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**Fast- and drift-ice communities
in the Bothnian Bay
and the impact of UVA radiation
on the Baltic Sea ice ecology**

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FAST- AND DRIFT-ICE COMMUNITIES IN THE BOTHNIAN BAY AND THE IMPACT OF UVA RADIATION ON THE BALTIC SEA ICE ECOLOGY

JONNA PIIPARINEN

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The seasonal sea ice cover is one of the main characteristics of the Baltic Sea. The structural similarity of Baltic Sea ice to polar sea ice with saline brine inclusions also enables the formation of active sympagic food webs in the brackish Baltic Sea ice. The sympagic communities generally show a clear succession with increasing light levels from the low productive winter stage to ice-algal blooms dominated by diatoms. The size and thus the community structure of the sympagic communities are significantly governed by the size of brine inclusions, which is a function of both salinity and temperature, initially of the parental water but later on of the ice. In the Baltic Sea, this translates to decreasing brine volumes from the northern Baltic Sea to the Bothnian Bay due to the increasing river water influence. The gradient is also accompanied by changes in other physical, chemical and biological features. However, the drift and fast ice in the Bothnian Bay may differ greatly in this sense, as drift ice may have been formed at more southern locations with higher salinities. The first theme of this thesis covers descriptive studies on sea-ice ecology from riverine-influenced fast ice to drift ice in the Bothnian Bay, which adds to knowledge of the ecological significance of the ice cover, which can last up to 7 months per year in this area.

Sea-ice food webs are to a large degree light-limited, and increasing light irradiances typically enhance the primary production in ice, fuelling the secondary production of other sea-ice organisms. However, any increase in solar radiation also includes an increase in harmful UVA radiation. The second theme of this thesis examines the sensitivity of sea-ice bacteria and algae to UVA and is based on two in situ experiments. These results are of importance when assessing the effects of thinning of the ice cover due to climate change.

Rafting and the formation of snow ice are common processes in the ice field of the Bothnian Bay and as was evidenced in this thesis, they markedly affect the chemical and biological properties. Rafting altered the vertical distribution of organisms and, for example, former bottom communities were trapped in the middle of the new rafted ice column or even displaced to the surface. Snow-ice formation, on the other hand, provided habitable space and centric diatoms in particular seemed to benefit from this increased habitat in the better-illuminated, nitrogen-rich surface layer. Due to the highly dynamic behaviour of the Bothnian Bay sea ice, no clear-cut division into drift and fast ice could be made based on the chemical and biological properties, and the similarity / dissimilarity of the two ice types depended on the developmental phase of the ice. The divergence became apparent at the more advanced stages, and a decreasing trend in chlorophyte biomass from fast to drift ice was observed, while the opposite held true for protozoan and metazoan biomass. The brine volumes affected the communities somewhat, and a higher percentage of flagellate species was generally linked to lower brine volumes, whereas chain-forming diatoms were mostly concentrated in layers with larger brine volumes. Close to the river mouth, the low brine volumes in the bottom layer resulting from a strong river water influence were counteracted by the build-up of a marked biomass in the surface layer with a notably higher brine volume. This emphasizes the significance of snow-ice formation and other large brine volume habitats for ice biomass in the Bothnian Bay.

The sensitivity of Baltic Sea ice microbial communities to UVA was clearly demonstrated in this thesis. The responses were strongly linked to the earlier light history, as well as to the solar irradiances they were exposed to. The increase in the biomass of chlorophytes and pennate diatoms following the clearance of the thin snow cover and exclusion of UVA indicates that their normally minor contribution to the biomass in the upper layers of sea ice might be partly dictated by UVA. However, when dark-acclimated algal communities thriving under a thick snow cover were exposed, UVA had only a minor effect and the decline in both the chlorophyll-a concentration and diatom abundance was mostly attributed to PAR. The effects of UVA on bacterial production in Baltic Sea ice were mostly indirect and followed the responses in algal growth. The results also demonstrated that UVA may occasionally enhance bacterial production independently of algal growth for reasons such as the photochemical degradation of organic matter and increased algal exudation under stress, producing labile carbon. The dominant bacterial class, Flavobacteria, seemed to be UVA-tolerant and was the only class to benefit from UVA exposure. On the contrary, all the Alpha-, Beta- and Gammaproteobacteria present in the surface layer showed UVA sensitivity. These results indicate that changes in the light field of ice may alter the community structure and affect the functioning of ice food webs.

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1. INTRODUCTION

1.1. Baltic Sea

By comprising an area of about 393 000 km² and having a mean salinity of 7, the Baltic Sea is the world's second largest brackish water body. The Baltic Sea has an average depth of 54 m and is strongly stratified with regards to salinity and temperature. Based on salinity, the Baltic Sea water column is divided into three layers (upper layer, halocline and lower layer), which are present around the year (Leppäranta & Myrberg 2009). Seasonal variation in air temperature results in the formation of additional transient sublayers in the upper layer in summer, when the presence of a thermocline and a colder diathermal layer can be observed. The salinity in the upper layer decreases from 18–26 in the Kattegat to 2–4 in the Bothnian Bay, which is attributable to the high inflow of fresh water from the numerous rivers in the surrounding catchment area of 1 720 000 km² and to the positive water balance, i.e. the river inflow and precipitation exceeds evaporation (see Leppäranta & Myrberg 2009). The river water not only affects the salinity, but brings nutrients, organic matter and contaminants to the Baltic Sea, the quantity and quality of the loads depending on the catchment area of the river. Due to the brackish nature and short evolutionary history of c. 10 000 years, the biodiversity of the Baltic Sea is low as the salinity is generally too low for marine species and too high for freshwater species.

One of the main characteristics of the Baltic Sea is the seasonal ice cover. The duration, extent and thickness of the sea ice depend on the severity of the winter and show large interannual variability. In very mild winters only a small part (minimum 12.5 % in 2008) of the Baltic Sea has an ice

cover, whereas during very cold winters, the whole Baltic Sea is ice-covered (Leppäranta & Myrberg 2009). The last severe winter was recorded in 1987 (max annual ice extent > 300 000 km²), after which the winters have been mild and on average less than 50 % of the Baltic Sea has been ice-covered (Vainio in Leppäranta & Myrberg 2009).

In the Baltic Sea, freezing starts from the Bothnian Bay in mid-November and proceeds to the Gulf of Finland, which becomes ice-covered approximately a month later. Melting proceeds in the opposite direction, starting in southern locations in March. The sea ice, which is formed along the coast and is attached to the shore or to the islands in the archipelago, is called fast ice. As the name implies, it remains on the same spot for most of the time. At a certain water depth, the ice becomes dynamic, i.e. it is moved by winds and currents, and is thus called drift ice. In the Baltic Sea, the boundary depth between fast and drift ice depends on the ice thickness and is generally between 5 and 15 m (Leppäranta 1981). However, in very cold winters such as 1985, the whole Bothnian Bay ice cover may become immobile fast ice (Leppäranta 1987). The prevailing southwest winds during Baltic winters transport the drift ice from more southern locations to the Bothnian Bay forming large pack-ice fields where rafting and ridging (i.e. dynamic ice growth) play significant roles in ice thickness growth (Omstedt 1985, Granskog et al. 2004b).

1.2. Structure of Baltic Sea ice

A typical feature of sea ice is inclusions of highly saline, liquid brine. Brine inclusions are formed during freezing, when a part of the salt, rejected from the forming ice crystal lattices, is concentrated between the ice crystals. Naturally, freshwater ice

do not contain brine inclusions, which start to form when the salinity of parent water exceeds 0.6, meaning that most of the Baltic Sea ice is structurally similar to polar sea ice (Palosuo 1961, Leppäranta & Manninen 1988, Weeks et al. 1990, Kawamura et al. 2001). Similarly to salt, part of the dissolved inorganic and organic matter is also expelled from the ice during the ice formation process, and only about 10–40 % is retained within the ice (Petrich & Eicken 2010). The dissolved organic matter (DOM) in sea ice is allochthonous, deriving from the parent water, or autochthonous, deriving from exudation and cell lysis in ice. In the Baltic Sea, the strong allochthonous influence can be observed in the sea ice as the presence of humic substances (Stedmon et al. 2007).

The brine in ice can be distributed in multiple separate brine pockets (typical for young ice) or it can be contained in a brine channel system, where brine pockets are interconnected (typical for older ice). The total volume of brine in ice depends on the temperature and salinity of the parent water and ice, as well as on desalination processes in ice such as gravity drainage, brine expulsion and flushing (e.g. Petrich & Eicken 2010). During ice formation, temperature inversely affects the volume of entrapped brine, i.e. the colder it is, the more brine gets trapped between ice crystals. In the formed ice sheet, the salinity and volume of brine vary with temperature: when temperature decreases, the brine concentrates into a smaller volume with higher salinity, and during melting the reverse happens. The air temperature varies more than the temperature of underlying water, and the variations in ice temperature are thus greater in the upper ice than in the lower part. The presence of snow moderates the temperature variations, as snow is an efficient insulator. The low ambient Baltic Sea salinity reflects to the bulk ice salinity, which rarely exceeds

2 (Kawamura et al. 2001, Meiners et al. 2002, Granskog et al. 2004a, 2006b). The total brine volume is also less than in polar ice, being mostly below 10 % of the total ice volume (Meiners et al. 2002). A value of 5 % is considered as the threshold for permeability, after which the brine pockets are assumed to be interconnected and fluids are transported more freely (Golden et al. 1998). However, due to the lower total brine volumes in the Baltic Sea, higher temperatures are needed to render the ice permeable in comparison to polar ice (Granskog et al. 2006a).

The fast ice in the Baltic Sea typically has two layers of structurally different ice (Kawamura et al. 2001, Granskog et al. 2003b, Uusikivi et al. 2011). The topmost layer is termed the granular layer and it reflects the initial stages of ice formation, when ice crystals grow equally in all directions (diameter 1-5 mm). After a solid ice cover has been formed, the ice crystals start to grow more downwards, producing columnar ice with crystals up to half a metre long. In dynamic conditions such as in the open sea, the ice grows via a pancake ice cycle and the ice is mostly granular and transitional (Omstedt 1985, Lange et al. 1989, Weeks et al. 1990, Granskog et al. 2003b). In addition to normal downwards growth, the granular layer on the top often increases in thickness during the ice-covered period in the Baltic Sea due to the formation of superimposed ice and snow ice (i.e. meteoric ice). In the first case, the fluctuating air temperatures result in melting-freezing events in the Baltic Sea, when the melted snow is incorporated in sea ice as superimposed ice (Granskog et al. 2006c, Pirazzini et al. 2006, Uusikivi et al. 2011). In the latter case, the freeboard is negative due, for instance, to a heavy snow load, and the underlying seawater floods on top of the ice via cracks or by filtration through the ice and is subsequently frozen

together with snow, forming snow ice (Seinä & Peltola 1991, Leppäranta & Myrberg 2009).

1.3. Baltic Sea ice biota

The sea-ice habitat, i.e. the brine inclusions, is cold (down to minus 20–30 °C), saline (up to 200), concentrated in elements and a spatially closed environment. Due to the brackish parent water, the brine salinity varies from 6 to 30 in Baltic Sea ice (Ikävalko & Thomsen 1997, Mock et al. 1997, Kaartokallio 2001) and the brine channels are smaller (< 200 µm) than in polar ice, which in turn sets an upper limit for the size of sea-ice organisms. Thus, the food web is microbial and consists of bacteria, algae and heterotrophic flagellates in nano- and micro-size classes (2–20 µm and 20–200 µm, respectively), ciliates, rotifers, and copepod nauplii stages, the last two representing the top predators (Meiners et al. 2002, Kaartokallio 2004, Kaartokallio et al. 2007). At the base of the microbial food web are the bacteria, which have a key role in returning the energy and material in the released DOM back to the food web. In addition to the typical size-based trophic interactions in a microbial food web (e.g. Azam et al. 1983), the food web in sea ice has 'shortcuts', meaning that bacteria are grazed by ciliates, DOM is taken up directly by heterotrophic flagellates, flagellates are grazed by rotifers and ciliates and heterotrophic flagellates also graze on algae (e.g. Delille et al. 2002, Kaartokallio 2004). Further complexity in the food web is elicited by mixotrophic protists, which obtain nutrition by combining autotrophic and heterotrophic mechanisms. Examples of mixotrophs include the chloroplast-bearing obligate phototrophic ciliate *Mesodinium rubrum*, heterotrophic dinoflagellates with

kleptochloroplasts and phagotrophic algae (Kaartokallio 2004, Gast et al. 2007, Moorthi et al. 2009). Viruses have not yet been studied in Baltic Sea ice, but their effect on bacteria as well as on eukaryotes cannot be excluded (Deming 2010).

The Baltic sea-ice bacterial communities show a close association with the polar phylotypes, and the majority of sequences belong to α -, β - and γ -proteobacteria and to the *Cytophaga-Flavobacteria-Bacteroidetes* group (Kaartokallio et al. 2005, 2008). Similarly to polar sea ice, diatoms are generally the dominant algal group in the Baltic Sea ice with representatives from both centric (e.g. *Chaetoceros wighamii*, *Melosira arctica* and *Thassiosira baltica*) and pennate species (e.g. *Achnanthes taeniata*, *Navicula vanhoeffenii* and *Nitzschia frigida*) (Haecky et al. 1998, Haecky & Andersson 1999, Kaartokallio et al. 2007). Dinoflagellates are also abundant in ice and typically dominated by *Scrippsiella hangoei* (Larsen et al. 1995, Meiners et al. 2002, Kuosa & Kaartokallio 2006), but the presence of *Peridiniella catenata* has also been reported (Norrman & Andersson 1994, Haecky & Andersson 1999, Kaartokallio et al. 2007). Small auto- and heterotrophic flagellates (< 20 µm) can form a substantial part of the biomass (Ikävalko & Thomsen 1997, Meiners et al. 2002, Kaartokallio 2004), but light microscopy is generally too imprecise to identify them. Chlorophytes (e.g. *Chlamydomonas* sp., *Dictyosphaerum* sp. and *Monoraphidium contortum*), cryptophytes and cyanobacteria also contribute to the ice-algal biomass in the Baltic Sea, but much less than diatoms and dinoflagellates (Huttunen & Niemi 1986, Ikävalko & Thomsen 1997, Meiners et al. 2002). Most of the ciliates belong to the genera *Bursaria*, *Strombidium* and *Lacrymaria* (Kaartokallio 2004, Kaartokallio et al. 2007). The rotifer biomass in ice mostly

consists of *Synchaeta* cf. *littoralis* / *baltica* (Norrman & Andersson 1994, Meiners et al. 2002, Kaartokallio 2004). Copepod nauplii stages are generally unidentified, but in the study of Werner & Auel (2004) they were identified as *Acartia biflosa* nauplii.

The typical succession of the ice-communities in Baltic Sea follows the annual cycle of solar radiation, and advances from a low productive mid-winter stage to ice-algal blooms in March-April, followed by a post-bloom heterotrophic phase (Norrman & Andersson 1994, Haecky et al. 1998, Haecky & Andersson 1999, Kaartokallio 2004, Kuosa & Kaartokallio 2006). Following ice formation, the initial community, which has been entrapped in ice either passively by enclosure or actively by scavenging (Ackley & Sullivan 1994), starts to change, as not all the entrapped organisms are able to tolerate the new habitat. Thus, an increasing divergence from the parent water community is observed over time (Meiners et al. 2002, Kaartokallio et al. 2007). This also indicates that most of the ice organisms are actively growing, although it has also been proposed that ice might act as an overwintering habitat for metazoans (Werner & Auel 2004). During ice melt, all the material inside the ice is released to the underlying water. The released nutrients may fuel a spring bloom and the ice diatoms may seed the phytoplankton community unless not settled on the bottom (Haecky et al. 1998).

The quantity and composition of ice biomass change along the south-north axis (Meiners et al. 2002). In the Gulf of Finland (GoF), the chlorophyll-*a* (chl-*a*) values are at maximum 5.5 mg m⁻², with diatoms as the dominant group, whereas in the Gulf of Bothnia (GoB) the maximum chl-*a* value is 2.2 mg m⁻² and the biomass is mainly composed of flagellates (Granskog et al. 2006a and references therein). Bacterial

production (BP) shows great variability and was only slightly higher in the GoF (0.0–2.4 µg C l⁻¹ h⁻¹) than in the GoB (0.0–1.1 µg C l⁻¹ h⁻¹), with the bacterial biomass showing the same trend (GoF: 0.6–56.3 µg C l⁻¹, GoB: 0–29.6 µg C l⁻¹) (Kaartokallio 2005).

1.4. UVA

Solar radiation comprises the part of electromagnetic radiation emitted by the sun that reaches the Earth's surface. It can be divided into ultraviolet radiation (UVR, 280–400 nm), photosynthetically active radiation (PAR, 400–700 nm) and infrared radiation (IR, 700–3000 nm). UVR is further divided to UVB (280–315 nm) and to UVA (315–400 nm), and as the energy content per photon decreases with increasing wavelength, UVB is biologically more harmful than UVA. However, the wider wavelength span of UVA may cause more damage to organisms, especially in aquatic environments, where UVB attenuates considerably faster than UVA, the attenuation coefficient depending on the concentration and optical qualities of dissolved and particulate (living and non-living) matter. In the dissolved fraction, chromophoric dissolved organic matter (CDOM), which mostly consists of humic substances, is mainly responsible for the absorption of solar UV and blue radiation and the attenuation is enhanced by the absorption by phytoplankton, especially in eutrophic waters (Wetzel 2003 and references therein).

1.4.1. UVA damage and repair mechanisms

In unicellular organisms, the radiation rapidly reaches vital components such as DNA, and they are therefore the most sensitive to UVR

(Garcia-Pichel 1994, Karentz et al. 1994, Jeffrey et al. 1996). Although the absorption peak of DNA (260 nm) is well below the UVA range, DNA can be damaged indirectly via non-DNA chromophores (e.g. riboflavin, porphyrins, NADH/NADPH), which absorb the UVA and pass the excitation energy on to DNA or molecular oxygen. This leads to the formation of reactive oxygen species (ROS), which in turn transfer the energy to organic molecules such as DNA, resulting DNA breaks and the formation of pyrimidine dimers and 6-4 adducts (Peak & Peak 1986). In addition, UVA is also known to damage cell membranes, cellular proteins and RNA. Thus, the effects of UVA vary from lethal to sublethal, depending on the UVA fluence rate and total dose, as well as on the presence of oxygen (Jagger 1981, Pizarro & Orce 1988).

In algae, one of the main effects of UVA is the inhibition of photosynthesis. This is due to the high sensitivity of the reaction centre of the photosystem II (PSII) protein-pigment complex and the Calvin cycle enzymes to ROS, which are formed in oxygenated conditions when the absorption of excessive PAR and UVA over-saturates the photosynthetic electron transport chain and chlorophyll-*a* becomes an alternative electron acceptor (Asada & Takahashi 1987, Salin 1988). In some cases, a threshold irradiance needs to be exceeded before photoinhibition takes place. Antarctic coastal phytoplankton had a threshold of 10 W m^{-2} , whereas Arctic deep-layer phytoplankton showed immediate photoinhibition when exposed to UVR (Booth et al. 1997, Helbling et al. 1996b). Algae are, however, able to recover from UVA exposure by increasing the chlorophyll-*a* content and reactivating the damaged photosystem (Kim & Watanabe 1994). In addition to damage in PSII, the presence of ROS may also lead to loss of chlorophyll-*a* in the chloroplasts,

i.e. photobleaching (Gerber & Häder 1995, Maske & Latasa 1997).

Organisms have developed different means to cope with solar UVR (see Banaszak 2003) and references therein), which usually come with energetic costs resulting in, for instance, decreased growth and survival rates (Vincent & Neale 2000). First in line are the physical barriers, which include cell size, shape, structures in the outer covering of a cell and migration patterns. Inside the cell, UV-absorbing compounds, pigments and different quenching mechanisms become important. Many organisms are able to produce mycosporine-like amino acids (MAAs), which are cyclohexenone or cyclohexenimine rings with varying amino acid side groups absorbing mostly in the UV range between 309 and 360 nm (Roy 2000 and references therein). Other absorptive compounds include scytonemin in the extracellular sheet of cyanobacteria and melanin in the skin of aquatic vertebrates and in the cuticle of cladocerans. Quenching, i.e. the neutralization of toxic photoproducts like ROS, is mediated by antioxidants such as superoxide dismutase, ascorbate peroxidase and carotenoid pigments.

Organisms also possess a variety of repair mechanisms to counteract the UVR-induced photodamage. This is especially vital for heterotrophic bacteria, which generally lack UVR-absorbing protective pigments and compounds (Karentz et al. 1994). Protein damage is mainly overcome by *de novo* synthesis, whereas DNA damage needs to be repaired. The most common damage in DNA (50–80 %) is dimers between two adjacent pyrimidine bases, resulting in a block in the DNA replication process (e.g. Banaszak 2003). The dimers can be removed via two repair pathways: photoreactivation and excision repair. Photoreactivation only involves the enzyme

photolyase, which recognizes the structural distortion in DNA, and by absorbing UVA and short-wavelength PAR, monomerizes it. Excision repair, on the contrary, does not need light and includes many enzymes, which recognize the structural distortion, remove it and resynthesize a segment of DNA to complete the DNA strand. In addition to repair pathways, organisms have dimer bypass and recombinational repair as tolerance pathways, which do not repair the damage but help organism to tolerate it (Banaszak 2003).

The effects of UVR on aquatic organisms show dependence on the latitude. In a study by Helbling et al. (1992), Antarctic phytoplankton were significantly more affected by UVR than tropical phytoplankton, and more than 50 % of the photoinhibition caused by UVR was due to UVA. The greater photoinhibitory effect of UVA on phytoplankton communities compared to UVB is supported by several studies reviewed by Mock and Thomas (2005). UVA also played a larger role in reducing bacterial viability in Antarctic waters (Helbling et al. 1995) and bacterial production in the surface water layer of the northern Adriatic Sea and an alpine lake (Sommaruga et al. 1997).

