Demers, S. 1992: Chlorophyll *a* biomass and growth of sea-ice microalgae along a salinity gradient (southeastern Hudson Bay, Canadian Arctic). – Polar Biol. 12: 445–453.

Leppäranta, M. 1981: On the structure and mechanics of pack ice in the Bothnian Bay. – Finnish Marine Research 248: 3–86.

Lignell, R., Kaitala, S. & Kuosa, H. 1992: Factors controlling phyto- and bacterioplankton in late spring on a salinity gradient in the Northern Baltic. – Mar. Ecol. Prog. Ser. 84: 121–131.

Lizotte, M. P. 2003: The microbiology of sea ice. – In: Thomas, D. N. & Dieckmann, G. S. (eds.), Sea Ice: An Introduction to its Physics, Chemistry, Biology and Geology, pp. 184–210. Blackwell Science, Oxford, UK.

Maranger, R., Bird, D. F. & Juniper, S. K. 1994: Viral and bacterial dynamics in Arctic sea ice during the spring algal bloom near Resolute, N.W.T., Canada. – Mar. Ecol. Prog. Ser. 111: 121–127.

Massana, R., Gasol, J. M., Bjørnsen, P. K., Blackburn, N. T., Hagström, Å., Hietanen, S., Hygum, B. H., Kuparinen, J. & Pedrós-Alió, C. 1997: Measurement of bacterial size via image analysis of epifluorescence preparations: description of an inexpensive system and solutions to some of the most common problems. – Sci. Mar. 61: 397–407.

McGrath Grossi, S., Kottmeier, S. T., Loe, R. L., Taylor, G. T. & Sullivan, C. W. 1987: Sea ice microbial communities VI. Growth and primary production in bottom ice under graded snow cover. – Mar. Ecol. Prog. Ser. 35: 153–164.

Meese, D. A. 1989: The chemical and structural properties of sea ice in the southern Beaufort Sea. – CRREL Rep. 89-25: 1–134.

Meier, H. E. M. 2002: Regional ocean climate simulations with a 3D ice-ocean model for the Baltic Sea. Part 2: results for sea ice. – Clim. Dyn. 19: 255–266.

Meiners, K., Fehling, J., Granskog, M. A. & Spindler, M. 2002: Abundance, biomass and composition of biota in Baltic sea ice and underlying water (March 2000). – Polar Biol. 25: 761–770.

Mock, T., Meiners, K. & Giesenhagen, H. 1997: Bacteria in sea ice and underlying brackish water at 54º26'50" N (Baltic Sea, Kiel Bight). – Mar. Ecol. Prog. Ser. 158: 23–40.

Mock, T. & Thomas, D.N. 2005: Recent advances in sea-ice microbiology. – Environ. Microbiol. 7: 605–619.

Muyzer, G., DeWaal, E. C. & Uitterlinden, A. G. 1993: Profiling of complex microbial-populations by denaturing gradient gel-electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S ribosomal-RNA. – *Appl. Environ. Microbiol.* 59: 695–700.

Nichols, D. S., Greenhill, A. R., Shadbolt, C. T., Ross, T. & McMeekin, T. A. 1999: Physicochemical parameters for growth of the sea ice bacteria *Glaciecola punicea* ACAM 611^T and *Gelidibacter* sp. Strain IC158. – Appl. Environ. Microbiol. 65: 3757–3760.

Nichols, D. S., Nichols, P. D. & McMeekin, T. A. 1995: Ecology and physiology of psychrophilic bacteria from Antarctic saline lakes and sea-ice. – Science Progress 78: 311–347.

Nichols, D. S., Olley, J., Garda, H., Brenner, R. R. & McMeekin, T. A. 2000: Effect of temperature and salinity stress on growth and lipid composition of *Shewanella gelidimarina*. – Appl. Environ. Microbiol. 66: 2422–2429.

Norrman, B. & Andersson, A. 1994: Development of ice biota in a temperate sea area (Gulf of Bothnia). – Polar Biol. 14: 531–537.

Oradiovskiy, S. G. 1974: Investigation of the chemical composition of Antarctic sea ice. – Oceanology 14: 50–54.

Pelegri, S. P., Dolan, J. & Rassoulzadegan, F. 1999: Use of high temperature catalytic oxidation (HTCO) to measure carbon content of microorganisms. – Aquat. Microb. Ecol. 16: 273–280.

Petri, R. & Imhoff, J. F. 2001: Genetic analysis of sea-ice bacterial communities of the western Baltic Sea using an improved double gradient method. – Polar Biol. 24: 252–257.

Pomeroy, L. R. & Wiebe, W. J. 2001: Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. – Aquat. Microb. Ecol. 23: 187–204.

Qian, J. & Mopper, K. 1996: Automated high performance, high-temperature combustion total carbon analyzer. – Anal. Chem. 68: 3090–3097.

