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Ice formation, growth and associated substrate supply determine sea-ice bacterial community dynamics

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- II** **Eronen-Rasimus, E.**, Lyra, C., Rintala, J.-M., Jürgens, K., Ikonen, V. and Kaartokallio, H. Ice formation and growth shape bacterial community structure in Baltic Sea drift ice. *FEMS Microbiology Ecology* 91: 1–9. **
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ICE FORMATION, GROWTH AND ASSOCIATED SUBSTRATE SUPPLY DETERMINE SEA-ICE BACTERIAL COMMUNITY DYNAMICS

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Sea ice, at its maximum extent, is one of the largest biomes on Earth. In addition to the polar oceans, it covers extensive sea areas at lower latitudes such as the Baltic Sea and the Sea of Okhotsk. During ice formation, organic and inorganic components in the parent seawater are concentrated into saline brines within the ice, which serve as a habitat for diverse auto- and heterotrophic organisms, including bacteria. Sea-ice bacteria are responsible for many biogeochemical processes, such as decomposition of particulate organic matter, recycling of dissolved organic matter and remineralization of nutrients, analogously to bacterially driven biogeochemical processes in the water column. Since bacterial groups vary by their metabolic traits and participation in biogeochemical processes, knowledge of the bacterial community structure and its seasonal variation is essential for an understanding of ice biogeochemistry.

This thesis characterises sea-ice bacterial communities during ice formation and during the winter/spring transition phase when the community composition is poorly known. Bacterial communities in Arctic and Baltic sea ice during the winter/spring transition were studied and compared. In addition, the effect of the dissolved organic matter regime on bacterial community formation was investigated in an experimental sea-ice system with North Sea water. The main methods applied were terminal-restriction fragment length polymorphism and/or Illumina Miseq sequencing together with bacterial production and abundance measurements.

During the early stages of sea-ice formation, the bacterial communities were similar to the parent water communities, suggesting that the parent water determines the initial sea-ice bacterial community composition. After congelment of the sea ice, the bacterial communities changed towards communities typical of sea ice in spring. During the winter/spring transition, members of the classes Flavobacteriia (formerly Flavobacteria), Gammaproteobacteria and Alphaproteobacteria were predominant both in Baltic and Arctic sea ice. The Baltic and Arctic sea-ice bacterial communities were significantly different; however, a few members of common sea-ice bacterial genera, such as *Polaribacter* and *Shewanella*, were closely related, pointing to similar selection in ice, regardless of differences in the prevailing environmental conditions.

In the experimental system, the bacterial communities were able to respond to altered substrate availability immediately after ice formation. This indicates successful adaptation of sea-ice bacteria to major shifts in temperature and salinity during ice formation. The results of this thesis suggest that sea-ice bacterial community formation and dynamics is

defined by a combination of changes in environmental conditions during sea-ice maturation and its associated substrate availability, as well as resource competition. The sea-ice habitat provides an example of the enormous capacity of bacteria to adapt to changing environments and how minor members of the bacterial community can become predominant when environmental conditions change.

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1. INTRODUCTION

1.1. Sea ice

Sea ice, at its maximum extent, is one of the largest biomes on Earth (Thomas and Dieckmann 2002). In addition to the polar oceans, sea ice covers large sea areas in sub-polar regions, such as the Baltic Sea and the Sea of Okhotsk (Granskog et al. 2010). Sea ice is not only a passive lid on top of the sea, but also an important component in Earth's climate and oceanic systems. Sea ice alters the energy flow between the atmosphere and sea, limits gas exchange and influences the transport of atmospheric deposition into the sea. In addition, it is an important habitat for diverse organisms from auto- and heterotrophic lineages (Mock and Thomas 2005; Arrigo et al. 2010; Caron and Gast 2010; Deming 2010) from bacteria to seals and polar bears. Sea-ice bacteria actively grow in ice and participate in biogeochemical cycles, especially in the spring and summer seasons. Since sea-ice-dwelling bacterial groups vary in their metabolic capabilities, understanding the bacterial community dynamics in sea ice is essential for assessing their effect on atmospheric systems (respiration) and nutrient and biomass fluxes (bacterial growth) to the underlying waters. Understanding these bacterially mediated processes will also aid in evaluating the possible future consequences of diminishing sea-ice cover for the biogeochemical cycling and food webs of the ice-covered seas.

It is crucial to understand physical processes in sea ice, since they determine the immediate environment of sea-ice organisms, e.g. habitable space, salinity, temperature and nutrient supply. In addition, the knowledge of these physical conditions will aid in better understanding of bacterial

community dynamics. Freezing temperature and initiation of ice formation are dependent on the salinity of the parent water. At a salinity of 5 ‰ (usually measured on a unitless practical salinity scale; however since it is essentially equal to ‰, it is used hereafter in this thesis; UNESCO, 1981), such as in the northern Baltic Sea, freezing begins approximately at $-0.3\text{ }^{\circ}\text{C}$, whereas at an oceanic salinity of 34 ‰ freezing begins at $-1.86\text{ }^{\circ}\text{C}$. When salinities are over 0.6 ‰, the ice formed has characteristics of sea ice, e.g. vertical orientation of ice crystals, allowing brine channel formation (Palosuo 1961). When sea ice forms, ice crystals aggregate on the surface of the water column, eventually congealing into a continuous ice sheet. Bacteria apparently become entrained in sea ice via phytoplankton (Grossmann and Gleitz 1993; Grossmann 1994; Grossmann and Dieckmann 1994; Helmke and Weyland 1995; Weissenberger and Grossmann 1998; Riedel et al. 2007b) or augmentation by gas vacuoles (Staley and Gosink 1999), ice-binding proteins (Raymond et al. 2007) and extracellular polymeric substances (EPS; Ewert and Deming 2011, 2013).

Under calm conditions, ice grows downwards, i.e. congelation growth, whereas under turbulent conditions, ice crystals aggregate first into centimetre-sized floes of ice that in turn accrete into decimetre-sized round ice discs. These pancake-ice floes (Figure 1) bump and grind against one another by the action of waves and ocean swell. Eventually, the pancakes adhere to each other, finally congealing into a continuous ice sheet. During dynamic growth, ice floes can slide over each other, resulting in rafted ice, as well as pile against each other, forming pack-ice fields. Sea-ice growth in the Arctic Ocean is dominated by congelation growth. In the open areas of the Baltic Sea, dynamic ice



Photo by: Anne-Mari Luhtanen

Fig. 1. Pancake ice in the Weddell Sea, Antarctica

growth conditions prevail, and the pancake ice cycle is important, similar to conditions in Antarctic waters (Lange et al. 1989; Granskog et al. 2006). During ice growth, only a small portion of the salt is retained in the ice, whereas a large fraction is rejected into the underlying water column (Petrich and Eicken 2010). The dissolved organic and inorganic constituents are concentrated into brines that form complex channels and pores at the ice-crystal junctions. The brine channels and isolated brine pockets serve as primary habitat for sea-ice organisms (Figure 2).