1.4.2. Solar radiation and sea ice

The incident radiation field consists of a direct beam component from the sun and of a diffuse component from the sky and clouds. Part of the incoming radiation is reflected from the surface either as specular (the angle of incidence is the same as reflected) or diffuse reflection (light is distributed in all directions independently of the angle), and the size of the reflected fraction, i.e. albedo, is dependent on many factors such as the wavelength, solar zenith angle, the surface

characteristics and the relative proportions of direct and diffuse irradiance (Perovich 1996). The ice albedo has large spatial and temporal variability. The total albedo (400–2400 nm) of snow-covered sea ice is higher (0.78) than that of bare melting ice (0.44) or ice with melt ponds (0.20) (Perovich et al. 1998). However, as the longer wavelengths have lower albedos, it is more informative to separate the total albedo into different wavelength regions. In the study of Perovich et al. (1998), the albedo for UVR wavelengths of 305–400 nm and PAR (400–700 nm) was quite uniform and decreased from 0.88 in the cold snow-covered ice to just below 0.6 in the melting ice, and further to approx. 0.3 when the melting ice had surface ponds.

The penetration and attenuation of radiation in sea ice depends on the snow and ice thickness, as well as on impurities (biota, particulate and dissolved organic matter) and inclusions (air bubbles and brine pockets). Impurities are generally strongly absorbing, showing great wavelength dependence, while inclusions are mostly scatterers, affecting the direction of the radiation but not the spectral quality (Perovich 1996 and references therein). The majority of the absorption by DOM is attributed to the chromophoric fraction absorbing between 300 and 800 nm, i.e. CDOM. The studies of Belzile et al. (2002) and Ehn et al. (2004) indicate that the CDOM in ice is qualitatively different from the CDOM in the water, which might be attributed to the freezing or degradation processes in ice. Particulate matter primarily attenuates light by absorbing it, but depending on the matter, it can also scatter light (e.g. Perovich 1998). The absorption by water molecules becomes significant above 700 nm.

Scattering is caused by the difference in the indices of refraction between an inclusion and the surrounding ice, and the

distribution of the brine and air bubbles in many small inclusions therefore enhances scattering compared to the presence of fewer larger inclusions. The direction of scattering is usually more forward than sideways or backwards, and once the ice is thick enough, the backscattering does no longer affects the albedo (Perovich 1996).

The portion that is not reflected or absorbed is transmitted through the ice and the physical properties of the ice and the wavelength of the light determine the relative scale of these processes. As the inclusions undergo both horizontal and vertical temporal and spatial variations, the optical properties of ice also exhibit large variation. The cold snow-covered ice and bare melting ice in Arctic were found to have an approximately equal transmittance of 0.25 % at 320 nm and 1.8–2.1 % at 400–700 nm, whereas for ponded ice of equal thickness, the respective values were 6.2 % and 14.4 %, the difference being strongly linked to the different bottom-ice algal biomasses (Perovich et al. 1998).

The transmittance of UVR varies greatly depending on the above-described factors. Similarly to other aquatic environments, UVB is attenuated more than either UVA or visible radiation. In the Weddell Sea, the mean transmittances for UVA and UVB in ice with an average thickness of 52 cm and approx. 8 cm snow cover were 4.2 % and 2.5 %, respectively (Perovich 1993). The UVR transmittance showed higher sensitivity to snow depth than the transmittance of PAR (Perovich 1993, 1995). Thus, even a thin snow cover can greatly reduce the amount of biologically harmful radiation in and under the ice, while the level of PAR is less affected. As ice is generally composed of different ice types (e.g. columnar, granular), the extinction coefficients in different layers vary accordingly (e.g. Grenfell & Maykut 1977). The changes in physical

and biological characteristics result in the dependence of transmittance on the age of the ice. For example, 33 cm thick young sea ice transmits roughly 20 % of UVR, while cold first-year ice of ≥ 1.25 m containing a greater bottom biomass transmits less than 2 % (Perovich 1995).

1.4.3. Optical properties of Baltic Sea ice

Compared to polar sea ice, the ice cover in the Baltic Sea is thin. The snow cover is also relatively thin, showing high spatial and temporal variations in its presence, thickness and density. The typically large snow-ice fraction in the surface ice increases albedo by scattering and the transmittance of ice is reduced due to atmospheric fallout and high concentrations of particulate and dissolved organic material derived from land and autochthonous production (Leppäranta et al. 2003, Ehn et al. 2004, Uusikivi et al. 2010). As a consequence of the relatively thin ice, backscattering from the ice and through the ice surface may occasionally play a substantial role. Milder periods with surface ice melting are frequent during the winters in the Baltic Sea and the resulting wet ice surfaces strongly reduce the albedo (Rasmus et al. 2002).

The optical properties of Baltic Sea ice have been examined in only a few studies, and most of them have been conducted in Santala Bay, Gulf of Finland. Consistently with observations from the Arctic (e.g. Perovich et al. 1998), the majority of the transmitted light in Baltic Sea ice is in the PAR band (Leppäranta et al. 2003, Ehn et al. 2004). In March 2000, the transmittance of PAR varied from 25 to 42 % increasing to 66–73 % during the melt phase and a parallel trend was also observed in UVA (Ehn et al. 2004). The ice surface conditions affected

the transmittance, especially of UVA, and the spectral transmittance of UVA in 27.5 cm thick snow-free ice with a wet surface was markedly higher (from 10 % at 315 nm to 30 % at 400 nm) than in the equally thick ice with a frozen surface (from 0 % to 15 %, respectively). In early April, the spectral transmittance of UVA for 30 cm thick ice with a 3 cm snow cover was between 5 and 11 %, while the transmittance of PAR was only 13.6 % (Rasmus et al. 2002).

The shape of the spectral albedo curve in the study of Ehn et al. (2004) was similar to the curve obtained in the Arctic, but the thinner ice, higher atmospheric fallout and CDOM concentration in Baltic Sea resulted in a lower albedo. The maximum transmittance of blue-white ice is at 470 nm, but the absorbing inclusions shift it to 500–550 nm (Perovich et al. 1998), and in the Baltic Sea this shift is further to 562–570 nm (Ehn et al. 2004, Uusikivi et al. 2010). The modelled results of Uusikivi et al. (2010) indicate that the transmission of UVR is mainly affected by CDOM, whereas the transmission of PAR is influenced more by particulate matter.

1.4.4. UVA and sea-ice organisms

The effects of UVR on sea-ice organisms have been studied far less than on plankton, and the focus has been mainly on UVB (e.g. Ryan & Beaglehole 1994, Schofield et al. 1995, McMinn et al. 1999), but the effects of UVA have not been investigated up to date. UVA damage to organisms in sea ice may be more substantial than in surface waters, for many reasons.

i) Dark-acclimated organisms are generally highly sensitive to PAR, but also to UVR (e.g. Villafañe et al. 1995, 2004), as the

photoinduction of screening compounds and activity of photorepair enzymes are directly dependent on light intensities (Vernet 2000). Thus, the light history plays an important role in determining the responses to UVA, and pre-acclimation under PAR enhances the recovery from the UVR exposure (Kim & Watanabe 1994, Vernet 2000 and references therein).

ii) The vertical movement of sympagic organisms is very limited, and unlike plankton they are not able to escape the UVR to deeper layers. Thus, sympagic organisms can be assumed to be more cumulatively exposed to solar radiation than plankton. This especially concerns heterotrophic bacteria, which unlike phytoplankton do not usually possess any UV-protective compounds or pigments.

iii) The actively growing cells are often more sensitive to inactivation than the cells in the stationary phase due to the shorter time for excision repair between subsequent rounds of replication (Harm 1980, Jagger 1985). Junge et al. (2002) and Martin et al. (2008) reported that 27–34 % of polar sea-ice bacteria were active (stained with 5-cyano-2,3-ditoyl tetrazolium chloride, CTC), which is markedly higher than the 1–10 % reported for bacterioplankton (Karner & Fuhrman 1997, Sherr et al. 1999).

iv) The extracellular formation of ROS in ice is favoured in hyperoxic ($> 300\text{--}350 \mu\text{mol O}_2 \text{ kg}^{-1}$) conditions, like those commonly observed in brine, causing oxidative damage to the cells in ice (Thomas et al. 2010 and references therein).

- v) The recovery rate in a cold environment like sea ice might be lower than at higher temperatures, as recovery is often driven by enzymatic reactions, which are directly related to temperature (Vernet 2000 and references therein).

2. OUTLINE OF THE THESIS

This thesis is divided into two main themes: the sea-ice communities in the Bothnian Bay (I, II) and the effect of UVA on Baltic sea-ice communities (III, IV). The first two papers are explorative in nature, and by covering sympagic communities from different kinds of drift ice (I) to fast ice (II) in the Bothnian Bay, an attempt is made to link the biotic to abiotic physico-chemical variables (e.g. nutrients and ice structure). The last two papers (III, IV) are based on an *in situ* experimental set-up aiming to assess the sensitivity of Baltic Sea ice algae and bacteria to solar UVA radiation when exposed to low versus increasing solar irradiance.

The Bothnian Bay sea ice has been a focus of many physico-chemical investigations (Palosuo 1961, Weeks et al. 1990, Rahm et al. 1995, Granskog et al. 2003b, 2004b, 2005a, 2006b, Granskog & Kaartokallio 2004). However, only a few ecological studies from drift ice (Ikävalko & Thomsen 1997, Ikävalko 1998, Meiners et al. 2002) and none on fast ice have been reported from the Bothnian Bay. For a more comprehensive view, multiple locations on drift ice were sampled in 2006 (I) to include ice of different origins, and fast-ice succession was monitored at three stations with varying degrees of river influence from February to April 2004 (II). These studies add to knowledge of the ecology of Bothnian Bay sea ice, which has earlier largely been neglected.

The effect of UVR on Baltic Sea ice communities has been an understudied subject to date. According to the climate change scenarios (Haapala et al. 2001, HELCOM 2007), UVR will have the most adverse effect on the winter time communities, especially in sea ice, as the thinner snow and ice cover will allow more radiation to penetrate into and through the ice, among other effects. Although UVB is more harmful per photon, UVA has been recognized to be the mainly responsible for the photoinhibition in the Antarctic and other aquatic environments (Bühlmann et al. 1987, Cullen et al. 1992, Helbling et al. 1992, Smith et al. 1992, Villafañe et al. 2003 and references therein, Villafañe et al. 2004), and similar observations have been reported on bacterioplankton viability and production (Helbling et al. 1995, Sommaruga et al. 1997). For the first time, the responses of ice algae and bacteria to UVA were investigated in the Baltic Sea (III, IV) providing novel information on the UVA sensitivity of the ice communities. The two major questions answered in these studies were: 1) Are the winter-time UVA irradiances high enough to have an impact on Baltic Sea ice algal and bacterial communities, and their processes and 2) does the timing and duration of UVA exposure matter?

3. STUDY AREA

The semi-enclosed Baltic Sea is separated into four major sub-basins with their own characteristics: the Baltic Proper, Gulf of Riga, Gulf of Finland and Gulf of Bothnia. All the studies presented in this thesis were conducted in the northern Baltic Sea (Fig. 1), where the surface water salinity decreases from 6 to < 1 with increasing latitude. This is due to the increasing river discharge towards the Bothnian Bay as well as the topography of the Baltic Sea.

3.1. Bothnian Bay

The northernmost part of the Baltic Sea, the Bothnian Bay, which is the focus of this thesis (I, II), is separated from the Bothnian Sea by two sills at 25 m depth in the Quark area. The sills prevent the intrusion of saline deep water, which together with the strong river discharge ($102 \text{ km}^3 \text{ yr}^{-1}$) from a catchment area of $260\,000 \text{ km}^2$ reduces the salinity of the Bothnian Bay and makes the vertical salinity gradient weak, varying from 4.0–4.5 in the deep to 0.0–3.8 in the surface layers (Mälkki & Tamsalu 1985). In addition, river waters are rich in nutrients and annually bring 45 755 tons of N and 2 400 tons of P to the basin (averages 1994–2004, Finnish Environment Institute), but due to the absorption of phosphate by humic and iron compounds, primary production is often P-limited in the Bothnian Bay (dissolved inorganic N:P > 130 in sea water) (Kankaala et al. 1984, Tamminen & Andersen 2007, Fleming-Lehtinen et al. 2008). The Bothnian Bay is also distinctively characterised by a long ice-covered period, which can last up to 7 months (Ice service, Finnish Meteorological Institute). Along the coast, fast ice is formed,

while the central part of the Bothnian Bay is covered by drift- and pack-ice fields. Due to the prevailing SW winds in winter, the ice packs and forms pressure ridges of several metres in height, hampering the wintertime navigation in the Bothnian Bay. Following the 5–15 m isobath, which separates fast and drift ice (Leppäranta 1981), one-third of the Bothnian Bay is fast ice and two-thirds is drift / pack ice.

In this thesis, samples were collected from both drift ice (I) and fast ice (II) in the Bothnian Bay. Different types of drift ice were sampled offshore from various locations, while the fast-ice samples were collected in a coastal area influenced by the inflowing waters of the regulated, eutrophicated River Siikajoki, NW Finland (Fig. 1). The brown colouration and pH of 6.4 (mean 2000–2004, Finnish Environment Institute) of the river water can be attributed to the high amount of humic substances draining from the forest- and peatland-dominated catchment area of $4\,318 \text{ km}^2$ (Ekholm 1993). The mean annual discharge is approx. $38 \text{ m}^3 \text{ s}^{-1}$ (1936–2004) and the highest values occur in April–May due to rain and the melting of ice and snow (Finnish Environment Institute) when the coast is still ice-covered, and the river water thus spreads as a freshwater lens under the ice further than during the open sea period (Granskog et al. 2005a).

3.2. Tvärminne

The UVA experiments (III, IV) were carried out in the Gulf of Finland, in the vicinity of the Tvärminne Zoological Station in the Hanko Peninsula, SW Finland (Fig. 1). The study site is sheltered by islands and the water depth is about 3.5 m. There are no major rivers flowing through the area and the surface water salinity is generally between 5

and 6. However, in winter a low salinity layer of 1–2 may form under the ice overlaying this normal salinity water (III). The ice coverage in the area is highly dependent on the coldness of the winter. During severe and moderate winters ice covers the area for 3

to 5 months, reaching thicknesses > 30 cm, while in the mild winters, the sea remains open. The currents and winds also affect the ice conditions, and strong winds may remove the whole ice cover within one day.

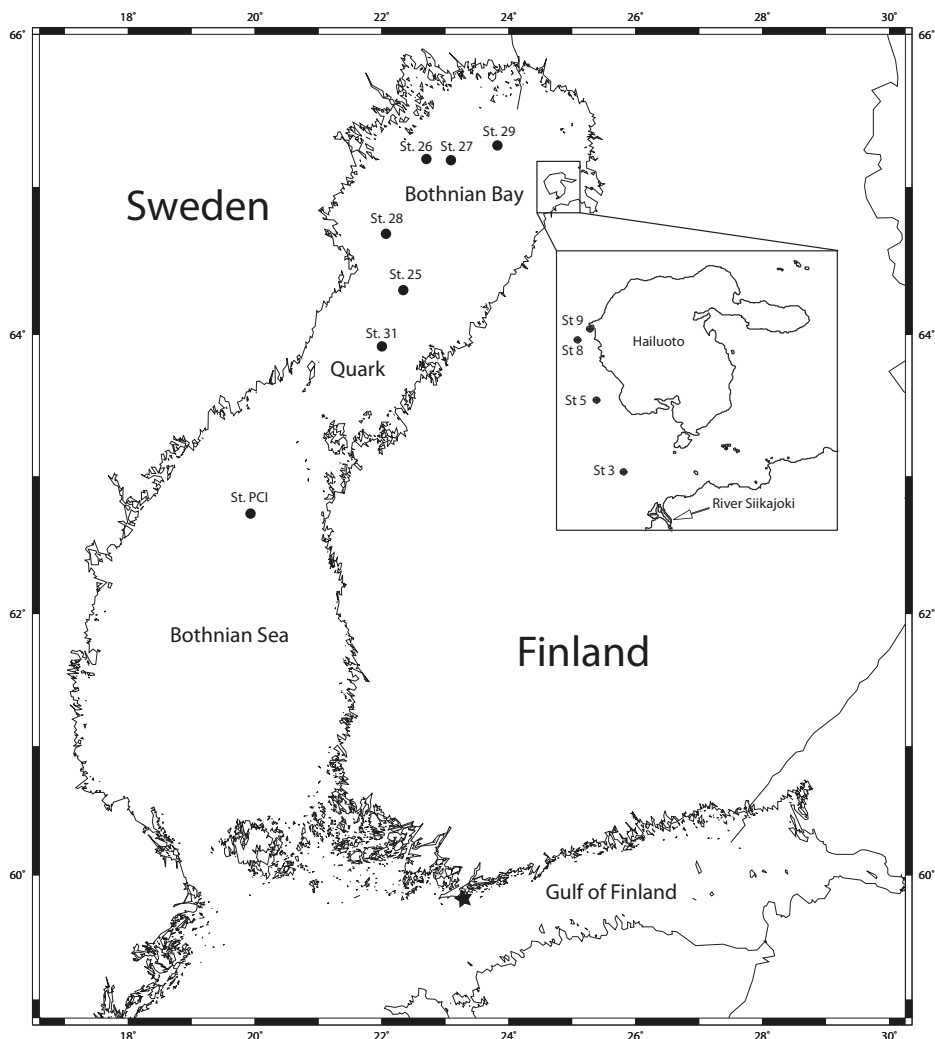


Fig. 1. Map of the study locations. The stations 3–9 and 25–31 are the fast- and drift-ice sampling locations in the Bothnian Bay, respectively (I, II). Pancake ice floes (PCI) were collected at the ice edge in the Bothnian Sea. The star denotes the location of the Tvärminne Zoological Station, the site of the UVA studies (III, IV).

4. MATERIALS AND METHODS

4.1. Sampling

The sea-ice sampling for the studies presented in this thesis, excluding pancake ice, followed the established procedure for sampling of sea ice with a cylindrical ice corer (Eicken 2009). The ice auger, which was used in all the studies, was a Mark II type with a 9 cm internal diameter (Kovacs Enterprises) driven by a two-stroke motor. The ice columns were cut into sections of varying thickness, and the brine that drained during the cutting was always collected. However, the drainage of brine was not generally substantial, especially in the Bothnian Bay (I, II), where the brine volumes and ice temperatures were low. Water samples were collected from the under-ice water (UIW) (I, II) and from the near-bottom water (NBW) (II) in the Bothnian Bay studies. The UIW samples were taken directly into plastic bottles from drill holes cleared of slush, while for NBW a Limnos water sampler was used. The reason for using a bottle for UIW sampling was to obtain the water samples from as close as possible to the ice bottom, and as undisturbed as possible. The use of a Limnos sampler requires the drilling of multiple adjacent ice holes to make a hole big enough for the sampler to fit through, concomitantly mixing the surface water layers and compromising undisturbed samples. This was of significance especially when the thin (< 0.5 m) under-ice plume was sampled (II).

Melting poses an acknowledged problem when working with ice samples. Not only is the habitat destroyed but the ongoing processes (e.g. nutrient uptake, excretion) alter the samples chemically and biologically during the long melting period affecting

accuracy of the results. In this thesis, the samples were allowed to melt, similarly to Ryan et al. (2004), instead of accelerating the melting by heating the samples, which could cause more damage to the organisms resulting in, for example, the release of intracellular nutrients and loss of chl-*a* and biomass. The collected ice samples were melted in Nalgene plastic containers at +4 °C for about 20 h with or without the addition of 0.2 µm filtered seawater (FSW), depending on the measured variables. In papers I and II, the directly melted ice samples were used for salinity and nutrient measurements, whereas biological variables were measured from samples with FSW. The addition of FSW reduces the melt-induced osmotic stress to sea-ice algae, especially to flagellates (Garrison & Buck 1986), and is necessary for obtaining precise biomass estimates, chl-*a* concentrations and photosynthetic activities when salinity changes are greater than seven. In the study of Kaartokallio (2004), the direct melting procedure did not cause any reductions in chl-*a* concentration or primary productivity, whereas in February 2006, a similar test with Baltic Sea fast-ice samples revealed a 0.5–4.7-fold difference in the chl-*a* concentration between buffered and directly melted samples. In an unpublished study from the same area, chl-*a* did not show any significant differences between direct and buffered melting, but primary production ($\mu\text{g C } (\mu\text{g chl-}a)^{-1} \text{ h}^{-1}$) was higher in the latter case (Rintala et al. in prep). This shows that more detailed experiments under varying salinities are still needed to concretize the significance of the melting procedure for the ice samples in Baltic Sea. The main focus in UVA studies (III, IV) was on bacteria, which are not affected by salinity changes (Helmke & Weyland 1995), and only direct melting was therefore applied (see below for details).

4.1.1. Bothnian Bay

The sampling of drift ice (I) was conducted onboard R/V Maria S. Merian (The Leibniz Institute for Baltic Sea Research, Warnemünde) from seven locations from the south of Quark to the NE Bothnian Bay in early March 2006 (Fig. 1). The aim was to sample over a large area and collect drift ice of different types and thicknesses within the boundaries set by navigability. At each station, three ice cores were obtained, one of which was used for nutrient, one for biological and one for structural analysis. The two first cores were cut into 1–6 pieces at approximately 10-cm intervals and melted while the last core was stored frozen (-20 °C) for later processing. One of the sliced cores was melted directly and the second with FSW addition (salinity 2.1–3.5). Pancake ice floes (3 replicates) were collected using a metal casket and melted directly. Otherwise, sampling of replicate ice samples was impossible due to limitations in sample processing (e.g. manpower, time and laboratory space). As the aim in this study was to link the abiotic to biotic factors in different ice types rather than to study the horizontal variability *per se*, the replicates were not necessary, although they would have provided more support for the observations.