Robinson, D. H., Arrigo, K. R., Kolber, Z., Gosselin, M. & Sullivan, C. W. 1998: Photophysiological evidence of nutrient limitation of platelet ice algae in McMurdo Sound, Antarctica. – J. Phycol. 34: 788–797.

Rysgaard, S. & Glud, R. N. 2004: Anaerobic N_2 production in Arctic sea ice. – Limnol. Oceanogr. 49: 86–94.

Salonen, K. 1979: A versatile method for the rapid and accurate determination of carbon by high temperature combustion. – Limnol. Oceanogr. 24: 177–129.

Seinä, A. & Palosuo, E. 1996: The classification of the maximum annual extent of ice cover in the Baltic Sea 1720–1995. – Meri 27: 79–91.

Seinä, A. & Peltonen, J. 1991: Duration of the ice season and statistics of fast ice thickness along

the Finnish Coast 1961–1990. – Finnish Mar. Res. 258: 1–43.

Sherr, E. B. 1988: Direct use of high molecular weight polysaccharide by heterotrophic flagellates. – Nature 335: 710–711.

Shiah, F. K. & Ducklow, H. W. 1997: Bacterioplankton growth responses to temperature and chlorophyll variations in estuaries measured by thymidine:leucine incorporation ratio. – Aquat. Microb. Ecol. 13: 151–159.

Sime-Ngando, T., Demers, S. & Juniper, K. S. 1999: Protozoan bacterivory in the ice and the water column of a cold temperate lagoon. – Microb. Ecol. 37: 95–106.

Sime-Ngando, T., Gosselin, M., Juniper, K.S. & Levasseur, M. 1997: Changes in phagotrophic microprotists (20–200 µm) during the spring algal bloom, Canadian Arctic Archipelago. – J. Mar. Syst. 11: 163–172.

Steemann Nielsen, E., 1952: The use of radioactive carbon (C14) for measuring organic production in the sea. – J. Cons. Int. Exp. Mer 18: 117–140.

Stewart, F. J. & Fritsen, C. H. 2004: Bacteria-algae relationships in Antarctic sea-ice. – Antarct. Sci. 16: 143–156.

Stigebrandt, A. 2001: Physical oceanography of the Baltic Sea. – In: Wulff, F. V., Rahm, L. A. & Larsson, P. (eds.), A Systems Analysis of the Baltic Sea, pp. 19–74. Springer-Verlag.

Stoecker, D., Buck, K. R. & Putt, M. 1993: Changes in the sea-ice brine community during the spring-summer transition, McMurdo Sound, Antarctica. II Phagotrophic protists. – Mar. Ecol. Prog. Ser. 95: 103–113.

Sullivan, C. W. & Palmisano, A. C. 1984: Sea ice microbial communities: distribution, abundance and diversity of ice bacteria in McMurdo Sound, Antarctica in 1980. – Appl. Environ. Microbiol. 47: 788–795.

Thomas, D. N., Engbrodt, R., Gianelli, V., Kattner, G., Kennedy, H., Haas, C. & Dieckmann, G. S. 2001: Dissolved organic matter in Antarctic sea ice. – Ann. Glaciol. 33: 297–303.

Thomas, D. N., Lara, R. J., Eicken, H., Kattner, G. & Skoog, A. 1995: Dissolved organic matter in Arctic multi-year sea ice during winter: major components and relationship to ice characteristics. – Polar Biol. 15: 477–483.

Thomas, D. N. & Papadimitriou, S. 2003: Biogeochemistry of Sea Ice. – In: Thomas, D. N. & Dieckmann, G. S. (eds.), Sea Ice: An Introduction to its Physics, Chemistry, Biology and Geology, pp. 267–302. Blackwell Science, Oxford.

Tiedje, J. M. 1988: Ecology of denitrification and dissimilatory nitrogen reduction to ammonium. – In: Zehnder, A. J. B. (ed.), Biology of Anaerobic Microorganisms, pp. 179–244. Wiley, New York.

Utermöhl, H. 1958: Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. – Mitt. Int. Ver. Theor. Angew. Limnol. 9: 1–38.

Vezina, A. F., Demers, S., Laurion, I., Sime-Ngando, T., Juniper, K. S. & Devine, L. 1997: Carbon flows through the microbial food web of the first-year ice in Resolute Passage (Canadian High Arctic). – J. Mar. Syst. 11: 173–189.

Voipio, A. 1981: The Baltic Sea. – Elsevier Oceanography Series 30. Elsevier. 418 pp.

Weissenberger, J., Dieckmann, G. S., Gradinger, R. & Spindler, M. 1992: Sea ice: a cast technique to examine and analyse brine pockets and channel structure. – Limnol. Oceanogr. 37: 179–183.

Werner, I. & Auel, H. 2004: Environmental conditions and overwintering strategies of planktonic metazoans in and below coastal fast ice in the Gulf of Finland (Baltic Sea). – Sarsia 89: 102–116.

Zumft, W. G. 1997: Cell biology and molecular basis of denitrification. – Microbiol. Mol. Biol. Rev. 61: 533–616.