Due to the lower salinity in the Baltic Sea, the habitable space in the brine is smaller than in the Arctic Ocean, where the ice salinity and brine channel volume are

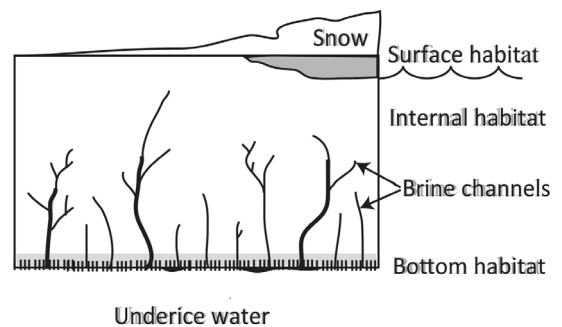


Fig. 2. Schematic illustration of sea ice habitats. Re-drawn after Arrigo (2003).

larger and 80 % of the brine channels are interconnected (Weissenberger et al. 1992). The brine salinity is inversely proportional to the temperature, whereas the volume is

directly proportional to the temperature, i.e. when the temperature decreases, the brine volume decreases and salinity increases. In polar sea ice, the brine salinity can be as high as 216 ‰ at -21 °C (Eicken et al. 2000). In the Arctic Ocean, bulk salinities (measured from melted ice) decrease during seasonal sea-ice development from 8–10 ‰ when freezing begins to approximately 5 ‰ in spring and finally 0–3 ‰ in late summer (Malmgren 1927; Petrich and Eicken 2010). A characteristic C-shaped salinity profile, with higher salinity in the upper and lower ice, is observed in young ice until melt water flushing in late summer (Malmgren 1927; Petrich and Eicken 2010). In the Baltic Sea,

the typical C-shaped appearance encountered in polar regions is lacking, because the bulk salinities are generally less than 2 ‰ (Granskog et al. 2006). Sea-ice salinity is also affected during sea-ice development by desalination processes, such as brine diffusion, brine expulsion, gravity drainage, and flushing with surface meltwater. However, sea ice can also obtain more salt by flooding and intrusion of underlying seawater into the ice column (Petrich and Eicken 2003). These processes are important, since they bring more nutrients to sea-ice microorganisms (Gradinger et al. 1992). Brine movement is linked to the interconnectivity between brine channels and pores that is determined by



Fig. 3. Frost flower on a sea-ice mesocosm (II).

permeability, i.e. the reciprocal resistivity of fluid in sea ice. According to the *Law of Fives* (Golden et al. 1998), sea ice is considered to be permeable when the temperature is above -5 °C at a bulk salinity of 5 ‰ and brine volume of 5 %. In the Baltic Sea, the bulk salinity rarely reaches 5 ‰; however, at -1 °C and a bulk salinity of 1 ‰, the brine volumes are large enough for ice to become permeable (Leppäranta and Manninen 1988; Granskog et al. 2006).

In addition to the internal habitats, microorganisms also occur on the ice surface and bottom (Figure 2). One relatively recently recognized surface habitat is the frost flower layer on newly formed ice. Frost flowers (Figure 3) are short-lived ice-crystal clusters that form almost exclusively on newly formed or young sea ice under cold and calm conditions, either from atmospheric deposition of water vapour onto the ice or when warm ice is sublimated or evaporated into a cold atmospheric boundary layer (Perovich and Richter-Menge 1994; Style and Worster 2009; Barber et al. 2014). The brine in frost flowers originates directly from sea-ice brine as it is expelled upward from the ice to the frost flowers or brine-wetted surface ice (Perovich and Richter-Menge 1994; Style and Worster 2009; Barber et al. 2014). The maximum bulk salinities in frost flowers are approximately 120 ‰, with modelled brine salinities as high as 200 ‰ at -10°C (Barber et al. 2014). Frost flowers can have three- to sixfold higher bacterial abundances than in the underlying sea ice (Bowman and Deming 2010), in which the bulk ice bacterial abundance ranges from 3.6×10^3 cells mL⁻¹ in cold Arctic winter ice (Collins et al. 2008) to 3×10^7 cells mL⁻¹ measured during ice algal mass growth (Smith et al. 1989; Thomas et al. 2001).

1.2. Abiotic and biotic factors influencing sea-ice bacterial community dynamics

Of the diverse auto- and heterotrophic microorganisms observed in sea ice (Arrigo et al. 2010; Mock and Thomas 2005; Caron and Gast 2010; Deming 2010), bacteria and microscopic ice algae are the most important groups in terms of biomass and production. Similar to that observed in open water, bacteria in ice drive a microbial loop, in which dissolved organic matter (DOM) is recycled back to the upper trophic levels as bacterial biomass. Sea-ice bacteria are also responsible for other essential biogeochemical functions, including decomposition of particulate organic matter and remineralization of nutrients.

Sea-ice bacterial community structure and activity are governed by abiotic and biotic factors, such as salinity, temperature, nutrients, light, protozoan grazing and viral lysis (Pomeroy and Wiebe 2001; Kaartokallio 2004; Kuosa and Kaartokallio 2006; Riedel et al. 2007a; Collins et al. 2008, 2010; Piiparinen and Kuosa 2011). Key factors likely driving growth and structure of bacterial communities are substrate supply and availability of multiple attachment sites including EPS, particles, brine channel walls and protist communities (Kottmeier et al. 1987; Helmke and Weyland 1995; Bowman et al. 1997a; Junge et al. 2002, 2004). Sea-ice viruses, presumably infecting both protists and bacteria, occur in sea ice from autumn (Gowing et al. 2002; Collins et al. 2011) through winter (Wells and Deming 2006) to mass growth of algae in spring (Maranger et al. 1994; Gowing 2003; Gowing et al. 2004). Bacterial mortality during winter may in part be virally mediated (Collins et al. 2008, 2010). However, the ecological

significance of viruses in sea ice remains to be determined.

1.2.1. Dissolved organic matter in sea ice

Bacterial activity in sea ice is associated with concentrated biological assemblages and high concentrations of DOM, which serves as energy and carbon sources for heterotrophic bacteria (Thomas et al. 2001; Junge et al. 2004; Mock and Thomas 2005; Riedel et al. 2008). Dissolved Organic Carbon (DOC) concentration can be as high as 23 300 $\mu\text{mol L}^{-1}$ in brine (Thomas et al. 2001). DOM is a mixture of labile and refractory compounds, including carbohydrates, amino acids, proteins and humic substances (Amon et al. 2001; Thomas et al. 2001; Thomas et al. 2010) of which a large proportion is derived from algal and bacterial EPS (Riedel et al. 2006, 2008; Collins et al., 2008; Ewert and Deming 2011, 2013; Underwood et al. 2010, 2013; Krembs et al. 2011; Aslam et al. 2012). In addition to EPS, DOM in Arctic and Baltic sea ice also contains allochthonous components introduced by riverine inputs from terrestrial systems (Dittmar and Kattner 2003; Stedmon et al. 2007; Deutsch et al. 2012; Hoikkala et al. 2015). During sea-ice formation, DOM is concentrated into brines (Müller et al. 2011, 2013), and its average molecular size is reduced while it becomes more bioavailable (Müller et al. 2011, 2013; Jørgensen et al. 2015).