The fast-ice samples in paper II were collected along a transect with declining riverine influence (St. 3, 5, 8) at three different stages of the ice season in February–April 2004. The original idea was to sample from the same stations as Granskog et al. (2005a), but the varying weather and ice conditions set logistical limitations, and stations 5 and 8 were consequently located to the north of their transect and station 3 was only sampled in March. In April, stations 3–8 were inaccessible due to advanced melting and samples were taken near the SW coast

of Hailuoto Island (St. 9, Fig. 1). At each sampling, 9 replicates were taken from an area of about 1 m² and cut into 1 to 3 equally thick pieces (II). Three of the ice cores were melted directly, while the rest were melted with autoclaved FSW (salinity 3.2).

4.1.2. UVA experiments

The experimental set-up in the *in situ* UVA experiments (III, IV) consisted of two tent-shaped wooden frames covering an area of 0.97 m² each, one of them being covered with UVA-transparent (75 %) plastic foil (PAR+UVA treatment) and the other with UVR-opaque foil (Roscolab Ltd) transmitting practically nothing below 390 nm (PAR treatment). Both of the foils allowed 82–86 % transmission of the visible wavelengths. The units were anchored onto snow-free sea ice at a site with minimum shading from nearby land. The untreated, snow-covered ice with varying snow depth and the treated ice (PAR+UVA, PAR) were sampled every seven days starting on 2nd March and ending 30th March in 2005, with one extra sampling after four days of incubation (III). The treated ice started to melt one week earlier than untreated ice due to the greenhouse effect of the tents, and the last sampling was thus excluded from the data analysis (Fig. 2A). In 2006, there were two 2-week incubations, one in February (7th to 21st) and one in March (14th to 28th) (IV). In these experiments, samples were also collected once a week. Short incubations of less than one day tend to give the worst case scenarios, as organisms do not have time to acclimatize, but in long-term incubations (1–2 weeks) the possible feedback mechanisms are accounted for and the results are more realistic (Bothwell et al. 1994, Villafañe et al. 1995).

Due to limited manpower, replicate units were not used in the UVA experiments. Similarly to polar sea ice, the variability in Baltic Sea ice properties is very high at all distances and many variables are correlated with ice thickness (Eicken et al. 1991, Steffens et al. 2006). Thus, in order to ensure similar conditions in terms of ice thickness and biological characteristics, the cores had to be taken close to each other and the practical solution was the use of one tent per treatment. For the same reason, the tents were placed close together, but so that the interference of one tent with another was minimal. An effort to reduce the effect of horizontal patchiness was made by random sampling of 2–3 ice cores from each unit area. Immediately after sampling, the ice samples were cut into 3 to 6 pieces, depending on the ice thickness, but always so that the uppermost ice was cut into 5-cm pieces. Part of each parallel ice sample was used for bacterial production measurement (see below), while the rest was directly melted and pooled to ensure sufficient material for further processing. The chlorophyll-*a* and algal biomass of the most sensitive species could have been affected by the direct melting, but this was taken into consideration when the data were analyzed.

4.2. Measurements

4.2.1. Physico-chemical variables

The air temperature data were obtained from the Finnish Meteorological Institute (II) and from the Tvärminne Zoological Station (III, IV). In the Bothnian Bay studies (I, II), the ice temperature profiles at each station were determined from one extra core using a Testo 110 thermometer. The salinity-temperature profiles in the underlying water were obtained in the fast-ice study (II) with a

YSI 63 meter (Yellow Springs Instruments). The same instrument was also used in all the studies for bulk salinity measurements of the melted ice samples. The brine volume estimates for Bothnian Bay samples (I, II) were calculated based on the ice temperature and salinity measurements (Cox & Weeks 1983, Leppäranta & Manninen 1988).

The ice crystal structure of drift ice samples (I) was determined by polarizing 10–20 cm long and 1 cm thick ice sections between two crossed polarization plates and classifying the ice as granular, columnar or transitional based on the crystal size and shape. The portion of meteoric ice (i.e. snow ice and superimposed ice) in the ice columns was determined from the melted ice samples with stable oxygen isotope ($\delta^{18}\text{O}$) analysis (Jeffries et al. 1994) using a Finnigan MAT Delta Plus XL mass spectrometer in continuous flow mode interfaced to a GasBench II.

The global radiation (= diffuse and direct) from 400 to 1100 nm during the UVA experiments in 2005 and 2006 (III, IV) was measured every half an hour with a GroWeather station at the Tvärminne Zoological Station. No instrumentation was available to continuously measure UVA radiation, but as UVA generally comprises a constant portion of the solar radiation, it can be approximated that UVA varied as a function of the global radiation. The downwelling radiation inside the untreated sea ice (snow-free ice, UNT) and treated ice (PAR, PAR+UVA) in the 2006 UVA experiments (IV) was measured with a Ramses-ACC Hyperspectral Irradiance Sensor (320–950 nm) attached to a metal casing and aimed at a diffusely reflecting target with 5 cm distance from the sensor (see paper IV for details). Measurements were carried out on three occasions (7th, 14th and 28th March) with 7.5 to 10.0 cm

intervals starting 10 cm below the ice surface by drilling a hole the size of the sensor probe and setting the sensor at the desired depths in the ice. Changes in the global radiation while taking the measurements in the ice were compensated by measuring the global incoming radiation with a Licor LI-200 pyranometer (400–1100 nm) at the ice surface during every measurement.

The inorganic nutrients (PO_4 , NH_4 , NO_3 , SiO_4) in the drift-ice study (I) were measured onboard R/V Maria S. Merian from thawed ice samples and UIW according to standard seawater protocols (Hansen & Koroleff 1999). In the fast-ice study (II), total nitrogen, phosphorus and organic carbon concentrations were determined in addition to inorganic nutrients (PO_4 , NH_4 , $\text{NO}_3 + \text{NO}_2$, SiO_2). The measurements were performed according to the standards of the Finnish Standards Association (SFS 3032, SFS-EN 1484, SFS-EN ISO 13395 and 11905-1) and the guidelines of the analyzers and of the National Board of Waters and the Environment (unpubl.) in the laboratory of the North Ostrobothnia Regional Environment Centre (Oulu, Finland).

4.2.2. *Chlorophyll-a and biomass*

Chlorophyll-*a* concentrations ($\mu\text{g l}^{-1}$) were measured in every study of this thesis. In papers I and II, the concentrations in ice were determined from samples melted with FSW and the results were corrected for the dilution caused by the FSW addition. The chl-*a* content in the $< 2 \mu\text{m}$ fraction was measured in the fast-ice study (II) by filtering the sample through a $2 \mu\text{m}$ polycarbonate membrane filter (Poretics). The chl-*a* concentrations in both UVA experiments (III, IV) were obtained from directly melted samples and the chl-*a* sample processing followed the

guidelines of HELCOM (1988), which have been adapted specifically for Baltic Sea water samples. Volumes of 50–200 ml of ice and UIW samples (2 aliquots per sample) were filtered on GF/F filters (Whatman), which were then placed in scintillation vials (II–IV) or plastic test tubes (I) with 10 ml of 96 % v/v ethanol and were left to extract in darkness at room temperature for 24 h. If measurements could not be undertaken in 24 hours, the samples were frozen (-20°C) and analysed later. After extraction, the extract was filtered through GF/F to reduce the turbidity, fluorescence was measured with a Jasco FP 750 spectrofluorometer (excitation 430 nm, emission 668 nm) calibrated with pure chl-*a* and the concentrations were calculated according to HELCOM (1988).

For the enumeration of algal species, 50 ml (I–III) / 10 ml (IV) of each sample, preserved with acidic Lugol's solution (II–III) or glutaraldehyde (1.25 % final concentration) (I, IV), was settled for 24 h (Utermöhl 1958). The algal cells were counted using a Leica inverted light microscope equipped with phase contrast, 12.5 \times oculars and 10, 20 and 40 \times objectives. Algal densities were converted to biomass in studies I, II and III. In the first two papers, the ice-algal biomass was determined for samples melted with FSW, while in paper III the direct melting of samples may have resulted in some loss of the flagellate biomass (Garrison & Buck 1986). However, as the focus in the paper III was more on the differences between the treatments and not on the absolute values, the biomass estimates were of value. In paper I, the biomasses were given as wet weight ($\mu\text{g ww l}^{-1}$) calculated using species-specific biovolumes (Olenina et al. 2006, K. Kivi, unpubl.), whereas in papers II–III and this thesis, the algal biomass is presented as carbon ($\mu\text{g C l}^{-1}$) calculated by using species- and size-specific carbon contents

(Menden-Deuer & Lessard 2000, Olenina et al. 2006). In paper IV, only diatom densities were presented, as glutaraldehyde fixation is not suitable for flagellated species.

The biomass of protozoa and metazoa in the Bothnian Bay samples (I, II unpubl.) was determined from the same samples with algal biomass and using the same counting protocol. In paper I, the biomass is given as a wet weight value ($\mu\text{g ww l}^{-1}$), whereas the unpublished data from Bothian Bay fast ice were converted to carbon ($\mu\text{g C l}^{-1}$) using a fixed percentage of 12 % as the carbon content of the wet weight (K. Kivi unpubl.).

4.2.3. Photosynthesis-irradiance measurements

Primary production in sea ice is a much-discussed topic, but to date no accurate methods have been available to measure it (Arrigo et al. 2010). One of the most common methods is to measure photosynthesis-irradiance responses (P-E), which yield estimates of photosynthetic efficiency, the maximum photosynthetic rate and photoinhibition. The P-E curves in this thesis (I–III) were obtained by measuring $\text{H}^{14}\text{CO}_3^-$ incorporation (Stemann Nielsen 1952) under 18 light levels from 0 to $577 \mu\text{E m}^{-2} \text{s}^{-1}$ using PE incubators equipped with green filters, resulting in a spectrum from 450 to 680 nm. Volumes of 3 ml per sample were incubated with 50 μl additions of $\text{NaH}^{14}\text{CO}_3$ (final concentration of $0.33 \mu\text{Ci ml}^{-1}$; DHI Water and Environment) for 2 h in incubators cooled by circulating ice-cold water through them. The incubations were stopped with the addition of 100 μl of formaldehyde (final concentration 1.23 %) and the samples were acidified with 1N HCl for 48 hours. After that, 4 ml of Insta-Gel scintillation cocktail (PerkinElmer) was added to each sample and

the radioactivity (DPM) of the samples was measured with a Wallac WinSpectral 1414 scintillation counter.

The chlorophyll-*a* normalized carbon uptake rates ($\mu\text{g C } (\mu\text{g chl-}a \text{ h})^{-1}$) were calculated using the following equation:

$$P = \frac{(DPM - DPM_{\text{dark}})TICk_1k_2}{DPM_{\text{added}}Tchl},$$

where DPM_{dark} is the average of two dark-incubated samples, TIC is the total inorganic carbon in the sample, k_1 is the metabolic carbon isotope discrimination correction factor (1.05) (Nielsen & Breista 1984), k_2 is the dilution factor for FSW addition (not in III), DPM_{added} is the radioactivity added to each sample (2 220 000 dpm), T is the incubation time (h) and chl is the chlorophyll-*a* concentration (mg l^{-1}). For water samples and FSW, TIC was obtained from salinity by using the equation of Buch (1945). The TIC concentrations (mg l^{-1}) of ice samples (+FSW) were calculated as volume-weighted averages of TIC in FSW and TIC in directly melted ice samples, which were obtained from the salinity-TIC regression curve (H. Kuosa, unpubl., $n = 50$, $R^2 = 0.88$). The equation of Buch (1945) works for seawater but gives incorrect values for melted Baltic sea-ice samples, and the salinity-TIC regression curve was therefore used (H. Kuosa, pers. comm.). The reasons for the unsuitability of the Buch's equation are unclear, but are probably related to the closed system and undersaturation of CO_2 in ice. The exponential model of Platt et al. (1980) was fitted to the data and the photosynthetic efficiency (α^B), maximum photosynthetic capacity (P_{max}^B), photoinhibition (β^B) and light saturation index ($E_k = P_{\text{max}}^B / \alpha^B$) were calculated.

4.2.4. Bacterial numbers and production

The total bacterial numbers (TBN) (III, IV) were obtained by filtering 1–5 ml of the thawed, glutaraldehyde-preserved samples (final concentration 1.25 %) on 0.2 µm polycarbonate filters (Osmonics), staining them with acridine orange (0.01 %) (Hobbie et al. 1977) and counting at least 200 bacteria from 20 fields in a counting grid (New Porton E11) under blue excitation light (I3 filter block) using an epifluorescence microscope (Leitz Aristoplan) equipped with a PL Fluotar 100 × 12.5 / 20 oil immersion objective (Leica).

Bacterial production in sea ice was measured as the total incorporation of ³H-thymidine (TTI) (III, IV) and ¹⁴C-leucine (TLI) (III) (Fuhrman & Azam 1982, Kirchman et al. 1985). The dual-labelling approach was chosen in order to detect the balance / imbalance in bacterial growth and the sensitivity of DNA and protein synthesis to UVA, as these processes may respond differently to different wavelengths (Simon 1990, Aas et al. 1996, Sommaruga et al. 1997). Instead of melting the ice samples, the samples were incubated as ice crush. This technique preserves the original habitat of the sea-ice bacteria and enables the immediate processing of the samples, and higher BP estimates are consequently yielded than with melted samples (Helmke & Weyland 1995, Kaartokallio 2004). In this protocol, ice samples are crushed with a spike and an electrical ice crusher, approx. 7.5 g (~10 ml) of ice crush is weighed in a scintillation vial and 2 ml of concentrated autoclaved FSW is added to simulate brine salinity and ensure even distribution of labelled thymidine and leucine. Adsorption blanks and replicates of each sample are then incubated in the ice-water phase with additions of labelled thymidine and leucine in the dark for about

16 h, after which the samples are fixed with formaldehyde and processed with the standard cold-trichloroacetic acid (TCA) extraction method (Fuhrman and Azam 1982) and filtered onto 0.2 µm MSE-filters. The filters are then dissolved in scintillation cocktail and the radioactivity is measured with scintillation counter.

In this thesis (III, IV), 1 blank and 2–3 replicates per sample were weighed using a Mettler PE 3600 laboratory balance and the added autoclaved FSW had been concentrated by evaporation to a salinity of 13 (~ 2x seawater salinity at the study site). The adsorption blanks were fixed with formaldehyde (final concentration 1.85 %) prior to additions of [methyl-³H]-thymidine and L-[¹⁴C(U)]-leucine (PerkinElmer) at final concentrations of 14 nM and 1000 nM, respectively. These saturating concentrations were based on earlier measurements from the area at various stages during winter (Kaartokallio 2004, Kuosa & Kaartokallio 2006, Piiparinen unpubl.). In most of the measurements, the labelled leucine was diluted with carrier leucine in the ratio of 1:4. The high saturation concentration of leucine in comparison to approx. 100 nM used in Baltic Sea open water studies (Piiparinen, unpubl.) could be linked to the need for amino acids, for example, in osmoregulation, or to the use of leucine as a substrate (Mock et al. 1997, Kaartokallio et al. 2007). Leucine has been reported to also be incorporated by cyanobacteria (Kamjunke & Jähnichen 2000, Hietanen et al. 2002), but as cyanobacteria are never a significant component in ice-algal communities, this was not considered as a problem with sea ice. The samples were incubated in an LMS 250 cooled incubator at approx. -0.5 °C for 16–18 h, after which they were fixed with formaldehyde. The incubation time for Baltic sea-ice samples was earlier determined

by Kaartokallio (2004), and the length is explained by the slow bacterial processes in sub-zero temperatures (Lizotte 2003 and references therein). After TCA extraction, the samples were filtered on 0.2 μm MSE filters (DHI Water & Environment) followed by scintillation counting using Insta-Gel Plus scintillation cocktail (PerkinElmer) and a Wallac WinSpectral 1414 counter. The values of thymidine blanks were generally high (1/3 of the replicate values), occasionally resulting in very low incorporation rates. The blank values of the water samples were equally high (data not shown), which points to higher adsorption of thymidine in freezing temperatures compared to summer temperatures in the Baltic Sea. The higher blank values may also be a result of the long incubation time. In leucine incorporation, this was not a problem and the replicates were at least ten times the blank values.

4.2.5. Molecular methods

The differences in bacterial community composition between untreated, PAR+UVA treatment and PAR treatment in paper IV were determined using terminal restriction fragment length polymorphism (T-RFLP) (Liu et al. 1997). In this method, bacterial species are ideally separated by their different restriction positions in a single gene, in this case 16S. The lengths and amplitudes of resulting fragments (TRFs) from the digestion of fluorescently labelled PCR products with a restriction enzyme are then determined with a DNA sequencer. The resulting fragment lengths are then compared to the TRF lengths of sequenced clones obtained from selected samples (i.e. clone library). The T-RFLP approach was chosen due to the insensitiveness of denaturing gradient gel electrophoresis (DGGE) in detecting the

differences in community composition in our samples (cf. Moeseneder et al. 1999). The whole protocol including the selection of the three restriction enzymes, was adapted for Baltic Sea ice samples by E. Eronen (in prep.). The digestions, cloning and sequencing were carried out at the Institute of Biotechnology, University of Helsinki.

The DNA of ice samples, which had been filtered on 0.2 μm sterile mixed cellulose ester filters (Whatman) and stored at -80°C , was extracted followed by checkups on agarose gels (1 % wt/vol). Fragments (1379 bp) of 16S rDNA (2 replicates / sample) were amplified with a BioRad Thermal Cycler using the primers and PCR protocol described in Eronen (in prep.). The success of PCR was checked by running 5 μl of each product on a 1 % agarose gel. The replicate PCR products were pooled and the purified products (24–108 $\text{ng } \mu\text{l}^{-1}$) were digested with the three restriction enzymes at the Institute of Biotechnology, University of Helsinki. The fragment size range varied from 26 bp and 50 bp to 1000 bp, depending on the enzyme. The TRFs were identified by the *in silico* analysis of clones and the TRFs obtained from the clone digestions (see below).

Two clone libraries were constructed of two surface layer samples (0–5 cm), one having a comprehensive and the other having a distinctively different T-RFLP electropherogram (14/2 PAR and 21/3 PAR+UVA, respectively). The unlabelled PCR products of the two samples were cloned with a QiaGen PCR cloning kit and sequenced with an ABI 3130 XL capillary sequencer by using primers pD' and pF' (Edwards et al. 1989). Altogether, 174 clones out of 196 were successfully sequenced, but after the assembling of the consensus sequences with the Gap 4.1. program (Staden 1996) and the subsequent chimera check

with Bellerophon (Huber et al. 2004), an additional 4 sequences were discarded. The remaining 170 sequences were aligned using ClustalW and BioEdit 7.0.5.3 (Hall 1999) and by using the Jukes-Cantor DNA distance algorithm, while the similarity between the clones was calculated with Phylip-3.69 (Felsenstein 2005) and the clones were assigned into OTUs (100 % sequence similarity) with DOTUR-1.53 (Schloss & Handelsman 2005). By using the same PCR protocol as in the first PCR, one clone from each OTU, was amplified and the products were purified and digested with the three restriction enzymes.

The clone sequences were classified to the family level with a 95 % confidence threshold in the Ribosomal Database Project (RDP) Naïve Bayesian rRNA Classifier (Version 2.2). The closest sequence to every clone was checked with RDP Seqmatch (Cole et al. 2007) using nomenclatural taxonomy with one match per sequence (*k*-nearest neighbour), and including both type and non-type strains and both uncultured and isolated good quality sequences of ≥ 1200 bp.

4.3. Statistics

All the statistical analyses in papers I–III were carried out using procedures in SPSS 15.0.1.

In the drift-ice study (I), the normalized data (weighted ice column averages and UIW) were first grouped based on the results from hierarchical cluster analysis using Ward's clustering method (Ward Jr 1963) and the squared Euclidean distance measure, which is the recommended distance measure for Ward's method (see Everitt et al. 2001). Then, backward stepwise discriminant analysis was run to identify the most significant selective variables (F to enter 3.84 and to remove 2.71)

between the different locations and sample types (i.e. water / UIW). The equality of group covariance matrices was tested with Box's M test.

In the fast-ice study (II), the parametric Students t-test and one-way ANOVA were run to test the differences between stations and sampling dates in water and integrated ice column chl-*a* values. The homogeneity of variances was assessed with Levene's test.

In the 2005 UVA study (III), the significance of the differences in chl-*a*, TTI, TLI and TBN in each layer between the two treatments (PAR+UVA, PAR) was tested with multiple regression analysis (Draper & Smith 1981). The global cumulative radiation (GR) at the surface (proxy for UVA radiation), bulk salinity (standardization for salinity difference), one dummy variable coding for treatments and the cross-product terms of GR and the dummy variable were used as independent variables in the models, and insignificant ones were eliminated with the backward regression technique (probability of F-to-remove: 0.10).

To detect the differences in the bacterial communities between the untreated and treated ice (PAR, PAR+UVA) in 2006 (IV), a principal coordinate analysis (PCO) using Bray-Curtis dissimilarity as the distance measure was performed on the fourth-root transformed TRFs with the PCO program (Anderson 2003). As the digested PCR-products also included chloroplast DNA, the TRFs clearly assigned to chloroplasts were first excluded, after which the PCO was run separately for the three TRF datasets representing the three restriction enzymes.

5. RESULTS AND DISCUSSION

5.1. Drift-ice and fast-ice communities in the Bothnian Bay (I, II)

5.1.1. Ice structure, physical processes and their significance for ice biota

The meteorological conditions at the time of ice formation may be traced back by determining the ice structure, which in turn may help in explaining the anomalies in ice communities. In paper I, the stations were classified into four groups based on the ice thickness: newly formed ice (St. 28), pancake ice (PCI), young ice (St. 25, 31) and thick ice (St. 26, 27, 29). Algal biomass generally increased with ice thickness, but despite the approximately equal ice thickness at drift-ice stations 25 and 31, the integrated algal biomass at station 25 was 5.3 times higher than at station 31. The reasons for the abnormality of station 31 might have partly lain within the turbulent conditions at the time of ice growth, which appeared as an atypical ice structure with irregular crystal boundaries and the presence of transitional ice (Eicken & Lange 1989), as opposed to the long columnar crystals with well-defined boundaries at station 25, which pointed to calm growth conditions. Most importantly, the average brine volumes differed notably (2.2 % St. 25 and 0.8 % St. 31, respectively), and a smaller habitable space may be one of the explanations for the low biomass (3.2 mg C m⁻²) and flagellate dominance (75 %) at station 31. The effect of physical constraints was also detectable in the fast ice (II) close to the river mouth (St. 3), where the bottommost layer was partly formed of nearly fresh plume water, similarly to the studies of Legendre et al. (1991), Granskog et al. (2005b) and Kaartokallio et al. (2007). This showed as

a steep decreasing vertical gradient in the brine volume from 2.0 % in the surface to 0.7 % in the bottom ice. This coincided with a decrease in the ice-algal biomass from 90 to 19 µg C l⁻¹ and an increase in the proportion of flagellates in the algal biomass from 22 % to 78 %.

The formation of meteoric ice is common in the Baltic Sea and its presence can be detected with δ¹⁸O-analysis (Jeffries et al. 1994, Granskog et al. 2003a, Uusikivi et al. 2011). A rough approximation of the origin can also easily be determined by measuring the bulk salinity of the granular ice layer: snow ice would show as a higher salinity, whereas superimposed ice would form a fresher layer. In the Bothnian Bay, flooding is frequent due to the heavy snow cover, and snow ice can comprise up to 50 % of the total ice thickness (Seinä & Peltola 1991, Leppäranta & Myrberg 2009). In the process, nutrients from snow and under-ice water become incorporated into the formed snow ice, and elevated concentrations of nutrients, especially nitrogen, can generally be detected from the surface ice. Snow-ice formation was detected in both drift ice (St. 26) and fast ice (St. 8), where nitrate and ammonium concentrations were manifold compared to the layers below and to the other ice columns (I, II). The significance of snow-ice formation for the biota in terms of the increase in habitable space is emphasized in Baltic Sea where the low salinity sets more strict size limits for organisms than in the polar regions (Krembs et al. 2000, Kaartokallio 2004, Werner & Auel 2004). Combined with higher light intensities and nutrient concentrations, this may be the explanation for the algal biomass peaks observed in the surface layer of the thick drift ice (St. 26, 27, 29) (I) and of the fast-ice station 3 (II). The heavy snow-cover of 16 cm at fast-ice station 8 (II) was most likely the

reason for the low observed algal biomass, despite the elevated nutrient concentrations and brine volume.

After ice formation, drift ice is subjected to deformation processes, which are reflected in the physical, chemical and biological properties of the ice. The dynamic growth of ice by rafting and ridging is typical for the Bothnian Bay area (Omstedt 1985, Granskog et al. 2004b).

The rafting of ice floes multiplies the ice thickness and places the surface community of the underlying ice floe in the middle of the new ice column. Rafting can be detected as internal snow-ice layers, but also as internal peaks in chlorophyll-*a* concentrations (I). The chlorophyll-*a* in these peaks may originate from the bottommost layer of the overlaying ice floe or from the topmost layer of the underlying ice column. The drift ice floes may also flip around when they are pressured against each other. Ice structure analysis revealed that the rafted ice column at station 29 (I) consisted of an inverted upper ice floe, which had frozen together with the underlying ice floe. This was evidenced by a 25 cm thick internal snow-ice layer and supported by coinciding peaks of chlorophyll-*a* and algal biomass. The consequence for the ice biota is the displacement of the original top and bottom communities to different light environments, but based on the results in paper I, it is impossible to determine whether the community structure started to change according to its new location in the ice column or whether the existing community simply adapted to the new conditions.

5.1.2. Comparison of the drift- and fast-ice communities

The drift- and fast-ice stations showed high variance in measured variables and no clear-

cut characterisations could be performed without multivariate analysis. Thus, the data presented in papers I and II were first grouped in hierarchical cluster analysis in the same manner as in paper I by using thickness-weighted ice column averages, after which discriminant analysis was performed (see 4.3. Statistics for details). The missing NO₃ value at station 29 was replaced with the minimum and maximum NO₃ values measured from other thick drift ice samples (see paper I for details).

The data grouped into four clusters in hierarchical cluster analysis separated by phosphate (PO₄), silicate (SiO₄) and the biomasses of protozoa and diatoms (Table 1). Groups 1 and 2, which included all the ice samples and most of the UIW samples, differed mostly in diatom biomass and silicate concentration. Group 1 consisted of all the drift- and fast-ice stations that were diatom-dominated, while all the UIW samples from the drift-ice stations together with newly formed ice and the deviant young ice at station 31, characterised by low biomasses and high silicate concentrations, fell into group 2. Also the fast-ice stations 5 and 8 (ice and water) in both February and March belonged to this group (Table 1). Thus, group 2 represented ice communities at earlier stage of development with low biomass consisting mostly of flagellates bearing similarities to under-ice water whereas the group 1 could be characterised more as the advanced stage of seasonal sea-ice development. The similarity of newly formed ice with UIW and the separation into different clusters with pancake ice gives a strong indication that ice habitat selects for organisms (e.g. diatoms) that are able to benefit from the more stable, highly concentrated habitat, and this selection process starts immediately after ice formation (I). Active scavenging (Ackley & Sullivan 1994, Gradinger & Ikävalko

1998) seemed to be the main mechanism in organism incorporation, as the salinity-normalised algal biomass was three times higher in new ice than in UIW (I).

Groups 3 and 4 consisted of water samples that were distinctively different from the other water samples (Table 1). The water column at fast-ice station 9 represented the early stage of a spring bloom with low phosphate concentrations and high biomasses (group 3), and the UIW at fast-ice station 3 had a strong riverine influence with high PO_4 and SiO_4 concentrations and very low biomasses (group 4). Despite the widely scattered locations around the Bothnian Bay, the SiO_4 and PO_4 concentrations in UIW beyond the plume were very similar ($33 \pm 5 \mu\text{M}$ and $0.08 \pm 0.04 \mu\text{M}$, respectively). The discriminating role of phosphate was mainly attributed to the plume sample, and when running the analyses without it, PO_4 became insignificant being in accordance with the general P limitation of the Bothnian Bay (e.g. Tamminen & Andersen 2007) and Baltic Sea ice ecosystems (e.g. Kuosa & Kaartokallio 2006).

Owing to the high input from the land, silicate concentrations in UIW generally

remain above $25 \mu\text{M}$ (Axe 2010), as was also found in the Bothnian Bay studies (I, II), and do not limit diatom growth. On the contrary, as silicate is not deposited from air like nitrogen, the ice silicate pools are not greatly replenished during the ice-covered season unless snow-ice formation takes place, like at drift-ice station 26 (I) and fast-ice station 8 in March (II). The impoverishment of SiO_4 in ice showed as lower salinity-normalized SiO_4 values compared to water values in both Bothnian Bay studies (I-II). The ability of the diatom-dominated community to take up the silicate in ice down to the limiting levels became evident at fast-ice station 9 in April, where the average concentration was $2.2 \mu\text{M}$ (II). This is between the values of $4 \mu\text{M}$ and $2 \mu\text{M}$, below which Si is considered to limit the algal growth in lacustrine systems and the sea, respectively (Reynolds 2006), matching the brackish environment. In the study on the Baltic spring bloom (Kuosa et al. 1997), the SiO_4 -Si concentration decreased to as low as $0.7 \mu\text{M}$, but the depletion of Si was strongly linked to the availability of nitrogen. In the Bothnian Bay (II), phosphate instead of dissolved inorganic nitrogen seems to be the co-limiting nutrient during ice-algal blooms.

Table 1. Discriminant analysis of the data from the Bothnian Bay (I–II). The ice values included in the analysis are thickness-weighted ice column averages. PCI = pancake ice, UIW = under-ice water, NBW = near-bottom water

| Group | Type | Stations | PO_4 (μM) | SiO_4 (μM) | Protozoa ($\mu\text{g C l}^{-1}$) | Diatoms ($\mu\text{g C l}^{-1}$) |
|-------|---|---|------------------------------------|-------------------------------------|--|---------------------------------------|
| 1 | Paper I: PCI/ young/thick ice Paper II: ice | I: PCI/ St. 25/ St. 26, 27, 29 II: St. 3, 9 | 0.14 | 4.77 | 0.39 | 33.26 |
| 2 | Paper I: UIW/ new ice/ young ice Paper II: UIW/ NBW/ ice | I: all/ St. 28/ St. 31 II: St. 3 (NBW), 5, 8 | 0.10 | 25.67 | 0.70 | 1.18 |
| 3 | Paper II: UIW/ NBW | II: St. 9 | 0.05 | 32.46 | 13.47 | 25.91 |
| 4 | Paper II: UIW | II: St. 3 | 0.97 | 166.44 | 0.34 | 0.43 |

However, diatoms are able to hydrolyse their intracellular phosphate reserves, and they may not therefore have been directly affected by the low PO_4 concentrations. Thus, as ice silicate concentrations decrease, the ice-diatom biomass tends to increase. This held true for the Bothnian Bay, and a significant inverse relationship was detected between the weighted ice column averages of diatom biomass and SiO_4 (Spearman $\rho = -0.646$, $p < 0.01$, $n = 15$).

When the analyses were run with integrated ice values and excluding water samples, silicate and phosphate became insignificant discriminating variables, and the three main groups were discriminated by the integrated chl-*a* concentration and biomass of protozoans and chlorophytes (Table 2). The brine volume was among the initial variables, but as the thickness-weighted averages of brine volume in drift and fast ice were equal (1.24 % and 1.28 %, respectively) in February–March, and only during the ice melt in April was there a marked increase to 5.84 % (II), the brine volume was a nonsignificant selective variable in discriminant analysis, and the analyses were run without it.

The division between early and advanced stages of ice community development became even more apparent than in the first hierarchical cluster analysis including the water samples, and all the new to young drift-ice stations (classification based on the ice thickness, paper I) grouped into the same group 1. Despite the increased ice thickness from February to March, the biomass remained low at fast-ice stations 5 and 8 (II) and they also belonged to group 1 at both samplings. This could be due to the heavy snow cover (16–25 cm) at these stations, which efficiently limited the light quantities whereas at the drift-ice stations the snow cover was variable with a maximum

thickness of 6 cm. However, the biomass at the fast-ice stations 5 and 8 was close to the values in the thick drift ice if rafting is excluded, i.e. the biomass per ice floe is calculated by dividing the biomass in rafted ice in half. Characteristic for this group was the low average biomass and high proportion (43 %) of unidentified flagellates ($< 20 \mu\text{m}$) in the algal biomass.

The advanced stages of drift and fast ice separated into their own groups 2 and 3 despite the equal algal biomass (approx. 25 and 28 mg C m^{-2}), the main reasons being the lower protozoan biomass and 230 times higher chlorophyte biomass in fast ice (Table 2). While no clear indication of a freshwater influence on species composition could be found in the fast-ice study (II), the combining of the datasets clearly revealed a strong decline in chlorophyte biomass from fast to drift ice (I–II). A similar but opposite trend was found in ice-rotifer biomass that declined from 31 to 1 mg C m^{-2} from offshore towards the coast. The same dominant centric diatom species (*Chaetoceros wighamii*, *Melosira arctica*) were encountered in both ice types (I–II) and they were generally associated with the surface layers, which also include the old surface layers of rafted drift ice (I). Pennate diatoms had a more even distribution or even peaked in the bottommost layer (I–II). The pennate diatom communities mostly consisted of unidentified species (median 77 % fast ice, 73 % drift ice), and from the identified species *Achnanthes taeniata* was generally the dominant one. Small unidentified flagellates formed roughly equal percentages in these drift- and fast-ice stations (25 and 28 %, respectively). The absence of copepod nauplii and adults from all the advanced stage ice samples was also conspicuous, which points to the earlier-mentioned size limitation of narrow brine channels and is consistent with the studies

Table 2. Discriminant analysis of the integrated drift- and fast-ice samples from the Bothnian Bay (I–II).

| Group | Type | Stations | Chl- <i>a</i> (mg m ⁻²) | Protozoa (mg m ⁻²) | Chlorophytes (mg m ⁻²) |
|-------|--|---|--|-----------------------------------|---------------------------------------|
| 1 | Paper I: new/ pancake/ young drift ice Paper II: fast ice | I: St. 28/PCI/ St. 25, 31 II: St. 5, 8 | 0.33 | 0.04 | 0.06 |
| 2 | Paper I: thick drift ice | I: St. 26, 27, 29 | 2.47 | 1.29 | 0.01 |
| 3 | Paper II: fast ice | II: St. 3, 9 | 1.66 | 0.60 | 2.29 |

of Meiners et al. (2002), Kaartokallio (2004) and Kaartokallio et al. (2007).

To conclude, all the physical (e.g. light, ice structure, brine volume) and chemical (e.g. nutrients) variables together played a role in the habitat setting for ice organisms, and no general division into drift and fast ice could be made based on the physical, chemical and biological characteristics. The similarity / dissimilarity of fast and drift ice depended on the developmental phase of the ice communities, and in the early stages, fast and drift ice resemble each other more than in later stages. This was not surprising, as both ice types are dynamic in the matter of rafting, ridging and meteoric ice formation, and for example, rafting can rapidly double the biomass in the ice column. River water plumes forming underneath the fast ice may also have a strong influence on the ice structure of the bottom ice and possibly also on the surface ice if the ice is flooded with the river water.

5.1.3. Succession of diatoms in Baltic Sea ice

The most conspicuous difference between under-ice water phytoplankton and ice-algal communities in the Bothnian Bay is the markedly higher diatom biomass in ice compared to water (I, II), which is also a

typical feature in other parts of Baltic Sea during winter (Haecky et al. 1998, Meiners et al. 2002, Kaartokallio et al. 2007). Earlier studies have implied that diatoms are already favoured at their incorporation into sea ice due to their size and surface characteristics, e.g. stickiness (Gradinger & Ikävalko 1998). The stability of the environment, better access to light, high nutrient concentrations and plethora of surfaces to glide on are keys for the success of diatoms in ice (e.g. Cota et al. 1991, Eicken 1992). The brine also offers marine diatoms also a habitat with salinities close to normal seawater in the otherwise less saline Baltic Sea (Arrigo & Sullivan 1992). In addition, the exclusion of their predators due to the size and geometry of brine channels may play a pivotal role (Krems et al. 2000), and the significance of this may increase along the gradient of decreasing surface water salinity from the southern to the northern Baltic Sea, or even within shorter scales from drift to fast ice. In the Bothnian Bay drift ice (I), the biggest heterotrophic organism in thicker ice was the rotifer *Synchaeta littoralis*, which had the reverse distribution to the biomass of centric diatoms and peaked in the bottommost ice layers. The same pattern was also observed in the Bothnian Bay fast ice (II) in March, but the biomass of *Synchaeta littoralis* was two times higher in the drift ice, similarly

to algal biomass. This indicates that the size and nutritional value of centric diatoms make them inedible for small *S. littoralis*, which are known to efficiently graze on small algae and flagellates (Egloff 1988, Dolan & Gallegos 1992), and in sea ice they might also graze on bacteria (Norrman & Andersson 1994, Haecky & Andersson 1999, Kaartokallio 2004). The exopolymeric substances (EPS) produced by diatoms are known to act as cryoprotectants (e.g. Meiners et al. 2003), but they may also act as a prevent mechanism against grazers and viral infections (Deming 2010).

In the polar sea ice, pennate diatoms generally dominate the diatom, as well as the total algal biomass, and this biomass is mostly found in the bottom-ice layer (Lizotte 2003 and references therein). In the Baltic Sea, the biomass of centric diatoms may even exceed the pennate biomass as the granular surface ice seems to favour centric species (I–II). However, the marked proportion of centric diatoms in the algal biomass is not unique to the Baltic Sea (Meiners 2002, Haecky et al. 1998) as similar observations exist from the Gulf of Lawrence (Dunbar & Acreman 1980) and the Okhotsk Sea (McMinn et al. 2008). The different vertical distribution of centric and pennate species in the ice column may be attributed to their different sensitivity to solar radiation. In centric diatoms, the low surface area to volume ratio and placement of multiple small chloroplasts around the cell periphery give better protection for the nucleus from the harmful effects of light than in pennate species, which typically have 2 chloroplasts and a higher surface area to volume ratio (Karentz et al. 1991). However, exceptions apply to this generalization, and for example, the carbon fixation of pennate *Pseudo-nitzschia* species is enhanced by wavelengths > 318 nm (Mengelt & Prézelin 2005).

5.1.4. Photosynthetic parameters

The photosynthetic parameter values presented in papers I and II were the first published values from Baltic Sea ice, so no earlier references existed, but compared to Antarctic annual pack ice, the values were in the same range (Lizotte & Sullivan 1991). The average P_{\max}^B in drift ice (excl. new ice and PCI) was exactly the same as in fast ice ($0.353 \mu\text{g C } [\mu\text{g chl-a}^{-1}] \text{ h}^{-1}$), but the α^B values in fast ice ($0.044 \mu\text{g C } (\mu\text{g chl-a}^{-1} \text{ h}^{-1}) (\mu\text{E m}^{-2} \text{ s}^{-1})^{-1}$) were half of those in drift ice, resulting in roughly twice as high E_k values in fast ice compared to drift ice (85 and 39 $\mu\text{E m}^{-2} \text{ s}^{-1}$, respectively). This indicates that the drift-ice communities were more dark-adapted, despite the thinner snow cover. One explanation for this discrepancy could be rafting, which had placed the communities of the underlying ice floe in a deeper and thus darker environment. Significant photoinhibition was only observed in two bottom-ice samples from drift ice, indicating that the Baltic Sea ice communities are not generally strongly dark-adapted (Michel et al. 1988). This is supported by the E_k values markedly higher than 10, as values below it generally denote strong dark-adaptation (Cota & Horne 1989, Kühl et al. 2001, Ban et al. 2006).

The notable biomass of unidentified species made it difficult in most cases to connect ice communities to the observed photosynthetic parameters, but some trend could be seen in centric diatoms. The drift-ice layers that had a dominance of centric diatoms (> 63 % of the total biomass) had higher P_{\max}^B (avg. $0.59 \mu\text{g C } (\mu\text{g chl-a}^{-1} \text{ h}^{-1})$) and α^B values (avg. $0.017 (\mu\text{g chl-a}^{-1} \text{ h}^{-1}) (\mu\text{E m}^{-2} \text{ s}^{-1})^{-1}$) than samples that were mostly composed of other groups (avg. P_{\max}^B 0.18, α^B 0.05). The comparison with fast ice was only possible in two cases as in other fast-ice

samples the proportion of centric diatoms in the algal biomass was well below 63 % (avg. 15 %), compared to drift ice (37 %). In the two centric diatom-dominated samples, P_{\max}^B was at the lower end of the drift-ice range (0.34–0.43), but α^B values were 10 times lower (0.0027–0.0028) compared to drift ice. As the dominant centric diatom species were the same in drift and fast ice, the difference points to better dark adaptation of centric diatoms in drift ice. However, these conclusions were based on a very limited number of samples, and further experiments are needed to verify this observation.

5.2. Effects of UVA on sea ice ecology (III, IV)

5.2.1. Physical environment and radiation

Following the annual cycle of solar radiation, the global irradiance (400–1100 nm) more than doubled from February to March in 2006 (Fig. 2B). The average daily irradiance during the February experiment in 2006 varied from 13 to 62 $W m^{-2}$ with a mean of 31 $W m^{-2}$, whereas the irradiance in the two March experiments was mostly of the time above 100 $W m^{-2}$ (2005: $120 \pm 32 W m^{-2}$, 2006: $121 \pm 41 W m^{-2}$). The proportion of UVA in the solar radiation is relatively stable, so UVA was expected to follow a similar increasing trend to the measured global radiation. This was supported by

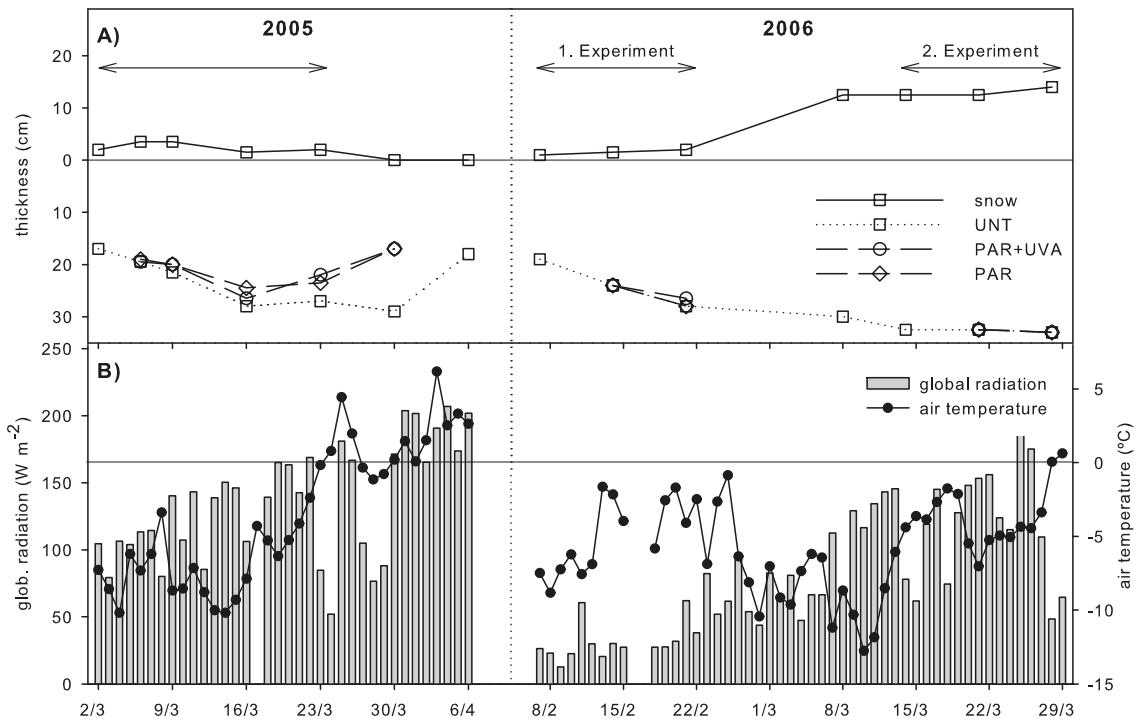


Fig 2. (A) The snow and ice thickness and (B) the global radiation (400-1100 nm) and air temperature during the UVA experiments in 2005 and 2006. Arrows indicate the duration of the experiments and the horizontal line in (B) denotes the 0 $^{\circ}C$ level.

the measurements in March 2006, as the measured UVA ($9.7\text{--}12.4\text{ W m}^{-2}$) constituted approx. 11 % of the integrated VIS (400–750 nm) + UVA irradiance, being equal to what have been reported for sea level by Moan (2004).