1.3. Open-water bacterial communities in the Arctic Ocean and Baltic Sea

Sea-ice bacterial communities are recruited from open water. Thus, knowledge of the open-water communities is needed to understand

the development of sea-ice bacterial communities. In general, oligotrophic bacteria, i.e. those adapted to grow best at low nutrient concentrations, such as the SAR11 clade of Alphaproteobacteria, dominate the ocean surface-water bacterioplankton worldwide (Morris et al. 2002). In association with elevated nutrient concentrations, e.g. during and after algal blooms, the abundance of copiotrophic bacteria, i.e. those adapted to grow best in nutrient-rich environments, belonging to the phylum Bacteroidetes, and classes Gammaproteobacteria and Alphaproteobacteria increases (Teeling et al. 2012). The substrate properties likely dictate the marine bacterial group dynamics (Teeling et al. 2012), since Gamma- and Alphaproteobacteria are mainly affiliated with labile low-molecular-weight (LMW) substances (Cottrell and Kirchman 2000; Pinhassi and Berman 2003; Elifantz et al. 2005; Malmström et al. 2005; Alonso-Saez and Gasol 2007; Teira et al. 2008, 2010; Gomez-Consarnau et al. 2012; Alonso-Saez et al. 2012; Teeling et al. 2012), whereas Bacteroidetes (e.g. Flavobacteriia) are able to decompose high-molecular-weight substances (Kirchman 2002; Elifantz et al. 2005, 2007; Alonso-Saez et al. 2012).

The Baltic Sea is a semi-enclosed brackish water basin characterized by its surface-water salinity gradient in a north-south direction (mean 7 ‰, range 0–26 ‰; Leppäranta and Myrberg 2009). The brackish nature is reflected in the bacterial communities, since it consists of both freshwater (e.g. Betaproteobacteria, Actinobacteria and Verrucomicrobia) and marine bacterial clades (Alphaproteobacteria, Bacteroidetes, Gammaproteobacteria) adapted to the prevailing low-saline conditions (Hagström et al. 2000; Pinhassi and Hagström 2000; Riemann et al. 2008; Andersson et al. 2010;

Herlemann et al. 2011; Laas et al. 2014). The Baltic Sea bacterial communities vary seasonally (Pinhassi and Hagström 2000; Riemann et al. 2008; Andersson et al. 2010) and Alphaproteobacteria and Actinobacteria (Laas et al. 2014) dominate the winter surface water bacterial communities in the Gulf of Finland.

The Arctic Ocean is the smallest, shallowest and least saline sea of the world's five oceanic divisions. Members of the classes Alphaproteobacteria, Gammaproteobacteria and phylum Bacteroidetes dominate the open-water bacterial communities in the Arctic Ocean during winter (Bano and Hollibaugh 2002; Alonso-Sáez et al. 2008; Kirchman et al. 2010) of which the oligotrophic Alphaproteobacteria SAR11 is the most abundant group. In addition to the predominant groups, there are less abundant groups such as Deltaproteobacteria, Verrucomicrobia and Betaproteobacteria (Bano and Hollibaugh 2002; Alonso-Sáez et al. 2008). Overall, seasonal variability in the Arctic Ocean bacterial communities seems to be limited (Kirchman et al. 2010).

In general, wintertime bacterial communities in the water column are low-productive in both the Arctic Ocean (Garneau et al. 2008; Seuthe et al. 2011) and the Baltic Sea (Väättänen 1981). However, bacteria in the Arctic Ocean are heterotrophically active throughout the winter, and the highest activities are observed in less abundant groups, such as Roseobacter (Alphaproteobacteria), Betaproteobacteria and Gammaproteobacteria compared with the most abundant SAR11 (Alphaproteobacteria) (Alonso-Sáez et al. 2008).

1.4. Development of sea-ice bacterial communities

In newly formed sea ice, bacterial activity is first suppressed and then restored after consolidation of the sea ice (Grossmann and Gleitz 1993; Grossmann 1994; Grossmann and Dieckmann 1994; Helmke and Weyland 1995; Kaartokallio 2004; Kaartokallio et al. 2008). Along the ice consolidation, psychrophilic bacteria (growth min. ≤ 0 °C, optimum ≤ 15 °C, max. 20 °C; Morita 1975) become more abundant than psychrotolerant bacteria (max. 25 °C, ability to grow at low temperatures; Junge et al. 2011; Helmke and Weyland 1995). However, the community composition at the early stages of sea-ice formation is not known except for frost flowers on top of the new ice. Based on the most recent finding, the frost flower bacterial community is very similar to that of the slush layer above the ice and is dominated by SAR11 Alphaproteobacteria, similar to communities in the underlying waters (Barber et al., 2014).

In Arctic sea ice, oligotrophic Alpha- and Gammaproteobacteria dominate the community throughout the winter (Collins et al. 2010). Since the upper section of cold winter sea ice and the underlying water bacterial communities are similar, selection during freezing appears to play a minor role in shaping the bacterial community (Collins et al. 2010). However, the phylum Bacteroidetes may dominate the sea ice bacterial communities during late winter (Junge et al. 2004). Members of the Bacteroidetes apparently favour cold temperatures and particles, which serve as a substrate for these high-molecular-weight organic matter-utilizing bacteria (Junge et al. 2004).

In the brackish Baltic Sea, copiotrophic bacteria predominate throughout the winter: Alphaproteobacteria (family Rhodobacteraceae) in early winter and Bacteroidetes in late winter and early spring (Kaartokallio et al. 2008). The predominance of copiotrophic bacteria is likely related to the higher ambient DOM concentrations in the Baltic Sea than in Arctic waters (Kuparinen et al. 2011) and/or the minor sea-ice algal ‘blooms’ that may occur before the main mass growth in spring (Kaartokallio et al. 2008).