The spectral transmission of UVA (1.7–7.3 %) in ice in March 2006 was lower than in earlier studies from the area, which report spectral transmittance percentages from 5 to 30 % (Rasmus et al. 2002, Ehn et al. 2004). Snow cover from 12.5 to 15 cm efficiently

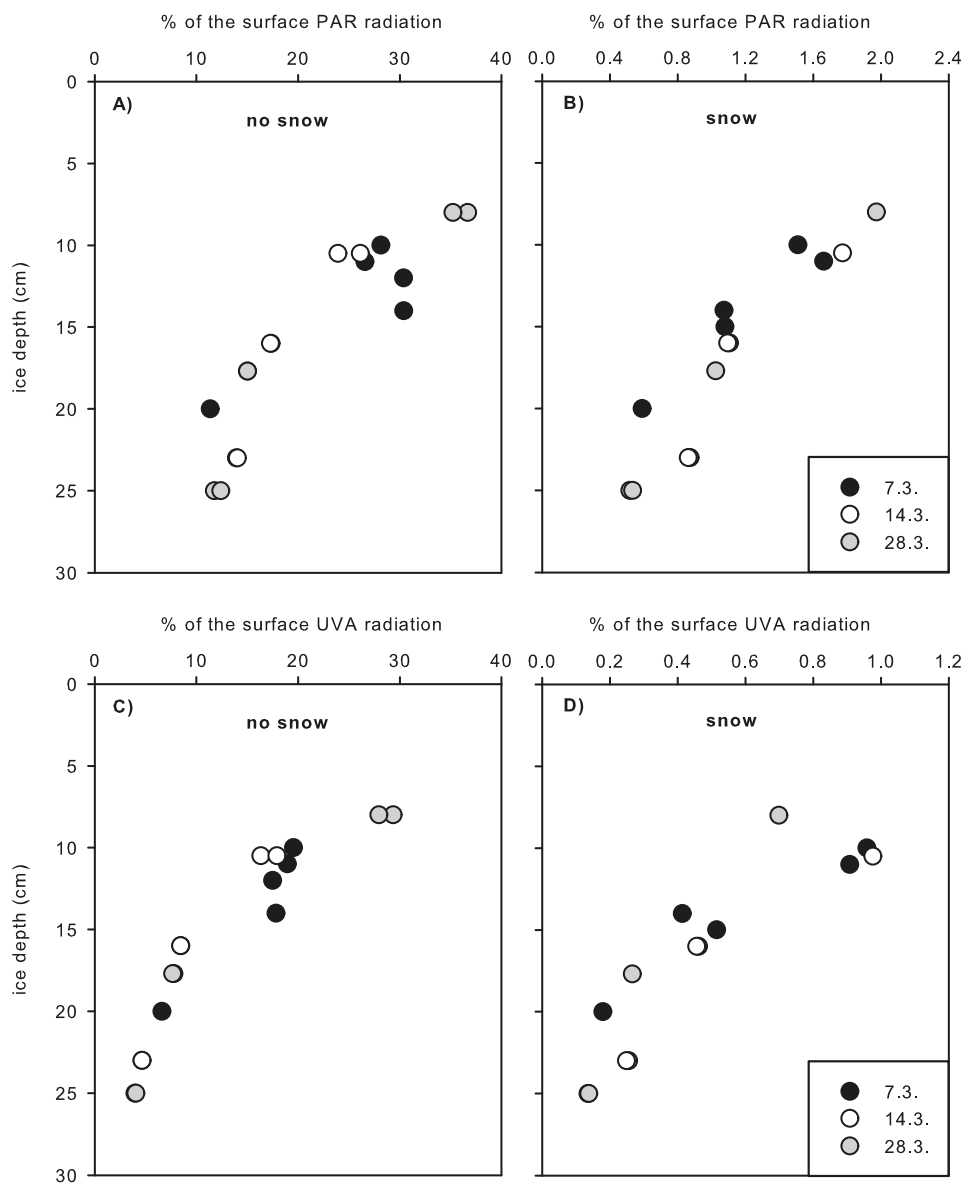


Fig. 3. Attenuation profiles of PAR (upper panel) and UVA (lower panel) in the untreated ice with the snow cover removed (A,C) and with the snow cover present (B,D) in March 2006.

attenuated downwelling light, and at 10 and 25 cm depth there was 1.0 % and 0.1 %, respectively, of the snow surface UVA radiation, whereas without the snow, the corresponding values were 18 % and 4 % (Figs. 3C-D). The lower attenuation of PAR resulted in respective values of 26 % and 12 % in snow-free conditions and 1.6 % and 0.5 % with the snow cover present (Figs. 3A-B). The different attenuation profiles of PAR in the untreated ice obtained on 7th, 14th and 28th March demonstrate that the algae had a slight effect on the attenuation and less PAR reached the depth of 25 cm when the integrated diatom abundance in the above layers was highest, i.e. on 7th March (Figs. 3A-B and 4A-B). The transmittance through the ice was probably further reduced by the algal biomass in the layer below 25 cm. The relationship between attenuation and algal biomass did not show in the UVA wavelengths (Figs. 3C-D). The highest attenuation of both PAR and UVA wavelengths in the PAR+UVA treatment at the end of the second experiment, despite the lowest biomass, suggest that the synthesis of MAA compounds / other protective pigments was induced by the UVA exposure, or that other algal groups were present.

5.2.2. *Algae*

The physical environment played a significant role in determining the ice algal abundance in the two UVA studies carried out in subsequent years (III, IV). One of the major reasons for the large variability in the vertical distribution of algal cells in March 2005 and 2006 was the different bulk salinity profiles, pointing to differences in ice structure. In 2005, the lower layers of ice were nearly fresh (bulk salinity 0.2 ± 0.07), and the algae were thus concentrated in the upper layers, whereas

in 2006 the corresponding bulk salinity was approximately double and algal abundance had a C-shaped distribution. Apparently, in 2005 there was a clear freshwater influence under the ice, whereas the salinity profile and the algal distribution in 2006 more closely represented the typical sea ice from the northern Baltic Sea (Haecky & Andersson 1999, Kaartokallio 2004, Rintala et al. 2006, Kaartokallio et al. 2007).

The ability of algal cells to change their cellular chlorophyll-*a* content as response to varying light intensity, i.e. photoacclimation, is well known (e.g. Falkowski & Raven 1997). This was also observed in the UVA studies III and IV. Removal of the snow cover and exposure of the ice to low PAR intensities in February 2006 had an enhancing effect on chl-*a* concentrations in the top 5 cm whereas exposure to higher intensities of PAR in March 2005 and 2006 resulted in a decrease in chl-*a*. The magnitude of the decrease in chl-*a* in March was directly dependent on the original snow cover thickness. The exposure of ice that had been covered with thin snow (< 5 cm) resulted in 22 % lower chl-*a* values, on average, in the top 10 cm (III), while an average reduction of 47 % in chl-*a* was observed when the snow cover had been thick (> 12 cm, IV). UVA caused an additional 35 % reduction in March 2005 (III), being similar to the value for the whole ice column (32 %), while in March 2006 the UVA effect was much less and the corresponding values were 6 % and 4 % (Fig. 4A). In the untreated ice, the chl-*a* concentration increased exponentially with diatom density, whereas in the treated ice, the chl-*a* concentration remained at lower and relatively steady level and was more or less independent of the diatom abundance. For example, 64 % of the measured values in the treated ice were < 5 $\mu\text{g l}^{-1}$, whereas in untreated ice, most of the values (61 %) were

above this value. This indicates that cells were indeed adjusting their cellular chl-*a* content according to available PAR with and without UVA. However, the additional decrease in the chl-*a* concentration due to UVA points to photobleaching (e.g. Maske & Latasa 1997) or to a switch in the flagellate community from auto- to heterotrophy (III). For example, the growth of the chloroplast-containing nanoflagellate *Ochromonas* sp. in northern Baltic Sea was mainly heterotrophic in the study of Andersson et

al. (1989), and autotrophy was speculated to be a survival strategy in low bacterial supply. The secondary role of autotrophy for *Ochromonas* sp. was supported by a higher carbon to chlorophyll ratio (i.e. lower chl-*a* content) than in other marine phytoplankton.

In studies from the Antarctic, chlorophyll-*a* synthesis showed no or only a slight UVR effect and the phytoplankton was able to recover from the exposure to UVR within two weeks (Helbling et al. 1992, Villafañe et al. 1995). The Baltic Sea ice algae, on the

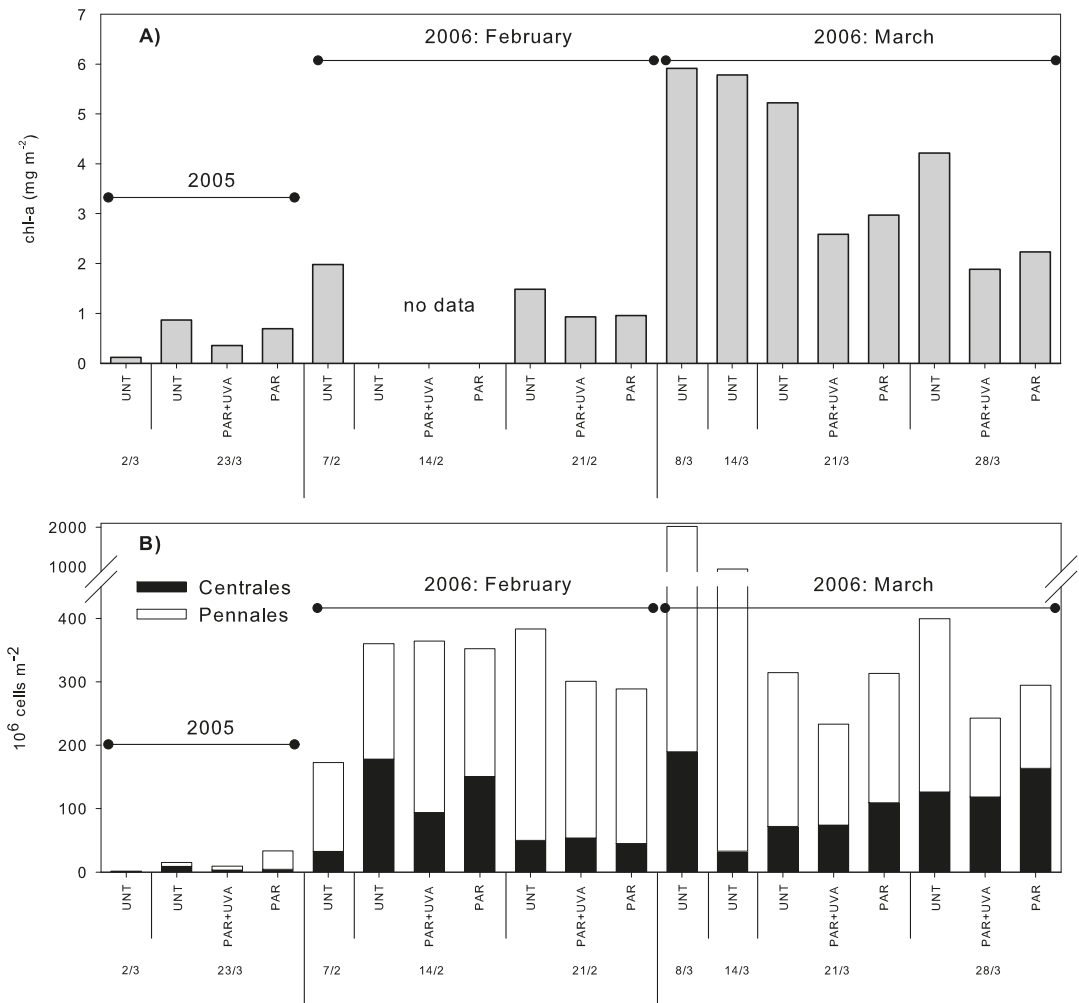


Fig 4. Integrated (A) chl-*a* (mg m⁻²) and (B) diatom abundance (10⁶ cells m⁻²) in the UVA experiments.

contrary, did not show any marked recovery in either chl-*a* or in biomass even after 3 weeks (III), despite the possible synthesis of MAAs, which have been reported to occur at high concentrations in Baltic Sea ice (Uusikivi et al. 2010). It must be kept in mind that dinoflagellates, which are a common group in Baltic sea ice (Kuosa & Kaartokallio 2006, Rintala et al. 2006, Kaartokallio et al. 2007, Uusikivi et al. 2010), were practically absent from the ice community in 2005, so further studies on the ability of ice algae to recover from UVR exposure are needed. The higher α^B and P^B_m values in the top 5 cm of the PAR+UVA treatment compared to PAR treatment indicate, however, that the photosynthetic capacity was not affected by UVA.

Similarly to chl-*a*, the timing of the snow-free ice conditions during the ice-covered period determined the effect of UVA on diatom densities. In February the exposure to low irradiance had no effect on the diatom densities, whereas the markedly higher irradiance in March (2005 and 2006) resulted

in clearly lower densities in the PAR+UVA treatment compared to the untreated ice and PAR treatment (Fig. 4B). There was evidence that the composition and vertical distribution of the Baltic Sea ice-algal communities were also shaped to some degree by UVA, and the exclusion of UVA specifically induced the growth of chlorophytes and pennate diatoms in the surface layers. In 2005 (III), the increased biomass mostly consisted of the flagellated chlorophyte *Chlamydomonas* spp. and a small unidentified pennate species (cell length 20–30 μm), whereas under UVA exposure the species composition was more similar to untreated ice with general flagellate dominance. The UVA sensitivity of *Chlamydomonas* agrees with the earlier observation of Maske (1984), and in the case of pennate diatoms, the possible explanations for the sensitivity include the low MAA production (Helbling et al. 1996a) and the typical 2-chloroplast configuration, which may not sufficiently shelter nucleic DNA (Karentz et al. 1991). The vertical distribution of centric diatoms

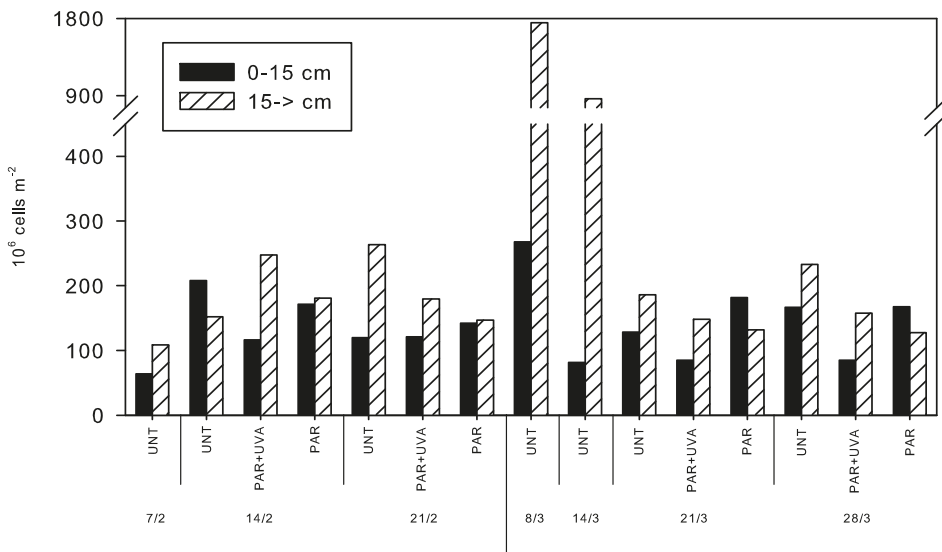


Fig. 5. Integrated diatom abundance above and under 15 cm in the untreated (UNT) and treated (PAR+UVA, PAR) ice columns in 2006.

seemed to be affected by PAR, whereas the biomass was also affected by UVA. In 2005, the centric diatom biomass (*Chaetoceros wighamii*, *Melosira arctica*, *Thalassiosira baltica*) in the snow-covered untreated ice (avg. 3.5 $\mu\text{g C l}^{-1}$) was mostly concentrated in the surface layers, whereas in the treated ice, the centric species were mostly found between 10 and 15 cm with a lower average biomass (PAR+UVA 1.5 $\mu\text{g C l}^{-1}$, PAR 2.6 $\mu\text{g C l}^{-1}$). Due to the glutaraldehyde fixation of 2006 samples (IV), only diatoms were well preserved, limiting the comparison between the studies in 2005 and 2006 to diatoms. The responses in the 2006 experiments were not as clear as in 2005, partially owing to difference in the initial vertical distribution of diatoms, but some similarities could be observed. These include a slight increase in pennate diatom (mainly *Achnanthes taeniata*) numbers in the top 15 cm of the PAR treatment in February and the marked reduction of centric diatom density (centric species with cell diameter 10–20 μm , *Chaetoceros wighamii*, *Melosira arctica*, *Thalassiosira baltica*) in the surface layer of treated ice and concentration in deeper layers (5–20 cm) in March. The important role of UVA in determining the vertical distribution of diatoms was supported by the results from the 2006 experiments (IV), as the exclusion of UVA in February led to a more even distribution of abundance, and in March to the concentration of the diatoms in the top 15 cm, differing from the diatom distribution in the untreated ice and the PAR+UVA treatment (Fig. 5).

These findings indicate that the generally observed low abundance of chlorophytes in sea ice and the abundance of pennate diatoms in the lower ice layers might be partly attributed to their sensitivity to UVA. Secondly, despite the typical preference of centric diatoms for the surface layers (I,

II), the centric diatoms do not tolerate well PAR and UVA in snow-free ice conditions lasting longer than one week. Thirdly, the high diatom abundances in the untreated ice compared to the treatments indicate that snow provides good shelter from UVA and excess PAR, but possibly also from rapid temperature and salinity fluctuations.

Larger cells have been stated to be less sensitive to UVR than smaller cells (Karentz et al. 1994), while in the study of Helbling et al. (1992) the microplankton abundance was enhanced by the exclusion of UVA with a concomitant decrease in the nanoplankton / microplankton ratio. The results from the 2005 experiment (III) agree with both, as the microplankton abundance was clearly higher in the PAR treatment than in the PAR+UVA treatment and untreated ice, and UVA caused a 52 % drop in nanoplankton abundance.

5.2.3. Bacterial production

In the Baltic Sea, the long-term effects of UVA on ice bacteria were closely linked to changes in algal biomass as well as in algal community composition, and lethal effects were not observed (III, IV). In 2005, the increase in the biomass of chlorophytes and pennate diatoms in the PAR treatment enhanced both bacterial DNA and protein synthesis (Figs. 6A-B), apparently via increased DOM release from the growing algae, as excreted carbon is a constant function of photosynthetic rates (Mague et al. 1980). In the flagellate-dominated untreated ice and PAR+UVA treatment, the emphasis was more on the TLI, pointing to less favourable conditions, as in unfavourable conditions protein synthesis may be favoured over DNA synthesis to ensure survival (Shiah & Ducklow 1997). The response in TTI to the exclusion of UVA in February 2006 was

similar to that in 2005, but as no increase was observed in the diatom densities, the increase in TTI in the PAR treatment may have been due to the growth of some other algal group. The response of bacterial activity to the treatments was most pronounced in the top 5 cm of ice, but the similar pattern in the integrated ice column values (Figs. 6A-B) highlights the extent of UVA-related effects.

As with algal responses, the timing of the exposure and the previous light history of the ice affected the response of bacteria. The marked enhancement of bacterial production in the surface layer of the

PAR+UVA treatment following the removal of the snow cover in March 2006 points to photochemical degradation of organic matter by UVA, producing labile dissolved organic compounds for bacteria (Lindell et al. 1995, Vähätalo et al. 2000), or to DOC release from algae under stress conditions (e.g. Hellebust 1965). However, as no difference was observed in the chl-*a* concentration between treatments (i.e. biomass was the same), and as the diatom densities in the surface layer declined in both treatments with no concomitant enhancement of TTI in the PAR treatment, photochemical degradation is

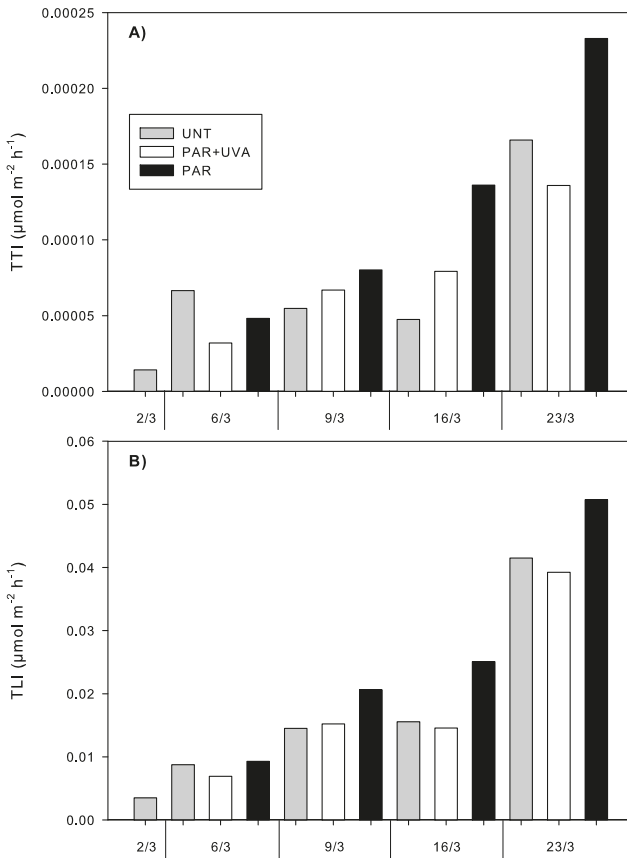


Fig. 6. The integrated total A) ^3H -thymidine incorporation (TTI) and B) ^{14}C -leucine incorporation (TLI) rates in the UVA experiment in 2005 (III).

a more plausible explanation. The thick snow cover prevailing for at least one week prior to the experiment had probably limited the primary production, and bacteria had used up most of the labile DOC. The enhancement was transitory and TTI declined to one third of the peak value within the second week of incubation, pointing to a decrease in photodegradable material and / or to harmful effects of UVA on bacteria.

The increase in TTI rates resulted in only moderate responses in TBN, which indicates that losses such as grazing or virus infections played a marked role in controlling the bacterial biomass, as in the studies of Kaartokallio (2004) and Kaartokallio et al. (2008).

While the effects of UVA on bacteria were predominantly through changes in algal biomass, the smaller protection from algae in the PAR+UVA treatment may have also induced some direct effects of UVA on bacteria. The natural bacterial communities from the Antarctic showed higher sensitivity to UVA than to UVB in 5–7 h *in situ* incubations, and ROS were speculated to be involved in the damage to the DNA replication process (Helbling et al. 1995). One UVA-related form of damage only involving RNA, is growth inhibition, consisting of growth delay and a subsequent slight depression in the growth rate (Jagger 1981). The damage appears as reduced protein synthesis, which would match the results from the surface layer of the PAR+UVA treatment in the 2005 experiment, but a separate experiment with algae excluded is needed to assess the direct effects of UVA on bacteria.

5.2.4. Bacterial community composition and the response to UVA (IV)

The PCO results demonstrated that ice-bacterial communities in 2006 underwent a clear succession from February to March. The responses to the treatments were generally observed after one week of incubation, after which they levelled off. The biggest changes were observed in the top 5 cm, where distinctively different communities were observed following the exclusion of UVA in February and the exposure to UVA in March.

Bacteria from three phyla (Bacteroidetes, Proteobacteria and Cyanobacteria) were present in the clone libraries (Table 3). The clones in the phyla Bacteroidetes and Proteobacteria could be further identified to class level; Bacteroidetes to Flavobacteria and Sphingobacteria and Proteobacteria to Alpha-, Beta- and Gammaproteobacteria. The presence of these classes in ice has also been observed in earlier studies from the Baltic Sea (Kaartokallio et al. 2005, 2008) and Antarctica (Bowman et al. 1997). The clones belonging to Cyanobacteria were classified as chloroplasts in SeqMatch, and they were therefore omitted from further analysis, including PCO.

Most of the clones (58.8 %) belonged to the class Flavobacteria and these clones had the closest matches to bacteria from the Arctic and Antarctic, and to psychrophile *Flavobacterium degerlachei* (Brinkmeyer et al. 2003, Van Trappen et al. 2004b, Srinivas et al. 2009, Miyashita et al. 2010). The relative peak areas of Flavobacteria sequences showed an increasing trend in February, but the thickening of the snow cover in early March resulted in markedly lower relative peak areas, pointing to the close connection of Flavobacteria with photosynthesis. Flavobacteria was the only bacterial class

Table 3. The identity of clones collected in the UVA experiment in 2006 (IV). The similarity score is obtained from SeqMatch and reports the percent sequence identity over all pairwise comparable positions when run with aligned sequences.

| Clone | | Closest sequences | | | | | |
|------------------------|-----------------------|-------------------|------------|----------|---------------------------------------|--|--------------------------|
| Phylum/Class | Genus | Count | Similarity | EMBL# | Closest match | Source | Reference |
| <i>Bacteroidetes/</i> | | | | | | | |
| Sphingobacteria | <i>Algoriphagus</i> | 1 | 99.6 | FJ196000 | <i>Algoriphagus</i> sp. | Antarctic sandy intertidal sediment | Yu et al. 2010 |
| Flavobacteria | <i>Flavobacterium</i> | 16 | 97.7–99.0 | AB455258 | <i>Flavobacterium degerlachei</i> (T) | Ace Lake microbial mat, Antarctic | Miyashita et al. 2010 |
| | | 11 | 97.8–98.9 | AJ557886 | <i>Flavobacterium degerlachei</i> (T) | | Van Trappen et al. 2004b |
| | | 1 | 97.9 | AY771760 | <i>Flavobacterium degerlachei</i> | Arctic | Lee et al., unpubl. |
| | | 49 | 97.7–99.6 | AF468432 | <i>Flavobacterium</i> sp. | Arctic sea ice–sea water interface | Brinkmeyer et al. 2003 |
| | | 1 | 98.2 | AF468434 | <i>Flavobacterium</i> sp. | Arctic sea-ice melt pond | Brinkmeyer et al. 2003 |
| | | 1 | 97.6 | AJ441000 | <i>Flavobacterium</i> sp. | Grace Lake microbial mat, Antarctic | Van Trappen et al. 2002 |
| | | 5 | 98.0–98.6 | DQ514307 | <i>Flavobacterium</i> sp. | Arctic Ocean sediment | Yu et al., unpubl. |
| | | 6 | 97.7–98.3 | EF628011 | <i>Flavobacterium</i> sp. | Arctic deep-sea sediment | Lin et al., unpubl. |
| | | 5 | 98.1–98.6 | FJ195988 | <i>Flavobacterium</i> sp. | Antarctic Ocean sediment | Ji et al., unpubl. |
| | | 4 | 94.7–98.6 | FN377738 | <i>Flavobacterium</i> sp. | Brownish sticky sediment, Kongsfjorden, Arctic | Srinivas et al. 2009 |
| | | 1 | 98.0 | FI946616 | Uncultured <i>Flavobacterium</i> sp. | Arctic meltwater | Larose et al. 2010 |
| <i>Proteobacteria/</i> | | | | | | | |
| Alphaproteobacteria | <i>Loktanella</i> | 3 | 99.1–100 | GQ472792 | Uncultured alpha proteobacterium | Surface water from northern Bering Sea | Zeng et al., unpubl. |
| Betaproteobacteria | <i>Polaromonas</i> | 1 | 94.2 | EU801408 | Uncultured bacterium | Chesapeake Bay | Shaw et al. 2008 |
| | <i>Rhodoferrax</i> | 6 | 98.7–99.6 | AB504935 | Uncultured bacterium | Iron-rich snow | Kojima et al. 2009 |
| | | 8 | 95.7–99.8 | AY250098 | Uncultured bacterium | Naphthalene-contaminated sediment | Jeon et al. 2003 |
| | | 1 | 99.1 | AY622248 | Uncultured beta proteobacterium | Acidic uranium-contaminated aquifer | Reardon et al. 2004 |
| | | 1 | 95.9 | FI946612 | Uncultured <i>Rhodoferrax</i> sp. | Arctic meltwater | Larose et al. 2010 |
| | | 2 | 99.1 | GQ390170 | Uncultured <i>Rhodoferrax</i> sp. | Oxic-anoxic interface of meromictic Lac Pavin | Bidre-Petit, unpubl. |
| | Unclassified | 1 | 96.0 | AM991264 | Uncultured bacterium | Karst spring water, Switzerland | Pronk et al. 2009 |
| | <i>Aquaspirillum</i> | 9 | 99.0–99.9 | AB074523 | <i>Aquaspirillum arcticum</i> | | Ding & Yokota 2002 |
| Gammaproteobacteria | <i>Colwellia</i> | 7 | 99.7–99.9 | AF468313 | Uncultured bacterium | Arctic sea ice, northern Fram Strait | Brinkmeyer et al. 2003 |

to show a positive response to UVA exposure in the surface layer, which indicates that they are apparently not harmed by the natural UVA intensities during the ice-covered season in the Baltic Sea, but their abundance is dictated more by the quality and quantity of DOM. This was not surprising, as these bacteria are known to take up and degrade organic matter, such as complex polysaccharides, in cold aquatic environments (Bernardet et al. 1996, Kirchman 2002). The one Bacteroidetes clone that did not belong to Flavobacteria was assigned to the genus *Algoriphagus* in the class Sphingobacteria, showing a close match to bacteria collected from Antarctic sandy intertidal sediment (Yu et al. 2010). The relative peak areas of Sphingobacteria increased from February to March, when they were among the dominant classes. The concentration of Sphingobacteria in the intermediate layers from 10 to 20 cm, which were generally low in chl-*a* and diatom abundance, indicate that these cold-adapted, marine and strictly aerobic heterotrophs (Bowman et al. 2003) were growing on some non-algal carbon source. No response to the treatments was observed, which may be due to their vertical placement in ice columns.

Alphaproteobacteria showed an increasing trend from February to March and was generally found in all but the bottommost layer. In February, the peaks were too few to observe any UVA-related pattern, but in March the exposure to UVA abolished Alphaproteobacteria from the topmost 5 cm in two weeks. However, the highest peak areas were observed in the layers from 5 to 20 cm in the same treatment, indicating that harmful levels of UVA irradiance were limited to the topmost layer. The sensitivity of Alphaproteobacteria to UVA has been reported earlier by Alonso-Sáez et al. (2006), who detected the inhibition of ATP and leucine uptake under UVA exposure. The

species were most likely (similarity 100 %) the same uncultured *Loktanella* species as collected from the surface water of the Bering Sea, most likely *L. salsilacus* (99.3–99.6 %). The Betaproteobacteria clones showed the closest relatedness to bacteria in the genera *Aquaspirillum*, *Polaromonas* and *Rhodofera*. All the clones in the genus *Aquaspirillum* were most likely the species *A. arcticum* (similarity ≥ 99 %), which was originally described from Arctic sediment (Butler et al. 1989) but has also been found from pack ice melt pools in the Arctic (Brinkmeyer et al. 2004). The one clone classified to the genus *Polaromonas*, which comprises psychrophilic, gas vacuolate, non-pigmented obligate aerobes, was closely related to uncultured bacteria from Chesapeake Bay (Shaw et al. 2008), but *Polaromonas* is also a common genus in Antarctic waters (Irgens et al. 1996). The relative peak area of *Aquaspirillum* sp. showed a declining trend during season, whereas that of *Polaromonas* sp. increased from February to March. *Polaromonas* species were mostly associated with the bottom layer, and no response to the treatments was observed, whereas *Aquaspirillum* sp. had a varying distribution and showed sensitivity to UVA in the surface layer in February. *Aquaspirillum* and *Polaromonas* were in a minor role compared to *Rhodofera*, which dominated the relative peak areas of Betaproteobacteria. Most of the closest matches to the clones classified as *Rhodofera* were from contaminated sediments or from iron-rich snow (Jeon et al. 2003, Kojima et al. 2009). The closest isolated relative was iron-reducing, psychrotolerant *R. ferrireducens*, which does not express photosynthetic growth like most *Rhodofera* species (Finneran et al. 2003), and has recently been reclassified as *Albidofera ferrireducens* (Ramana & Sasikala 2009). The presence of 18 clones in

the genus *Rhodoferrax* in the clone libraries was probably due to the iron emissions from the nearby Koverhar steel plant, which occasionally appear as a red colouration on the snow and ice. The relative peak areas of *Rhodoferrax* sp. in the surface ice were affected by the treatments, and the exclusion of PAR in February resulted in a marked increase in the areas, whereas in March the exposure to UVA gradually diminished the *Rhodoferrax* species from the surface layer by the end of the experiment. This points again to the role of light history, as no difference was observed in the surface layer between untreated ice and the PAR+UVA treatment in February (thin snow cover), but in March the removal of the thick snow cover was fatal for surface-dwelling *Rhodoferrax* species.

Gammaproteobacteria was mostly associated with the bottom layers and showed a declining trend from February to March. In February the exclusion of UVA led to a marked increase in the relative peak area in the top 5 cm, whereas exposure to UVA resulted in the lowest peak areas. In March the relative peak area was lower in treated ice than in untreated ice, the difference mainly being attributed to PAR. The seven clones in the class Gammaproteobacteria were classified to the genus *Colwellia* showing high similarities (99.8 %) to an uncultured bacterium from the Arctic sea ice in northern Fram Strait (Brinkmeyer et al. 2003) and to the isolate *C. piezophila* (99.7–99.9 %) from Arctic sea ice (H.K. Lee unpubl.). The members of the psychrophilic *Colwellia* genus are known to degrade particulate material (Méthé et al. 2005 and references therein), and their presence in highly saline brine is expected. *Colwellia* species require at least a salinity of 9 for growth (Bowman et al. 1998), which also explains why they are not encountered in the water column of the northern Baltic Sea (Hagström et al.

2000, Pinhassi & Hagström 2000). Based on these observations, the TTI peaks in the surface layer of the PAR treatment in February were attributed to Beta- and Gammaproteobacteria, whereas the TTI peak in the PAR+UVA treatment in March was most likely due to an increased abundance of Flavobacteria species. It was recently discovered that a group of Betaproteobacteria incorporated ³H-thymidine poorly (< 10 %), whereas ³H-leucine was incorporated more readily (80–90 %) (Pérez et al. 2010). Thus, it is uncertain to what degree the Betaproteobacteria are included in the TTI measurements of these UVA studies (III,IV), but the unequal thymidine and leucine incorporation of Betaproteobacteria could be one good explanation for the high TLI:TTI ratios (III) and the inverse relationship between TBN and TTI (III,IV). However, the ability for thymidine incorporation may vary between Betaproteobacteria lineages (see Cottrell & Kirchman 2003, Salcher et al. 2008). These varying findings certainly call for more detailed studies on the applicability of the thymidine and leucine incorporation methods to various bacterial groups found in Baltic Sea ice.

According to the relative peak areas, the abundances of different bacterial classes changed within the bacterial communities from February to March and also as response to the treatments, possibly affecting the energy and material fluxes. These changes were not seen in TBN, which seems to have been a poor variable to reflect the responses to UVA treatments. The TRFLP method combined with cloning is a good method to identify the dominant bacterial groups down to class level, but at lower taxonomical levels the identical restriction sites of different genera within a class makes more detailed taxonomical description of the community generally impossible. To obtain sequences

of the less-abundant bacterial species, other methods such as 454 sequencing have to be applied.

5.2.5. Significance in future climate scenarios

The future climate scenarios for the Baltic Sea predict an overall reduction in the ice-covered area, but also thinning of the winter ice and snow cover (Haapala et al. 2001, HELCOM 2007). Thus, more solar radiation will penetrate the ice and UVR will have a greater role, affecting the sympagic organisms as well as the plankton underneath the ice. The responses will be similar to what was observed in the surface layers of the PAR+UVA treatment in the UVA studies of 2005 and 2006, but probably somewhat more adverse due to the presence of UVB. In addition to the direct effects on organisms, the indirect effects will extend through the whole food web (Karentz 1991, Bothwell et al. 1994, Roy 2000 and references therein). For example, a reduction in algal biomass and changes in algal species composition will cascade to upper trophic levels and, as seen in the 2005 experiment, will also affect bacterial production and therefore remineralization processes. In addition, the nutritional quality of ice algae (e.g. the fatty acid composition) might be reduced (Leu et al. 2010). Acclimation, adaptation or changes in the species composition towards UVR-tolerant species may, however, change these predictions and new trophic interactions in food webs may be formed.

6. CONCLUSIONS

The sea ice communities in the Bothnian Bay thrive in a very dynamic environment. Drift ice is subjected to rafting and ridging, which change the vertical placement of sea-ice organisms and also affect the amount of available light, and fast ice is influenced by inflowing river water. As for all ice communities, light is the most important limiting factor for ice communities in the Bothnian Bay. However, the significance of habitable space expressed as brine volume increases from the more saline southern Baltic Sea to the low saline Bothnian Bay, and habitable space plays a key role in dictating the community composition, as larger species (e.g. chain-forming centric diatoms) tend to habit layers with a larger brine volume. The formation of snow ice, which is induced by flooding, is a frequent phenomenon in the Bothnian Bay and provides more habitats for organisms in a well-illuminated environment. This is especially important in fast ice close to river mouths, which might otherwise be severely limited by habitable space.

Algal community succession in Bothnian Bay drift ice advances with increasing ice thickness from a low biomass flagellate stage to a diatom-dominated community with increased biomass. The top ice layers in the thick ice, including snow ice, are dominated by centric diatoms, whereas pennate diatoms are distributed more evenly or concentrated in the bottom layers. However, exceptional conditions at the time of ice formation seen as deviant ice structure may hinder the normal succession from flagellate to diatom dominance, and the community may remain in the flagellate stage, despite the increasing ice thickness. Thus, determination of the ice structure is important in explaining the observed differences in community composition and biomass between ice samples. The algal biomass in Bothnian Bay

fast ice is comparable to the biomass in drift ice, considering that the latter is often composed of two overlapping ice floes. However, the proportion of diatoms is lower in fast ice than in drift ice, and chlorophytes constitute a significant amount of biomass for most of the season, while only during ice melt do diatoms form the majority of the ice-algal biomass.

The Baltic Sea ice communities are controlled to some degree by solar UVA, and the exclusion of UVA favours chlorophytes and pennate diatoms. The effect of UVA on bacteria is mostly indirect and follows the changes in algal biomass, as well as in community composition. Depending on the light history, UVA may occasionally be beneficial for bacteria, as it induces photochemical degradation of organic matter and / or increased algal excretion, which both provide labile DOC for the growth of Flavobacteria, among others. The abundance of Flavobacteria, the dominant class of the ice bacterial community in the northern Baltic Sea, seems to be linked to autochthonous DOM production, as thickening of snow cover results in a drop in their abundance. The other Bacteroidetes class, Sphingobacteria, is mainly present in deeper ice layers and is not therefore subjected to the harmful levels of UVA. While Bacteroidetes seem to be tolerant to UVA, Alpha-, Beta- and Gammaproteobacteria are negatively affected by UVA, which shows as the reduction / elimination of these classes in the surface layer under UVA exposure. The response is dependent on the light history, and exposure to PAR+UVA after a dark period results in a decrease attributed more to PAR. The light history also plays a role in algal responses, and when dark-acclimated communities are exposed to PAR+UVA, the effect of PAR might be greater than that of UVA. The lack of a marked recovery of algae is contrary to earlier studies from the Antarctic and might indicate that Baltic Sea ice communities are very sensitive to the consequences of climate change.