In spring, after increasing solar radiation induces a mass growth of sea-ice algae that provide ample supplies of autochthonic substrates for bacteria, copiotrophic bacteria begin to dominate the bacterial community both in polar and Baltic Sea ice. Commonly, the most predominant classes in the spring bacterial communities at both poles and in the Baltic Sea are Gammaproteobacteria (e.g. genera *Colwellia*, *Shewanella*, *Psychrobacter* and *Glaciecola*), Flavobacteriia (e.g. genera *Polaribacter* and *Flavobacterium*) and Alphaproteobacteria (e.g. genus *Octadecabacter*; Bowman et al. 1997b; Brown and Bowman 2001; Junge et al. 2002; Brinkmeyer et al. 2003; Kaartokallio et al. 2008; Deming 2010; Bowman et al. 2012). In addition to the dominant classes, less abundant bacterial groups belonging to Actinobacteria, Betaproteobacteria, Planctomycetes and Verrucomicrobia occur regularly in sea ice and/or melt ponds in polar and Baltic sea ice (Bowman et al. 1997a, b; Junge et al. 1998; Brown and Bowman 2001; Brinkmeyer et al. 2003, 2004; Kaartokallio et al. 2008; Collins et al. 2010; Piiparinen 2011; Bowman et al. 2012; Hatam et al. 2014). Since some of these groups are common in freshwater and terrestrial habitats, e.g. Betaproteobacteria and Actinobacteria, they

may have originated from riverine inputs (Brinkmeyer et al. 2003; Kaartokallio et al. 2008; Collins et al. 2010).

In addition to bacteria, Archaea form a minor but persistent part of prokaryotic communities throughout the winter in Arctic sea ice (fluorescent in situ hybridization (FISH) < 3.4 %, Junge et al. 2004; Collins et al. 2010). However, their abundance further decreases in spring/summer (FISH < 1 %, Brinkmeyer et al. 2003), analogously to their occurrence in Arctic waters (Alonso-Saez et al. 2008). Sea-ice Archaea belong to Thaumarchaeota (formerly Marine Group I Crenarchaeota), which is the dominant group in the Arctic Ocean (Galand et al. 2009), and Marine Group II Euryarchaeota (Collins et al. 2010).

Previously published work on bacterial communities in the Arctic has focused on fast ice (Junge et al. 2004; Collins et al. 2010) and multiyear ice (Brinkmeyer et al. 2003; Bowman et al. 2012; Hatam et al. 2014), whereas first-year drift ice has been studied only once (Junge et al. 2002). Seasonally, previous studies were confined to the winter and summer periods but the transition phase from winter to spring is largely unknown. Few reports are available on the bacterial community dynamics in coastal fast ice in the Baltic Sea, (Kaartokallio et al. 2008; Piiparinen 2011), but knowledge of drift-ice bacterial communities is completely lacking.

2. AIMS OF THE THESIS

This thesis is based on three Articles (I–III), focusing on different stages of sea ice bacterial community development, and an additional meta-analysis (IV) that combines bacterial diversity data [16S rRNA gene sequences and terminal-restriction

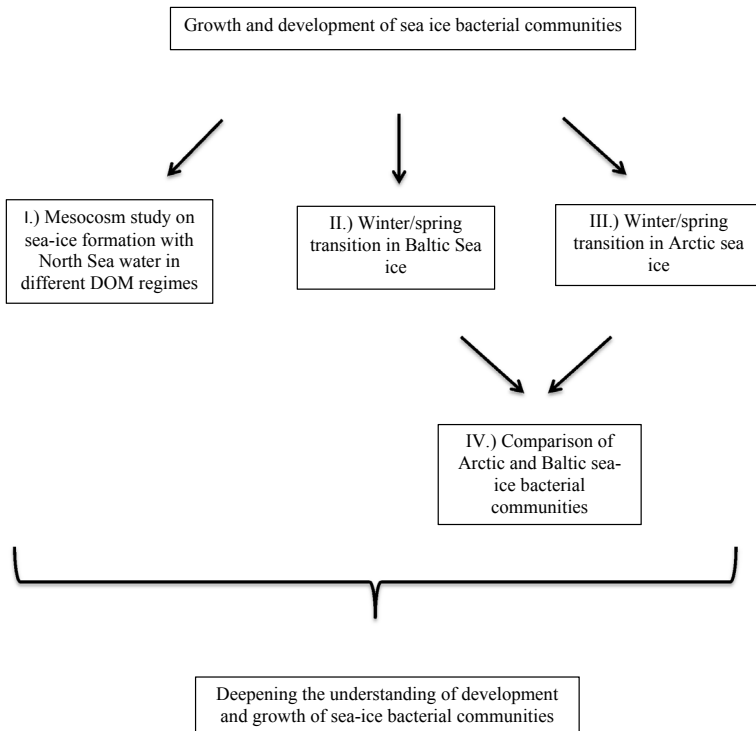


Fig. 4. Schematic flow chart of the aims of the thesis.

fragments (T-RFs)] from Articles II and III. Since sea-ice bacterial groups vary in their metabolic capabilities, understanding of bacterial community dynamics in the sea ice is needed to fully understand their part in the gas fluxes (respiration) and fluxes of nutrients and carbon (bacterial growth) to the underlying waters.

Sea-ice bacterial communities in the early stages of freezing have low productivity. However, bacterial production increases in the weeks after freezing begins, and psychrophilic bacteria become more abundant during sea-ice growth (Grossmann and Gleitz 1993; Grossmann 1994; Grossmann and Dieckmann 1994; Helmke and Weyland 1995; Kaartokallio 2004; Kaartokallio et

al. 2008). In the Arctic fast ice bacterial communities remain unchanged during winter and are dominated by oligotrophic bacteria, whereas the spring and summer communities are more active and dominated by copiotrophic bacteria (Bowman et al. 1997b; Brown and Bowman 2001; Junge et al. 2002; Brinkmeyer et al. 2003; Collins et al. 2010; Deming 2010; Bowman et al. 2012). In contrast to the Arctic, the Baltic Sea fast-ice communities already begin to change a month after freezing (Kaartokallio et al. 2008). In addition, there is evidence that the nutritional status of the parent water may affect bacterial communities (Helmke and Weyland 1995).

A schematic workflow of this thesis is shown in Figure 4. The specific aims of the Articles (I-III) and meta-analysis were:

Article I) Bacterial community dynamics and activity in relation to dissolved organic matter availability during sea-ice formation in a mesocosm experiment

In Article I, the aim was to examine the sea-ice bacterial community dynamics as the community formed and to evaluate the effect of DOM content of the parent water on the developing communities in a sea-ice mesocosms with North Sea water (Figure 5). An additional aim was to determine whether the bacterial communities formed during freezing reflected those of the parent water. The bacterial communities were studied with the 16S rRNA gene T-RFLP and cloning, together with bacterial abundance and production measurements.



Fig. 5. Sea-ice mesocosms in the Hamburg Ship Model Basin (HSVA), Germany (II).

Article II) Ice formation and growth shape bacterial community structure in Baltic Sea drift ice

In Article II, the aim was to examine the bacterial community structure in various developmental stages of drift ice, from open water to consolidated sea ice, in the Gulf of Bothnia, Baltic Sea during the winter/spring transition. The bacterial communities were studied with the same methods as in Article I.