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8. REFERENCES

- Aas, P., Lyons, M. M., Pledger, R., Mitchell, D. L. & Jeffrey, W. H. 1996: Inhibition of bacterial activities by solar radiation in nearshore waters and the Gulf of Mexico. – *Aquat. Microb. Ecol.* 11: 229-238.
- Ackley, S. F. & Sullivan, C. W. 1994: Physical controls on the development and characteristics of Antarctic sea ice biological communities – a review and synthesis. – *Deep-Sea Res.* 41: 1583-1604.
- Alonso-Sáez, L., Gasol, J. M., Lefort, T., Hofer, J. & Sommaruga, R. 2006: Effect of natural sunlight on bacterial activity and differential sensitivity of natural bacterioplankton groups in north-western Mediterranean coastal waters. – *Appl. Environ. Microbiol.* 72: 5806-5813.
- Andersson, A., Falk, S., Samuelsson, G. & Hagström, Å. 1989: Nutritional characteristics of a mixotrophic nanoflagellate, *Ochromonas* sp. – *Microb. Ecol.* 17: 251-262.
- Anderson, M. J. 2003: PCO: a FORTRAN computer program for principal coordinate analysis. – Department of Statistics, University of Auckland, New Zealand.
- Arrigo, K. R., Mock, T. & Lizotte, M. P. 2010: Primary producers and sea ice. – In: Thomas, D. N. & Dieckmann, G. S. (eds.), *Sea ice*, 2nd ed., pp. 283-324. Wiley-Blackwell, Oxford.
- Arrigo, K. R. & Sullivan, C. W. 1992: The influence of salinity and temperature covariation on the photophysiological characteristics of Antarctic sea ice microalgae. – *J. Phycol.* 28: 746-756.
- Asada, K. & Takahashi, M. 1987: Production and scavenging of active oxygen in photosynthesis. – In: Kyle, D. J., Osmond, C. B. & Arntzen, C. J. (eds.), *Photoinhibition*, pp. 227-287. Elsevier, Amsterdam.
- Axe, P. 2010: Spatial distribution of the winter nutrient pool. – HELCOM Indicator Fact Sheets, online, 20/05/2011. http://www.helcom.fi/BSAP_assessment/ifs/ifs2010/en_GB/Winter-NutrientPool/
- Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L. A. & Thingstad, F. 1983: The ecological role of water-column microbes in the sea. – *Mar. Ecol. Prog. Ser.* 10: 257-263.
- Ban, A., Aikawa, S., Hattori, H., Sasaki, H., Sampei, M., Kudoh, S., Fukuchi, M., Satoh, K. & Kashino, Y. 2006: Comparative analysis of photosynthetic properties in ice algae and phytoplankton inhabiting Franklin Bay, the Canadian Arctic, with those in mesophilic diatoms during CASES 03-04. – *Polar Biosci.* 19: 11-28.
- Banaszak, A. T. 2003: Photoprotective physiological and biochemical responses of aquatic organisms. – In: Helbling, E. W. & Zagarese, H. (eds.), *UV effects in aquatic organisms and ecosystems*, pp. 329-356. Royal Society of Chemistry, Cambridge.
- Belzile, C., Gibson, J. A. E. & Vincent, W. F. 2002: Colored dissolved organic matter and dissolved organic carbon exclusion from lake ice: Implications for irradiance transmission and carbon cycling. – *Limnol. Oceanogr.* 47: 1283-1293.
- Bernardet, J.-F., Segers, P., Vancanneyt, M., Berthe, F., Kersters, K. & Vandamme, P. 1996: Cutting a Gordian knot: emended classification and description of the genus *Flavobacterium*, emended description of the family Flavobacteriaceae, and proposal of *Flavobacterium hydatis* nom. nov. (basonym, *Cytophaga aquatilis* Strohl and Tait 1978). – *Int. J. Syst. Bacteriol.* 46: 128-148.
- Booth, C. R., Morrow, J. H., Coohill, T. P., Cullen, J. J., Frederick, J. E., Hader, D. P., Holm-Hansen, O., Jeffrey, W. H., Mitchell, D. L., Neale, P. J., and others 1997: Impacts of solar UVR on aquatic microorganisms. – *Photochem. Photobiol.* 65: 252-269.
- Bothwell, M. L., Sherbot, D. M. J. & Pollock, C. M. 1994: Ecosystem response to solar ultraviolet-B radiation: influence of trophic-level interactions. – *Science* 265: 97-100.
- Bowman, J. P., Gosink, J. J., McCammon, S. A., Lewis, T. E., Nichols, D. S., Nichols, P. D., Skerratt, J. H., Staley, J. T. & McMeekin, T. A. 1998: *Colwellia demingiae* sp. nov., *Colwellia hornerae* sp. nov., *Colwellia rossensis* sp. nov. and *Colwellia psychrotropica* sp. nov.: psy-

- chrophilic Antarctic species with the ability to synthesize docosahexaenoic acid (22:ω63). – *Int. J. Syst. Evol. Microbiol.* 48: 1171-1180.
- 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.
- Bowman, J. P., Nichols, C. M. & Gibson, J. A. E. 2003: *Algoriphagus ratkowskyi* gen. nov., sp. nov., *Brumimicrobium glaciale* gen. nov., sp. nov., *Cryomorpha ignava* gen. nov., sp. nov. and *Crocinitomix catalasitica* gen. nov., sp. nov., novel flavobacteria isolated from various polar habitats. – *Int. J. Syst. Evol. Microbiol.* 53: 1343-1355.
- Brinkmeyer, R., Glöckner, F. O., Helmke, E. & Amann, R. 2004: Predominance of β-proteobacteria in summer melt pools on Arctic pack ice. – *Limnol. Oceanogr.* 49: 1013-1021.
- Brinkmeyer, R., Knittel, K., Jürgens, J., Weyland, H., Amann, R. & Helmke, E. 2003: Diversity and structure of bacterial communities in Arctic versus Antarctic pack ice. – *Appl. Environ. Microbiol.* 69: 6610-6619.
- Buch, K. 1945: Kolsyrejämvikten i Baltiska havet. – *Fennia* 68: 29-81.
- Bühlmann, B., Bossard, P. & Uehlinger, U. 1987: The influence of longwave ultraviolet radiation (u.v.-A) on the photosynthetic activity (¹⁴C-assimilation) of phytoplankton. – *J. Plankton Res.* 9: 935-943.
- Butler, B. J., McCallum, K. L. & Inniss, W. E. 1989: Characterization of *Aquaspirillum arcticum* sp. nov., a new psychrophilic bacterium. – *Syst. Appl. Microbiol.* 12: 263-266.
- Cole, J. R., Chai, B., Farris, R. J., Wang, Q., Kulam-Syed-Mohideen, A. S., McGarrell, D. M., Bandela, A. M., Cardenas, E., Garrity, G. M. & Tiedje, J. M. 2007: The ribosomal database project (RDP-II): introducing myRDP space and quality controlled public data. – *Nucleic Acids Res.* 35: D169-D172.
- Cota, G. F. & Horne, E. P. W. 1989: Physical control of Arctic ice algal production. – *Mar. Ecol. Prog. Ser.* 52: 111-121.
- 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.
- Cottrell, M. T. & Kirchman, D. L. 2003: Contribution of major bacterial groups to bacterial biomass production (thymidine and leucine incorporation) in the Delaware estuary. – *Limnol. Oceanogr.* 48: 168-178.
- Cox, G. F. N. & Weeks, W. F. 1983: Equations for determining the gas and brine volumes in sea-ice samples. – *J. Glaciol.* 29: 306-316.
- Cullen, J. J., Neale, P. J. & Lesser, M. P. 1992: Biological weighting function for the inhibition of phytoplankton photosynthesis by ultraviolet radiation. – *Science* 258: 646-650.
- Delille, D., Fiala, M., Kuparinen, J., Kuosa, H. & Plessis, C. 2002: Seasonal changes in microbial biomass in the first-year ice of the Terre Adélie area (Antarctica). – *Aquat. Microb. Ecol.* 28: 257-265.
- Deming, J. W. 2010: Sea ice bacteria and viruses. – In: Thomas, D. N. & Dieckmann, G. S. (eds.), *Sea ice*, 2nd ed., pp. 247-282. Wiley-Blackwell, Oxford.
- Ding, L. & Yokota, A. 2002: Phylogenetic analysis of the genus *Aquaspirillum* based on 16S rRNA gene sequences. – *FEMS Microbiol. Lett.* 212: 165-169.
- Dolan, J. R. & Gallegos, C. C. 1992: Trophic role of planktonic rotifers in the Rhode River Estuary, spring–summer 1991. – *Mar. Ecol. Prog. Ser.* 85: 187-199.
- Draper, N. R. & Smith, H. 1981: *Applied regression analysis*, 2nd ed. – Wiley, New York, 709 pp.
- Dunbar, M. J. & Acreman, J. C. 1980: Standing crops and species composition of diatoms in sea ice from Robeson Channel to the Gulf of St. Lawrence. – *Ophelia* 19: 61-72.
- Edwards, U., Rogall, T., Blocker, H., Emde, M. & Bottger, E. C. 1989: Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. – *Nucleic Acids Res.* 17: 7843-7853.

- Egloff, D. A. 1988: Food and growth relations of the marine microzooplankter, *Synchaeta cecilia* (Rotifera). – *Hydrobiologia* 157: 129-141.
- Ehn, J., Granskog, M. A., Reinart, A. & Erm, A. 2004: Optical properties of melting landfast sea ice and underlying seawater in Santala Bay, Gulf of Finland. – *J. Geophys. Res.* 109, C09003, doi:10.1029/2003JC002042.
- Eicken, H. 1992: The role of sea ice in structuring Antarctic ecosystems. – *Polar Biol.* 12: 3-13.
- Eicken, H. 2009: Ice sampling and basic sea ice core analysis. – In: Eicken, H., Gradinger, R., Salganek, M., Shirasawa, K., Perovich, D. K. & Leppäranta, M. (eds.), *Field techniques for sea-ice research*, pp. 117-140. University of Alaska Press, Fairbanks.
- Eicken, H. & Lange, M. 1989: Development and properties of sea ice in the coastal regime of the southeastern Weddell Sea. – *J. Geophys. Res.* 94: 8193-8206.
- 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.
- Ekholm, M. 1993: Suomen vesistöalueet. – Vesi- ja ympäristöhallituksen julkaisuja, sarja A 126. Painatuskeskus Oy, Helsinki, 166 pp.
- Everitt, B. S., Landau, S. & Leese, M. 2001: *Cluster analysis*, 4th ed. – Arnold Publishers, London, 248 pp.
- Falkowski, P. G. & Raven, J. A. 1997: *Aquatic photosynthesis*. – Blackwell Science, Massachusetts, 375 pp.
- Felsenstein, J. 2005: PHYLIP (phylogeny inference package). – Department of Genome Sciences, University of Washington, Seattle.
- Finneran, K. T., Johnsen, C. V. & Lovley, D. R. 2003: *Rhodoferrax ferrireducens* sp. nov., a psychrotolerant, facultatively anaerobic bacterium that oxidizes acetate with the reduction of Fe (III). – *Int. J. Syst. Evol. Microbiol.* 53: 669-673.
- Fleming-Lehtinen, V., Laamanen, M., Kuosa, H., Hahti, H. & Olsonen, R. 2008: Long-term development of inorganic nutrients and chlorophyll α in the open northern Baltic Sea. – *Ambio* 37: 86-92.
- 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.
- Garcia-Pichel, F. 1994: A model for internal self-shading in planktonic organisms and its implications for the usefulness of ultraviolet sunscreens. – *Limnol. Oceanogr.* 39: 1704-1717.
- 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.
- Gast, R. J., Moran, D. M., Dennett, M. R. & Caron, D. A. 2007: Kleptoplasty in an Antarctic dinoflagellate: caught in evolutionary transition? – *Environ. Microbiol.* 9: 39-45.
- Gerber, S. & Häder, D. P. 1995: Effects of enhanced solar irradiation on chlorophyll fluorescence and photosynthetic oxygen production of five species of phytoplankton. – *FEMS Microbiol. Ecol.* 16: 33-42.
- Golden, K. M., Ackley, S. F. & Lytle, V. I. 1998: The percolation phase transition in sea ice. – *Science* 282: 2238-2241.
- Gradinger, R. & Ikävalko, J. 1998: Organism incorporation into newly forming Arctic sea ice in the Greenland Sea. – *J. Plankton Res.* 20: 871-886.
- 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. – *Wat. Air Soil Pollut.* 154: 331-347.
- Granskog, M. A., Kaartokallio, H., Kuosa, H., Thomas, D. N., Ehn, J. & Sonninen, E. 2005b: Scales of horizontal patchiness in chlorophyll a , chemical and physical properties of landfast sea ice in the Gulf of Finland (Baltic Sea). – *Polar Biol.* 28: 276-283.

- Granskog, M., Kaartokallio, H., Kuosa, H., Thomas, D. N. & Vainio, J. 2006a: Sea ice in the Baltic Sea – a review. – *Est. Coast. Shelf Sci.* 70: 145-160.
- Granskog, M. A., Kaartokallio, H. & Shirasawa, K. 2003a: 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., Leppäranta, M., Kawamura, T., Ehn, J. & Shirasawa, K. 2004a: 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.
- Granskog, M. A., Martma, T. A. & Vaikmäe, R. A. 2003b: Development, structure and composition of land-fast sea ice in the northern Baltic Sea. – *J. Glaciol.* 49: 139-148.
- Granskog, M. A., Uusikivi, J., Sequeiros, A. B. & Sonninen, E. 2006b: Relation of ice growth rate to salt segregation during freezing of low-salinity sea water (Bothnian Bay, Baltic Sea). – *Ann. Glaciol.* 44: 134-138.
- Granskog, M. A., Vihma, T., Pirazzini, R. & Cheng, B. 2006c: Superimposed ice formation and surface energy fluxes on sea ice during the spring melt-freeze period in the Baltic Sea. – *J. Glaciol.* 52: 119-127.
- Granskog, M. A., Virkkunen, K., Thomas, D. N., Ehn, J., Kola, H. & Martma, T. 2004b: Chemical properties of brackish water ice in the Bothnian Bay, the Baltic Sea. – *J. Glaciol.* 50: 292-302.
- Grenfell, T. C. & Maykut, G. A. 1977: The optical properties of ice and snow in the Arctic basin. – *J. Glaciol.* 18: 445-463.
- Haapala, J., Meier, M. H. E. & Rinne, J. 2001: Numerical investigations of future ice conditions in the Baltic Sea. – *Ambio* 30: 237-244.
- Haecy, P. & Andersson, A. 1999: Primary and bacterial production in sea ice in the northern Baltic Sea. – *Aquat. Microb. Ecol.* 20: 107-118.
- Haecy, 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., Pinhassi, J. & Zweifel, U. L. 2000: Biogeographical diversity among marine bacterioplankton. – *Aquat. Microb. Ecol.* 21: 231-244.
- Hall, T. A. 1999: BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. – *Nucl. Acids. Symp. Ser.* 41: 95-98.
- Hansen, H. P. & Koroleff, F. 1999: Determination of nutrients. – In: Grashoff, K., Kremling, K. & Ehrhardt, M. (eds.), *Methods of seawater analysis*, 3rd ed., pp 159-228. Wiley-VCH, Weinheim.
- Harm, W. 1980: Biological effects of ultraviolet radiation. – Cambridge University Press, Cambridge, 216 pp.
- Helbling, E. W., Chalker, B. E., Dunlap, W. C., Holm-Hansen, O. & Villafaña, V. E. 1996a: Photoacclimation of Antarctic marine diatoms to solar ultraviolet radiation. – *J. Exp. Mar. Biol. Ecol.* 204: 85-101.
- Helbling, W. E., Eilertsen, H. C., Villafaña, V. E. & Holm-Hansen, O. 1996b: Effects of UV radiation on post-bloom phytoplankton populations in Kvalsund, North Norway. – *J. Photochem. Photobiol.* 33: 255-259.
- Helbling, E. W., Marguet, E. R., Villafaña, V. E. & Holm-Hansen, O. 1995: Bacterioplankton viability in Antarctic waters as affected by solar ultraviolet radiation. – *Mar. Ecol. Prog. Ser.* 126: 293-298.
- Helbling, E. W., Villafaña, V., Ferrario, M. & Holm-Hansen, O. 1992: Impact of natural ultraviolet radiation on rates of photosynthesis and on specific marine phytoplankton species. – *Mar. Ecol. Prog. Ser.* 80: 89-100.
- HELCOM 1988: Guidelines for the Baltic monitoring programme for the third stage; part D. biological determinants. – *Balt. Sea Environ. Proc.* 27D: 16-23.
- HELCOM 2007: Climate change in the Baltic Sea area. – *Balt. Sea Environ. Proc.* 111: 1-54.
- Hellebust, J. A. 1965: Excretion of some organic compounds by marine phytoplankton. – *Limnol. Oceanogr.* 10: 192-206.

- 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.
- Hietanen, S., Lehtimäki, J. M., Tuominen, L., Sivonen, K. & Kuparinen, J. 2002: *Nodularia* spp. (Cyanobacteria) incorporate leucine but not thymidine: importance for bacterial-production measurements. – *Aquat. Microb. Ecol.* 28: 99-104.
- Hobbie, J. E., Daley, R. J. & Jasper, S. 1977: Use of nucleopore filters for counting bacteria by fluorescence microscopy. – *Appl. Environ. Microbiol.* 33: 1225-1228.
- Huber, T., Faulkner, G. & Hugenholtz, P. 2004: Bellerophon: a program to detect chimeric sequences in multiple sequence alignments. – *Bioinformatics* 20: 2317-2319.
- Huttunen, M. & Niemi, Å. 1986: Sea-ice algae in the northern Baltic Sea. – *Memoranda. Soc. Fauna Flora Fennica* 62: 58-62.
- Ikävalko, J. 1998: Further observations on flagellates within sea ice in northern Bothnian Bay, the Baltic Sea. – *Polar Biol.* 19: 323-329.
- Ikävalko, J. & Thomsen, H. A. 1997: The Baltic Sea ice biota (March 1994): a study of the protistan community. – *Eur. J. Protistol.* 33: 229-243.
- Irgens, R. L., Gosink, J. J. & Staley, J. T. 1996: *Polaromonas vacuolata* gen. nov., sp. nov., a psychrophilic, marine, gas vacuolate bacterium from Antarctica. – *Int. J. Syst. Evol. Microbiol.* 46: 822-826.
- Jagger, J. 1981: Near-UV radiation effects on microorganisms. – *Photochem. Photobiol.* 34: 761-768.
- Jagger, J. 1985: Solar-UV actions on living cells. – Praeger, New York, 202 pp.
- Jeffrey, W. H., Aas, P., Lyons, M. M., Coffin, R. B., Pledger, R. J. & Mitchell, D. L. 1996: Ambient solar radiation-induced photodamage in marine bacterioplankton. – *Photochem. Photobiol.* 64: 419-427.
- Jeffries, M. O., Shaw, R. A., Morris, K., Veazey, A. L. & Krouse, H. R. 1994: Crystal structure, stable isotopes ($\delta^{18}\text{O}$), and development of sea ice in the Ross, Amundsen, and Bellingshausen seas, Antarctica. – *J. Geophys. Res.* 99: 985-995.
- Jeon, C. O., Park, W., Padmanabhan, P., DeRito, C., Snape, J. R. & Madsen, E. L. 2003: Discovery of a bacterium, with distinctive dioxygenase, that is responsible for *in situ* biodegradation in contaminated sediment. – *Proc. Natl. Acad. Sci. USA* 100: 13591-13596.
- Junge, K., Imhoff, F., Staley, T. & Deming, W. 2002: Phylogenetic diversity of numerically important Arctic sea-ice bacteria cultured at subzero temperature. – *Microb. Ecol.* 43: 315-328.
- Kaartokallio, H. 2001: Evidence for active microbial nitrogen transformations in sea ice (Gulf of Bothnia, Baltic Sea) in midwinter. – *Polar Biol.* 24: 21-28.
- Kaartokallio, H. 2004: Food web components, and physical and chemical properties of Baltic Sea ice. – *Mar. Ecol. Prog. Ser.* 273: 49-63.
- 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.
- Kaartokallio, H., Kuosa, H., Thomas, D. N., Granskog, M. A. & Kivi, K. 2007: Biomass, composition and activity of organism assemblages along a salinity gradient in sea ice subjected to river discharge in the Baltic Sea. – *Polar Biol.* 30: 183-197.
- Kaartokallio, H., Laamanen, M. & Sivonen, K. 2005: Responses of Baltic Sea ice and open-water natural bacterial communities to salinity change. – *Appl. Environ. Microbiol.* 71: 4364-4371.
- Kaartokallio, H., Tuomainen, J., Kuosa, H., Kuparinen, J., Martikainen, P. J. & Servomaa, K. 2008: Succession of sea-ice bacterial communities in the Baltic Sea fast ice. – *Polar Biol.* 31: 783-793.
- Kamjunke, N. & Jähnichen, S. 2000: Leucine incorporation by *Microcystis aeruginosa*. – *Limnol. Oceanogr.* 45: 741-743.
- Kankaala, P., Alasaarela, E. & Sundberg, A. 1984: Phytoplankton and zooplankton production in the north-eastern and central Bothnian Bay – a

- review of studies carried out in 1968–1978. – *Ophelia* Suppl. 3: 69-88.
- Karentz, D. 1991: Ecological considerations of Antarctic ozone depletion. – *Antarctic Sci.* 3: 3-11.
- Karentz, D., Bothwell, M. L., Coffin, R. B., Hanson, A., Herndl, G. J., Kilham, S. S., Lesser, M. P., Lindell, M., Moeller, R. E., Morris, D. P., Neale, P. J., Sanders, R. W., Weiler, C. S. & Wetzel, R. G. 1994: Impact of UV-B radiation on pelagic freshwater ecosystems: report of working group on bacteria and phytoplankton. – *Arch. Hydrobiol. Beih. Ergebn. Limnol.* 43: 31-69.
- Karentz, D., Cleaver, J. E. & Mitchell, D. L. 1991: Cell survival characteristics and molecular responses of Antarctic phytoplankton to ultraviolet-B radiation. – *J. Phycol.* 27: 326-341.
- Karner, M. & Fuhrman, J. A. 1997: Determination of active marine bacterioplankton: a comparison of universal 16S rRNA probes, autoradiography, and nucleoid staining. – *Appl. Environ. Microbiol.* 63: 1208-1213.
- Kawamura, T., Shirasawa, K., Ishikawa, N., Lindfors, A., Rasmus, K., Granskog, M. A., Ehn, J., Leppäranta, M., Martma, T. & Vaikmäe, R. 2001: Time-series observations of the structure and properties of brackish ice in the Gulf of Finland. – *Ann. Glaciol.* 33: 1-4.
- Kim, D. S. & Watanabe, Y. 1994: Inhibition of growth and photosynthesis of freshwater phytoplankton by ultraviolet A (UVA) radiation and subsequent recovery from stress. – *J. Plankton Res.* 16: 1645-1654.
- Kirchman, D. L. 2002: The ecology of Cytophaga-Flavobacteria in aquatic environments. – *FEMS Microbiol. Ecol.* 39: 91-100.
- Kirchman, D. L., K'Neas, E. & Hodson, R. E. 1985: Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic ecosystems. – *Appl. Environ. Microbiol.* 49: 599-607.
- Kojima, H., Fukuhara, H. & Fukui, M. 2009: Community structure of microorganisms associated with reddish-brown iron-rich snow. – *Syst. Appl. Microbiol.* 32: 429-437.
- 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.
- Kühl, M., Glud, R. N., Borum, J., Roberts, R. & Rysgaard, S. 2001: Photosynthetic performance of surface-associated algae below sea ice as measured with a pulse-amplitude-modulated (PAM) fluorometer and O₂ microsensors. – *Mar. Ecol. Prog. Ser.* 223: 1-14.
- Kuosa, H., Autio, R., Kuuppo, P., Setälä, O. & Tanskanen, S. 1997: Nitrogen, silicon and zooplankton controlling the Baltic spring bloom: an experimental study. – *Est. Coast. Shelf Sci.* 45: 813-821.
- Kuosa, H. & Kaartokallio, H. 2006: Experimental evidence on nutrient and substrate limitation of Baltic Sea sea-ice algae and bacteria. – *Hydrobiologia* 554: 1-10.
- Lange, M. A., Ackley, S. F., Wadhams, P., Dieckmann, G. S. & Eicken, H. 1989: Development of sea ice in the Weddell Sea. – *Ann. Glaciol.* 12: 92-96.
- Larose, C., Berger, S., Ferrari, C., Navarro, E., Dommergue, A., Schneider, D. & Vogel, T. M. 2010: Microbial sequences retrieved from environmental samples from seasonal Arctic snow and meltwater from Svalbard, Norway. – *Extremophiles* 14: 205-212.
- Larsen, J., Kuosa, H., Ikävalko, J., Kivi, K. & Hällfors, S. 1995: A redescription of *Scrippsiella hangoei* (schiller) comb. nov. – a 'red tide' dinoflagellate from the northern Baltic. – *Phycologia* 34: 135-144.
- Legendre, L., Aota, M., Shirasawa, K., Martineau, M. J. & Ishikawa, M. 1991: Crystallographic structure of sea ice along a salinity gradient and environmental control of microalgae in the brine cells. – *J. Mar. Syst.* 2: 347-357.
- Leppäranta, M. 1981: On the structure and mechanics of pack ice in the Bothnian Bay. – *Finn. Mar. Res.* 248: 3-86.
- Leppäranta, M. 1987: Ice observations. Field experiment SEA ICE-85 in mid winter in the Bay of Bothnia. – *Meri* 15: 15-34.
- Leppäranta, M. & Manninen, T. 1988: The brine and gas content of sea ice with attention to low