Article III) Bacterial communities in Arctic first-year drift ice during the winter/spring transition

In Article III, the aim was to examine the bacterial communities in Arctic first-year drift ice to capture the spatial and vertical variability in the ice column during the winter/spring transition phase, when the community composition is less well known. Samples were collected along a north-south transect in the western Fram Strait, which is the main passage to sea-ice outflow from the Arctic Ocean (Serreze et al. 2007; Hansen et al. 2013). The transect allowed us to potentially capture ice floes with different developmental stages from more colder winter-type ice to warmer and more permeable spring-type ice. The bacterial communities were studied with both T-RFLP and MiSeq sequencing techniques together with bacterial production measurements.

Meta-analysis IV) Comparison of Arctic and Baltic drift ice communities

In addition to Articles I–III, meta-analysis was done with the sequence and T-RF data derived from the Articles II and III. The aim was to determine whether the Arctic and Baltic sea-ice bacterial communities differed

from each other during the winter/spring transition and to evaluate the phylogenetic relationships between the most common sea-ice bacterial classes.

The hypotheses of this thesis were:

1. bacterial communities are limited by physical factors when freezing begins and they need time to adapt afterwards (I, II)
2. bacterial community dynamics in the consolidated ice communities are mainly controlled by sea-ice algal growth and adjacent fresh DOM supplies (II, III).
3. bacterial communities in Arctic and Baltic sea ice are different, with similarities only at higher phylogenetic levels (IV).

3. MATERIALS AND METHODS

3.1. Study area

3.1.1. *The Baltic Sea*

The Baltic Sea is one of the Earth's largest brackish water basins, characterized by its vertical and surface-water salinity gradient (surface salinities: Bay of Bothnia: 2–4 ‰, Sea of Bothnia: 5–6 ‰, Bornholm basin: 7.3–8.5 ‰, Kattegat: 18–6 ‰; Leppäranta and Myrberg 2009). The brackish nature of the Baltic Sea is maintained by a combination of restricted water exchange with the North Sea through the Danish Straits, a large catchment area with high freshwater input and small water volume (mean depth 54 m). Rivers transport DOM from terrestrial sources (Deutsch et al. 2012; Hoikkala et al. 2015) which is also incorporated into the sea ice (Stedmon et al. 2007).

The Baltic Sea is annually covered by sea ice that lasts on average from October/November to May/June. During an average winter approximately 45 % (range 12.5 %–100 %) of the Baltic Sea is ice-covered between mid-February and mid-March. However, in the recent last decade the coverage of ice cover has decreased dramatically (Leppäranta and Myrberg 2009). Samples for Article II were collected aboard research vessel Maria S Merian in February–March 2006, in the Gulf of Bothnia, the northernmost part of the Baltic Sea, before the ice algal mass growth (Figure 6).

3.1.2. *The Arctic Ocean*

The Arctic Ocean is a land-locked polar sea and, like the Baltic Sea, it is characterized by high freshwater inputs, with Arctic rivers contributing almost 10 % of the global river discharge (Thomas et al. 2010). Due to the high freshwater input, Arctic Ocean surface salinities are lower (approximately 30 ‰) than in other marine systems. River waters transport nutrients, DOM and particulate matter (Dittmar and Kattner 2003) that are incorporated into sea ice forming over coastal areas and shelves. A large proportion of this organic material is biologically refractory (Amon and Benner 2003) and thus not available for bacterial growth.

In contrast to the Baltic Sea, perennial (multiyear) sea ice exists in the Arctic Ocean. However, the proportion of multiyear ice is decreasing (Serreze et al. 2007; Perovich et al. 2014). The ice extent in the Arctic Ocean varies from a maximum (16×10^6 km²) in March to a minimum extent (7×10^6 km²) in September (Serreze et al. 2007). The Arctic Ocean is connected to the global oceans by three topographic depressions,



Fig. 6. Map of the study sites in the Arctic Ocean (III, Figure 1) and Baltic Sea (II, Figure 1). Map of the earth courtesy of Majaneva, (2014).

of which the Fram Strait is the deepest and most significant. The Fram Strait is located between Greenland and Svalbard and is a mixing point for the East Greenland Current which brings cold Arctic waters to the south and the West Spitsbergen Current which brings warm Atlantic waters north. In addition, the Fram Strait is the main route for sea-ice outflow in spring from the Arctic Ocean (Serreze et al. 2007; Hansen et al., 2013). For Article III, samples were collected from the Fram Strait (Figure 6) aboard the Norwegian Coast Guard icebreaker Svalbard in May 2008 in the East Greenland Current, denoting that the stations provided data from late winter/early spring conditions.

3.2. Summary of the methods (I-III)

The methods used in this study are presented in Table 1 and described in detail in the Articles (I-III).

3.3. Meta-analysis (IV): Comparison of Arctic and Baltic sea-ice bacterial communities

BsuRI restricted T-RF data from Articles II and III were combined to compare the bacterial communities in Arctic and Baltic sea ice. The T-RFs were cleared of background noise and normalized as

Table 1. Summary of the methods used in Articles I–III. The numbers denote 1= first author, 2= other authors and 3=external service, - not used in article.

Parameter	Method/Analyses	I	II	III
Bacterial communities	DNA extraction	3	1	1
	PCR for T-RFLP and cloning	1	1	1
	T-RFLP	3	3	3
	MiSeq (incl. PCR)	-	-	3
Statistics	Principal coordinate analysis	1	-	1
	Generalized discriminate analysis	1	1	1
	PERMANOVA	-	-	1
	Kruscal-Wallis and Wilcoxon rank sum test	2	-	-
Bioinformatics	Mothur	1	1	1
	UPARSE pipeline	-	-	1, 2
Phylogeny	Neighbor-joining tree of 16S rRNA genes	1	1	1
Bacterial production	Thymidine (TdR) and/or Leucine (Leu) incorporation method	2	2	2
Bacterial abundance	Flow cytometry	2	-	-
	Acridine orange staining	-	2	-
Environmental parameters	Temperature, salinity, brine volume and chlorophyll-a	-	-	2

described in Articles II and III. To visualize the differences between the Arctic and Baltic sea-ice bacterial communities, principal coordinate (PCO) analysis was performed ($n = 49$).

To determine whether bacterial communities differed significantly in Arctic and Baltic sea ice (fixed factor; Arctic: $n = 22$, Baltic: $n = 27$), a one-way permutational multivariate analysis of variance (PERMANOVA; Anderson 2001; McArdle and Anderson 2001) was performed. Since the data were unbalanced, type III sums of squares was used. A total of 9999 permutations, using unrestricted permutation of raw data (Manly 1997) were performed, which is recommended for one-way designs and relatively small sample sizes (Anderson et al. 2008). The homogeneity of dispersion was tested with permutational analysis of dispersion (PERMDISP, Anderson 2006), using the distance to the centroids. Homogeneity of dispersion is an assumption in PERMANOVA and thus needed to discriminate whether the variation in the bacterial communities is explained by location, dispersion or both. For the multivariate analysis, Plymouth Routines In Multivariate Ecological Research (PRIMER) v. 6 software (Clarke and Gorley 2006) with the add-on package PERMANOVA+ (Anderson et al. 2008) was used.

The most abundant sequences from Articles II and III affiliated to the common sea-ice bacterial classes Flavobacteriia, Alphaproteobacteria, Gammaproteobacteria, Betaproteobacteria and Actinobacteria were combined and analysed. A phylogenetic, bootstrapped (1000), NJ tree with the Jukes-Kantor evolution model was constructed, using the Phylogeny Inference Package (Phylip 3.695; Felsenstein 2005). Approximately 450-bp-long sequences were

aligned with the Ribosomal Database Project RDPipeline aligner (Inference of RNA Alignment (INFERNAL), version 1.1rc4; Nawrocki et al. 2009). A sequence from the archaeon *Sulfolobus tokodaii* (AB022438) was used as an outgroup in the alignment. The tree was visualized with the Interactive Tree of Life (Letunic and Bork 2007).

Taxonomic classification of the 16S rRNA genes was done with the naïve Bayesian Classifier tool (v. 2.10, RDP training set 10; Wang et al. 2007) by applying a 80 % threshold and the Seqmatch tool (release 11.3, v. 3) with default options except that < 1200 bp sequences were included in the analysis. The reference sequences were selected based on the Seqmatch results. analysis of variance

4. RESULTS

4.1. Environmental factors affecting the development of sea-ice bacterial communities

The sea-ice temperatures varied from -7.3 °C (I: experiment), -5 °C (II: Baltic Sea) and -3.6 °C (III: Arctic) in the top ice layer to -2.4 °C (I: experiment), -1.5 °C (II: Baltic Sea) and -2 °C (III: Arctic) in the bottom ice. The bulk sea-ice salinity was highest during the early stages of freezing in the experiment (I, average salinity in experimental North Sea water ice: 11.2 ‰) and approximately half of that in the Arctic (average salinity: 4.7 ‰), following the expected trends based on the literature (Petrich and Eicken 2010). The bulk ice salinity in consolidated sea ice in the Baltic Sea was on average 0.6 ‰, which is typical for brackish Baltic Sea ice (Granskog et al. 2006).

The samples were collected before the onset of ice algal mass growth both in the Baltic Sea and the Arctic Ocean. The chlorophyll-*a* (chl-*a*) concentrations (bulk ice measures) were comparable to each other with maximum values in the bottom ice varying from 0.13 to 22.2 $\mu\text{g L}^{-1}$ in the Baltic sea ice and from 0.21 to 28.10 $\mu\text{g L}^{-1}$ in the Arctic sea ice. The experiment (I) was conducted in darkness in the absence of sea-ice protists (diatoms < 0.2 cells mL^{-1} , *Phaeocystis* < 30 cells mL^{-1} , < 60 cells of heterotrophic flagellates mL^{-1} , ciliates not detected; data not shown).

4.2. Bacterial abundance and activity during sea-ice formation and growth

Bacteria were enriched during ice formation regardless of the substrate status of the parent water (I), with the highest bacterial abundance (unnormalized bulk abundance) in frost flowers (I; unenriched mesocosms: 2.4×10^6 cells mL^{-1} and DOM-enriched mesocosms: 3.5×10^6 cells mL^{-1}) compared with ice (I; unenriched mesocosms: 4.4×10^5 and DOM-enriched mesocosms: 9.6×10^5). In the following stages, new ice and pancake ice, bacterial abundance decreased from an average of 6.3×10^5 cells mL^{-1} to 4.6×10^5 cells mL^{-1} . In consolidated sea ice, (II; young and thick ice) the abundance ranged from an average of 2.4×10^5 cells mL^{-1} to 3.9×10^5 cells mL^{-1} .

In the experimental study, bacterial production was approximately six times higher under DOM-enriched conditions on the first ice-sampling day (I; TdR = 0.0044 and 0.024 $\text{nmol L}^{-1} \text{h}^{-1}$ in ice, respectively). In the DOM-enriched mesocosms, bacterial production increased throughout

the experiment, while in the unenriched mesocosms the change in bacterial production compared with the initial water was negligible (I).

In the field studies (II, III), bacterial production was low in new ice and young ice (II; average = 0.002 $\text{nmol L}^{-1} \text{h}^{-1}$ in ice) and increased in thick ice (II; average = 0.018 $\text{nmol L}^{-1} \text{h}^{-1}$ in ice). In the Baltic Sea thick ice, the maximum value of bacterial production was 1000 times higher than the maximum value in the Arctic (II; TdR = 0.04 $\text{nmol L}^{-1} \text{h}^{-1}$, III; TdR = 0.000041 $\text{nmol L}^{-1} \text{h}^{-1}$ in ice, respectively).

4.3. Changes in bacterial communities associated with sea-ice formation and growth

4.3.1. Sea-ice formation

In the early stages of sea-ice formation, the bacterial communities resembled those in open water (I, II), except when DOM was introduced into the parent water (I). The classes Alphaproteobacteria, Flavobacteriia and Gammaproteobacteria predominated in the unenriched mesocosms (I; North Sea water), whereas Gammaproteobacteria predominated in DOM-enriched mesocosms (I). Baltic Sea pancake ice was dominated by class Actinobacteria and SAR11 Alphaproteobacteria (II).

Despite the insignificant changes in the early stages of ice formation, the bacterial diversity decreased after sea-ice formation (I, Table 4). In addition, common sea-ice bacterial genera, such as *Flavobacterium* (II), *Polaribacter* (I and II), *Psychromonas* (II), *Shewanella* (I and II) and *Glaciecola* (I) which were not detected with cloning

and sequencing in seawater, appeared after sea-ice formation (I and II: Table 3).

Based on the T-RF data, the frost flower bacterial communities were similar to those in the underlying sea ice and water (I; Figure 3 and 4).