- salinities and high temperatures. – Finnish Institute of Marine Research Internal Report 88 (2), Helsinki.
- Leppäranta, M. & Myrberg, K. 2009: Physical oceanography of the Baltic Sea. – Springer, Chichester, 378 pp.
- Leppäranta, M., Reinart, A., Erm, A., Arst, H., Husainov, M. & Sipelgas, L. 2003: Investigation of ice and water properties and under-ice light fields in fresh and brackish water bodies. – *Nordic Hydrol.* 34: 245-266.
- Leu, E., Wiktor, J., Søreide, J. E., Berge, J. & Falk-Petersen, S. 2010: Increased irradiance reduces food quality of sea ice algae. – *Mar. Ecol. Prog. Ser.* 411: 49-60.
- Lindell, M. J., Granéli, W. & Tranvik, L. J. 1995: Enhanced bacterial growth in response to photochemical transformation of dissolved organic matter. – *Limnol. Oceanogr.* 40: 195-199.
- Liu, W. T., Marsh, T. L., Cheng, H. & Forney, L. J. 1997: Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. – *Appl. Environ. Microbiol.* 63: 4516-4522.
- 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. Wiley-Blackwell, Oxford.
- Lizotte, M. P. & Sullivan, C. W. 1991: Photosynthesis-irradiance relationships in microalgae associated with Antarctic pack ice: evidence for in situ activity. – *Mar. Ecol. Prog. Ser.* 71: 175-184.
- Mague, T. H., Friberg, E., Hughes, D. J. & Morris, I. 1980: Extracellular release of carbon by marine phytoplankton; a physiological approach. – *Limnol. Oceanogr.* 25: 262-279.
- Mälkki, P. & Tamsalu, R. 1985: Physical features of the Baltic Sea. – *Finn. Mar. Res.* 252: 1-110.
- Martin, A., Hall, J. A., O'Toole, R., Davy, S. K. & Ryan, K. G. 2008: High single-cell metabolic activity in Antarctic sea ice bacteria. – *Aquat. Microb. Ecol.* 52: 25-31.
- Maske, H. 1984: Daylight ultraviolet radiation and the photoinhibition of phytoplankton carbon uptake. – *J. Plankton Res.* 6: 351-357.
- Maske, H. & Latasa, M. 1997: Solar ultraviolet radiation dependent decrease of particle light absorption and pigments in lake phytoplankton. – *Can. J. Fish. Aquat. Sci.* 54: 697-704.
- McMinn, A., Ashworth, C. & Ryan, K. 1999: Growth and productivity of Antarctic sea ice algae under PAR and UV irradiances. – *Bot. Mar.* 42: 401-407.
- McMinn, A., Hattori, H., Hirawake, T. & Iwamoto, A. 2008: Preliminary investigation of Okhotsk Sea ice algae; taxonomic composition and photosynthetic activity. – *Polar Biol.* 31: 1011-1015.
- 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.
- Meiners, K., Gradinger, R., Fehling, J., Civitarese, G. & Spindler, M. 2003: Vertical distribution of exopolymer particles in sea ice of the Fram Strait (Arctic) during autumn. – *Mar. Ecol. Prog. Ser.* 248: 1-13.
- Menden-Deuer, S. & Lessard, E. J. 2000: Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. – *Limnol. Oceanogr.* 45: 569-579.
- Mengelt, C. & Prézelin, B. B. 2005: UVA enhancement of carbon fixation and resilience to UV inhibition in the genus *Pseudo-nitzschia* may provide a competitive advantage in high UV surface waters. – *Mar. Ecol. Prog. Ser.* 301: 81-93.
- Méthé, B. A., Nelson, K. E., Deming, J. W., Momen, B., Melamud, E., Zhang, X., Moulton, J., Madupu, R., Nelson, W. C., Dodson, R. J., and others 2005: The psychrophilic lifestyle as revealed by the genome sequence of *Colwellia psychrerythraea* 34H through genomic and proteomic analyses. – *Proc. Natl. Acad. Sci. USA* 102: 10913-10918.
- Michel, C., Legendre, L., Demers, S. & Therriault, J. C. 1988: Photoadaptation of sea-ice microalgae in springtime: photosynthesis and carboxylating enzymes. – *Mar. Ecol. Prog. Ser.* 50: 177-185.

- Miyashita, M., Fujimura, S., Nakagawa, Y., Nishizawa, M., Tomizuka, N., Nakagawa, T. & Nakagawa, J. 2010: *Flavobacterium algicola* sp. nov., isolated from marine algae. – Int. J. Syst. Evol. Microbiol. 60: 344-348.
- Moan, J. 2004: Visible light and UV radiation. – 23/02/2011 <http://www.uio.no/studier/emner/matnat/fys/FYS3610/h04/undervisningsmateriale/Moan7.pdf>
- Mock, T., Meiners, K. M. & Gisenhagen, H. C. 1997: Bacteria in sea ice and underlying brackish water at 54° 26'50" 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.
- Moeseneder, M. M., Arrieta, J. M., Muyzer, G., Winter, C. & Herndl, G. J. 1999: Optimization of terminal-restriction fragment length polymorphism analysis for complex marine bacterioplankton communities and comparison with denaturing gradient gel electrophoresis. – Appl. Environ. Microbiol. 65: 3518-3525.
- Moorthi, S., Caron, D. A., Gast, R. J. & Sanders, R. W. 2009: Mixotrophy: a widespread and important ecological strategy for planktonic and sea-ice nanoflagellates in the Ross Sea, Antarctica. – Aquat. Microb. Ecol. 54: 269-277.
- Nielsen, G. Æ. & Bresta, A. M. 1984: Guidelines for the measurement of phytoplankton primary production. – Balt. Mar. Biol. 1: 1-23.
- Norrmann, B. & Andersson, A. 1994: Development of ice biota in a temperate sea area (Gulf of Bothnia). – Polar Biol. 14: 531-537.
- Olenina, I., Hajdu, S., Edler, L., Andersson, A., Wasmund, N., Busch, S., Göbel, J., Gromisz, S., Huseby, S., Huttunen, M., Jaanus, A., Kokkonen, P., Ledaine, I. & Niemkiewicz, E. 2006: Biovolumes and size-classes of phytoplankton in the Baltic Sea. – HELCOM Balt. Sea Environ. Proc. 106, 144 pp.
- Omstedt, A. 1985: An investigation of the crystal structure of sea ice in the Bothnian Bay. – SMHI Report 40, Norrköping.
- Palosuo, E. 1961: Crystal structure of brackish and freshwater ice. – Int. Assoc. Sci. Hydrology 54: 9-14.
- Peak, M. J. & Peak, J. G. 1986: Molecular photobiology of UVA. – In: Urbach, F. & Gange, R. W. (eds.), The biological effects of UVA radiation, pp. 42-53. Praeger Publishers, New York.
- Pérez, M. T., Hörtnagl, P. & Sommaruga, R. 2010: Contrasting ability to take up leucine and thymidine among freshwater bacterial groups: implications for bacterial production measurements. – Environ. Microbiol. 12: 74-82.
- Perovich, D. K. 1993: A theoretical model of ultraviolet light transmission through Antarctic sea ice. – J. Geophys. Res. 98: 22579-22587.
- Perovich, D. K. 1995: Observations of ultraviolet light reflection and transmission by first-year sea ice. – Geophys. Res. Lett. 22: 1349-1352.
- Perovich, D. K. 1996: The optical properties of sea ice. – CRREL Monogr. 96-1: 1-31.
- Perovich, D. K. 1998: The optical properties of sea ice. – In: Leppäranta, M. (ed.), Physics of ice covered seas, vol 1, pp. 195-230. Helsinki University Press, Helsinki.
- Perovich, D. K., Roesler, C. S. & Pegau, W. S. 1998: Variability in Arctic sea ice optical properties. – J. Geophys. Res. 103: 1193-1208.
- Petrich, C. & Eicken, H. 2010: Growth, structure and properties of sea ice. – In: Thomas, D. N. & Dieckmann, G. S. (eds.), Sea ice, 2nd ed., pp. 23-78. Wiley-Blackwell, Oxford.
- Pinhassi, J. & Hagström, Å. 2000: Seasonal succession in marine bacterioplankton. – Aquat. Microb. Ecol. 21: 245-256.
- Pirazzini, R., Vihma, T., Granskog, M. A. & Cheng, B. 2006: Surface albedo measurements over sea ice in the Baltic Sea during the spring snowmelt period. – Ann. Glaciol. 44: 7-14.
- Pizarro, R. A. & Orce, L. V. 1988: Membrane damage and recovery associated with growth delay induced by near-UV radiation in *Escherichia coli* K-12. – Photochem. Photobiol. 47: 391-397.
- Platt, T., Gallegos, C. L. & Harrison, W. G. 1980: Photoinhibition of photosynthesis in natural as-

- semblages of marine phytoplankton. – J. Mar. Res. 38: 687-701.
- Pronk, M., Goldscheider, N. & Zopfi, J. 2009: Microbial communities in karst groundwater and their potential use for biomonitoring. – Hydrogeol. J. 17: 37-48.
- Rahm, L., Håkansson, B., Larsson, P., Fogelqvist, E., Bremle, G. & Valderrama, J. 1995: Nutrient and persistent pollutant deposition on the Bothnian Bay ice and snow fields. – Wat. Air Soil Pollut. 84: 187-201.
- Ramana, C. V. & Sasikala, C. 2009: *Albidoferax*, a new genus of Comamonadaceae and reclassification of *Rhodoferrax ferrireducens* (Finneran et al., 2003) as *Albidoferax ferrireducens* comb. nov. – J. Gen. Appl. Microbiol. 55: 301-304.
- Rasmus, K., Ehn, J., Granskog, M., Kärkäs, E., Lepäranta, M., Lindfors, A., Pelkonen, A., Rasmus, S. & Reinart, A. 2002: Optical measurements of sea ice in the Gulf of Finland. – Nordic Hydrol. 33: 207-226.
- Reardon, C. L., Cummings, D. E., Petzke, L. M., Kinsall, B. L., Watson, D. B., Peyton, B. M. & Geesey, G. G. 2004: Composition and diversity of microbial communities recovered from surrogate minerals incubated in an acidic uranium-contaminated aquifer. – Appl. Environ. Microbiol. 70: 6037-6046.
- Reynolds, C. S. 2006: Ecology of phytoplankton. – Cambridge University Press, Cambridge, 552 pp.
- Rintala, J. M., Piiparinen, J., Ehn, J., Autio, R. & Kuosa, H. 2006: Changes in phytoplankton biomass and nutrient quantities in sea ice as responses to light/dark manipulations during different phases of the Baltic winter 2003. – Hydrobiologia 554: 11-24.
- Roy, S. 2000: Strategies for the minimisation of UV-induced damage. – In: de Mora, S. J., Demers, S. & Vernet, M. (eds.), The effects of UV radiation in the marine environment, pp. 177-205. Cambridge University Press, Cambridge.
- Ryan, K. G. & Beaglehole, D. 1994: Ultraviolet radiation and bottom-ice algae: laboratory and field studies from McMurdo Sound, Antarctica. – In: Weiler, C. S. & Penhale, P. A. (eds.), Ultraviolet radiation and biological research in Antarctica, pp. 229-242. American Geophysical Union, Washington.
- Ryan, K. G., Ralph, P. & McMinn, A. 2004: Acclimation of Antarctic bottom-ice algal communities to lowered salinities during melting. – Polar Biol. 27: 679-686.
- Salcher, M. M., Pernthaler, J., Zeder, M., Psenner, R. & Posch, T. 2008: Spatio-temporal niche separation of planktonic Betaproteobacteria in an oligo-mesotrophic lake. – Environ. Microbiol. 10: 2074-2086.
- Salin, M. L. 1988: Toxic oxygen species and protective systems of the chloroplast. – Physiol. Plant. 72: 681-689.
- Schloss, P. D. & Handelsman, J. 2005: Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. – Appl. Environ. Microbiol. 71: 1501-1506.
- Schofield, O., Kroon, B. M. A. & Prézelin, B. B. 1995: Impact of ultraviolet-B radiation on photosystem II activity and its relationship to the inhibition of carbon fixation rates for Antarctic ice algae communities. – J. Phycol. 31: 703-715.
- Seinä, A. & Peltola, J. 1991: Duration of the ice season and statistics of fast ice thickness along the Finnish coast 1961–1990. – Finn. Mar. Res 258: 1-46.
- SFS 3032, 1976: Determination of ammonia nitrogen of water. – Finnish Standards Association, Helsinki, Finland.
- SFS-EN 1484, 1997: Water analysis. Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC). – Finnish Standards Association, Helsinki
- SFS-EN ISO 13395, 1997: Finnish standard for nitrite and nitrate and their sum with spectrophotometric method and CFA- and FIA-techniques. – Finnish Standards Association, Helsinki
- SFS-EN ISO 11905-1, 1998: Water quality. Determination of nitrogen. Part 1: Method using oxidative digestion with peroxodisulfate. – Finnish Standards Association, Helsinki

- Shaw, A. K., Halpern, A. L., Beeson, K., Tran, B., Venter, J. C. & Martiny, J. B. H. 2008: It's all relative: ranking the diversity of aquatic bacterial communities. – *Environ. Microbiol.* 10: 2200-2210.
- Sherr, B. F., del Giorgio, P. & Sherr, E. B. 1999: Estimating abundance and single-cell characteristics of respiring bacteria via the redox dye CTC. – *Aquat. Microb. Ecol.* 18: 117-131.
- 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.
- Simon, M. 1990: Improved assessment of bacterial production: combined measurements of protein synthesis via leucine and cell multiplication via thymidine incorporation. – *Ergeb. Limnol.* 34: 151-155.
- Smith, R. C., Prezelin, B. B., Baker, K. S., Bidigare, R. R., Boucher, N. P., Coley, T., Karentz, D., MacIntyre, S., Matlick, H. A. & Menzies, D. 1992: Ozone depletion: ultraviolet radiation and phytoplankton biology in Antarctic waters. – *Science* 255: 952-959.
- Sommaruga, R., Obernosterer, I., Herndl, G. J. & Psenner, R. 1997: Inhibitory effect of solar radiation on thymidine and leucine incorporation by freshwater and marine bacterioplankton. – *Appl. Environ. Microbiol.* 63: 4178-4184.
- Srinivas, T. N. R., Nageswara Rao, S. S. S., Vishnu Vardhan Reddy, P., Pratibha, M. S., Sailaja, B., Kavya, B., Hara Kishore, K., Begum, Z., Singh, S. M. & Shivaji, S. 2009: Bacterial diversity and bioprospecting for cold-active lipases, amylases and proteases, from culturable bacteria of Kongsfjorden and Ny-Ålesund, Svalbard, Arctic. – *Curr. Microbiol.* 59: 537-547.
- Staden, R. 1996: The Staden sequence analysis package. – *Mol. Biotechnol.* 5: 233-241.
- Stedmon, C. A., Thomas, D. N., Granskog, M., Kaartokallio, H., Papadimitriou, S. & Kuosa, H. 2007: Characteristics of dissolved organic matter in Baltic coastal sea ice: allochthonous or autochthonous origins? – *Environ. Sci. Technol.* 41: 7273-7279.
- Stemann Nielsen, E. 1952: The use of radioactive carbon (^{14}C) for measuring organic production in the sea. – *J. Cons. Int. Explor. Mer* 18: 117-140.
- Steffens, M., Granskog, M. A., Kaartokallio, H., Kuosa, H., Luodekari, K., Papadimitriou, S. & Thomas, D. N. 2006: Spatial variation of biogeochemical properties of landfast sea ice in the Gulf of Bothnia, Baltic Sea. – *Ann. Glaciol.* 44: 80-87.
- Tamminen, T. & Andersen, T. 2007: Seasonal phytoplankton nutrient limitation patterns as revealed by bioassays over Baltic Sea gradients of salinity and eutrophication. – *Mar. Ecol. Prog. Ser.* 340: 121-138.
- Thomas, D. N., Papadimitriou, S. & Michel, C. 2010: Biogeochemistry of sea ice. – In: Thomas, D. N. & Dieckmann, G. S. (eds.), *Sea ice*, 2nd ed., pp. 425-467. Wiley-Blackwell, Oxford.
- Utermöhl, H. 1958: Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. – *Mitt. Int. Ver. Theor. Angew. Limnol.* 9: 1-38.
- Uusikivi, J., Granskog, M. A. & Sonninen, E. 2011: Meteoric ice contribution and influence of weather on landfast ice growth in the Gulf of Finland, Baltic Sea. – *Ann. Glaciol.* 52: 57-91.
- Uusikivi, J., Vähätalo, A. V., Granskog, M. A. & Sommaruga, R. 2010: Contribution of mycosporine-like amino acids and colored dissolved and particulate matter to sea ice optical properties and ultraviolet attenuation. – *Limnol. Oceanogr.* 55: 703-713.
- Vähätalo, A. V., Salkinoja-Salonen, M., Taalas, P. & Salonen, K. 2000: Spectrum of the quantum yield for photochemical mineralization of dissolved organic carbon in a humic lake. – *Limnol. Oceanogr.* 45: 664-676.
- Van Trappen, S., Mergaert, J. & Swings, J. 2004a: *Loktanella salsilacus* gen. nov., sp. nov., *Loktanella fryxellensis* sp. nov. and *Loktanella vestfoldensis* sp. nov., new members of the *Rhodobacter* group, isolated from microbial mats in Antarctic lakes. – *Int. J. Syst. Evol. Microbiol.* 54: 1263-1269.
- Van Trappen, S., Mergaert, J., Van Eygen, S., Dawyndt, P., Cnockaert, M. C. & Swings, J.

- 2002: Diversity of 746 heterotrophic bacteria isolated from microbial mats from ten Antarctic lakes. – *Syst. Appl. Microbiol.* 25: 603-610.
- Van Trappen, S., Vandecandelaere, I., Mergaert, J. & Swings, J. 2004b: *Flavobacterium degerlachei* sp. nov., *Flavobacterium frigoris* sp. nov. and *Flavobacterium micromati* sp. nov., novel psychrophilic bacteria isolated from microbial mats in Antarctic lakes. – *Int. J. Syst. Evol. Microbiol.* 54: 85-92.
- Vernet, M. 2000: Effects of UV radiation on the physiology and ecology of marine phytoplankton. – In: de Mora, S. J., Demers, S. & Vernet, M. (eds.), *The effects of UV radiation in the marine environment*, pp. 237-278. Cambridge University Press, Cambridge.
- Villafañe, V. E., Buma, A. G. J., Boelen, P. & Helbling, E. W. 2004: Solar UVR-induced DNA damage and inhibition of photosynthesis in phytoplankton from Andean lakes of Argentina. – *Arch. Hydrobiol.* 161: 245-266.
- Villafañe, V. E., Helbling, E. W., Holm-Hansen, O. & Chalker, B. E. 1995: Acclimatization of Antarctic natural phytoplankton assemblages when exposed to solar ultraviolet radiation. – *J. Plankton Res.* 17: 2295-2306.
- Villafañe, V. E., Sundbäck, K., Figueroa, F. L. & Helbling, E. W. 2003: Photosynthesis in the aquatic environment as affected by UVR. – In: Helbling, E. W. & Zagarese, H. (eds.), *UV effects in aquatic organisms and ecosystems*, pp. 357-397. Royal Society of Chemistry, Cambridge.
- Vincent, W. F. & Neale, P. J. 2000: Mechanisms of UV damage to aquatic organisms. – In: de Mora, S. J., Demers, S. & Vernet, M. (eds.), *The effects of UV radiation in the marine environment*, pp. 149-176. Cambridge University Press, Cambridge.
- Ward Jr, J. H. 1963: Hierarchical grouping to optimize an objective function. – *J. Am. Stat. Assoc.* 58: 236-244.
- Weeks, W. F., Gow, A. J., Kosloff, P. & Digby-Argus, S. 1990: The internal structure, composition and properties of brackish ice from the Bay of Bothnia. – *CRREL Monogr.* 90-1: 5-15.
- 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.
- Wetzel, R. G. 2003: Solar radiation as an ecosystem modulator. – In: Helbling, E. W. & Zagarese, H. (eds.), *UV effects in aquatic organisms and ecosystems*, pp. 3-18. Royal Society of Chemistry, Cambridge.
- Yu, Y., Li, H., Zeng, Y. & Chen, B. 2010: Phylogenetic diversity of culturable bacteria from Antarctic sandy intertidal sediments. – *Polar Biol.* 33: 869-875.