4.3.2. Consolidated sea ice and under-ice water

The bacterial communities in consolidated sea-ice (II; young and thick ice) communities were significantly different (II; Bonferroni-corrected $P < 0.005$, Table 2) from the

open-water communities and from each other (II). Gammaproteobacteria (II: genus *Acinetobacter*, Figure 2) predominated in young ice in the Baltic Sea. Flavobacteriia (II: genus *Flavobacterium*, Figure 2; III, genus *Polaribacter*, Figure 4) and Gammaproteobacteria (II; e.g. genera *Psychromonas* and *Shewanella*; III: genus *Glaciecola*, Figure 4) predominated in both the Baltic (thick ice) and Arctic drift-ice bacterial communities during the winter/spring transition (II, III). A large proportion (42 %) of the Arctic Gammaproteobacteria could not be identified below the class level. In the Arctic, the third most predominant

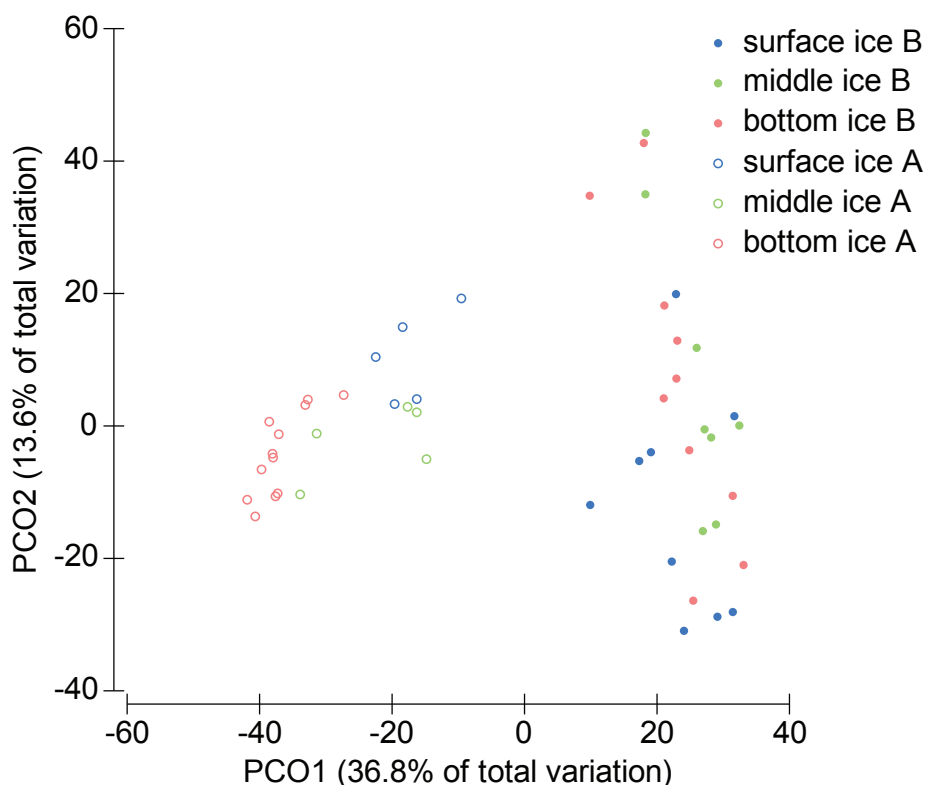


Fig. 7. Principal coordinate (PCO) analysis plot showing the differences in Arctic (open circle) and Baltic (filled circle) sea ice bacterial communities. A = Arctic and B = Baltic.

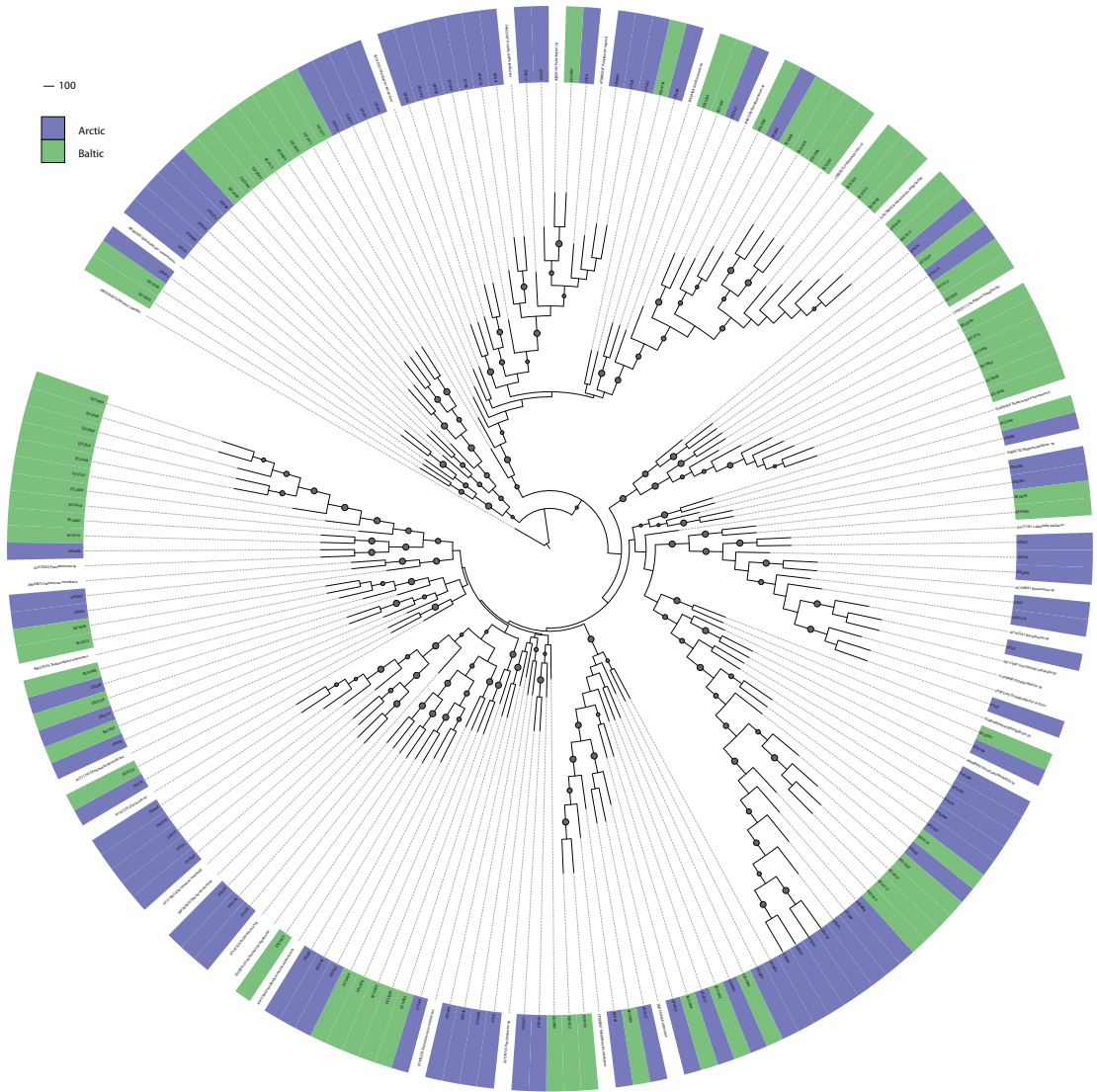


Fig. 8. Bootstrapped (1000) phylogenetic NJ tree of 16S rRNA gene sequences (~450 bp) derived from the sea ice in the Arctic (III) and Baltic (II). Bootstrap values > 50% are shown in grey circles. *Sulfolobus tokodaii* (AB022438) was used as an outgroup in the alignment.

class was Alphaproteobacteria (III: genus *Octadecabacter*, Figure 4) whereas in the Baltic Sea, Betaproteobacteria (II: genus *Albidiferax*, Figure 2) was equally as abundant as Gammaproteobacteria.

The Arctic sea-ice bacterial communities varied vertically (III: Figure 3, IV: Figure 7), whereas in the Baltic sea ice no clear vertical structure was observed (II: Figure 3; IV: Figure 7). The under-ice water bacterial communities were significantly different from the open-water bacterial communities (II) and discriminated in their own group in both the Baltic (II: Figure 5) and the Arctic (III: Figure 3).

4.3.3. *Minor members of sea ice bacterial communities*

In addition to the predominant bacterial classes, less abundant (< 15 %) classes, such as Actinobacteria (II: Figure 2; III: Figure 4), Betaproteobacteria in the Arctic (III: Figure 4), Phycisphaerae (II: Figure 2; III: Figure 4) and Opitutae (II: Figure 2; III: Figure 4) occurred in both Baltic and Arctic sea ice. Phycisphaerae predominated in the Baltic and Actinobacteria and Opitutae in Arctic sea ice.

4.4. **Meta-analysis (IV): Arctic vs. Baltic Sea ice bacterial communities**

At the community level, the Baltic and Arctic sea-ice bacterial communities were significantly different (IV: $P = 0.0001$, Figure 7). No differences in dispersion were detected among sites (PERMDISP: $P > 0.05$), indicating that the differences

between bacterial communities were solely explained by the location effect.

In the phylogenetic NJ tree, the Baltic and Arctic sequences were mostly intermixed (Figure 8). A few sequences belonging to the genera *Polaribacter* (97.7 %), *Flavobacterium* (97.7 %), *Shewanella* (99.5%), *Candidatus Pelagibacter* (98.6 %) and *Acidovorax* (99.8 %) were very closely related. The similarities were based on pairwise sequence alignments (data not shown).

5. DISCUSSION

5.1. **Development of bacterial communities associated with sea-ice formation and growth**

5.1.1. *Sea-ice formation*

In the early stages of sea-ice formation, without DOM-enrichment, the bacterial communities resembled those in the open water (I, II), suggesting that the parent water determines the initial bacterial communities that develop in sea ice (I, II). The bacterial cells were enriched in ice immediately after ice formation, regardless of the nutrient and substrate regime of the parent water (I). Previously, bacterial entrainment and enrichment in sea ice have been associated with algae and/or particles (Grossmann and Gleitz 1993; Grossmann 1994; Grossmann and Dieckmann 1994; Helmke and Weyland 1995; Weissenberger and Grossmann 1998; Riedel et al. 2007b) as well as EPS (Ewert and Deming, 2011, 2013), gas vacuoles (Staley and Gosink 1999) and ice binding proteins (Raymond et al. 2007). Since our experiment (I) was conducted in the absence of protists, they were not likely involved in

the enrichment observed, pointing to possible enrichment caused by other factors such as gas vacuoles, EPS and ice-binding proteins. The frost flower bacterial communities were similar to those in the underlying sea ice and water (I). The result is in accordance with a recent study from the Arctic (Barber et al., 2014), in which the frost flower community was very similar to that of the slush layer above the ice.

During the following stages after sea-ice formation, new ice and pancake ice, bacterial abundance decreased (II) and bacterial production remained low. Bacterial diversity was reduced non-selectively during sea-ice formation, whereas common sea-ice bacterial genera (I: genera *Colwellia*, *Glaciecola* and *Polaribacter*; II: genera *Psychromonas* and *Shewanella*) appeared only after sea-ice formation also in North Sea water that does not typically freeze and produce sea ice. Due to the non-selective loss of bacteria and low bacterial production (Suttle et al. 2007) observed in this study (II), the most likely explanation is the adjustment of the bacterial community to a changing environment. However, since high virus-to-bacteria ratios are known to occur at early stages of freezing (Collins and Deming 2011), and since bacterial stress responses may induce lysogenic viruses (Fuhrman 1999), virally mediated lysis may also have been responsible. The grazing effect is highly unlikely, since sea-ice protists grow more slowly than ice bacteria and also require adaptation to translocation from open water to ice (e.g. Kuparinen et al. 2011). The changes in the bacterial communities during the early stages of freezing, both in experimental North Sea water and in Baltic sea ice, represent examples of the rare biosphere concept (I, II; Sogin et al. 2006; Pedros-Alio 2006; 2012), showing how previously minor

community members can become dominant under changing environmental conditions.

Unlike in unmanipulated conditions, DOM-enrichment induced overwhelming gammaproteobacterial predominance (I), showing that Gammaproteobacteria are capable of opportunistic growth in sea ice, similar to their growth in open-water communities (Eilers et al. 2000; Fuchs et al. 2000; Pinhassi and Berman 2003; Allers et al. 2007; Alonso-Saez et al. 2008; Teira et al. 2008, 2010; Gomez-Consarnau et al. 2012). In sea ice, Gammaproteobacteria are commonly present in late spring/summer, likely due to the high availability of organic substrates originating from sea-ice algae (Junge et al. 2002; Brinkmeyer et al. 2003; Kaartokallio et al. 2008; Deming et al. 2010; Bowman et al. 2012). In addition, Gammaproteobacteria in the Arctic Ocean are known to have high single-cell activity, despite the low production values (Alonso-Saez et al. 2008; Garneau et al. 2008), implying their capability to respond rapidly to changing substrate supplies. This thesis (I, II) and a previous study (Helmke and Weyland 1995) show the importance of availability and quality of organic matter in sea ice.

5.1.2. Consolidated sea ice

The bacterial community composition changed significantly after consolidation of the ice (young ice and thick ice, II). Bacterial abundance further decreased from pancake to consolidated ice (II). However, bacterial production increased in thick ice together with the chl-*a* values, implying that the recovery of the bacterial communities was related to new substrate input by sea-ice algae (II, III). The recovery of the bacterial

communities after congelment of the sea ice was also reported previously (Grossmann and Gleitz 1993; Grossmann 1994; Grossmann and Dieckmann 1994; Helmke and Weyland 1995; Kaartokallio 2004; Kaartokallio et al. 2008). Even though the chl-*a* concentration was equal in Arctic and Baltic sea ice, bacterial production was negligible in the Arctic compared with the Baltic sea ice. The higher bacterial production values in the Baltic Sea ice are likely related to the higher ambient DOM concentrations and thinner ice cover in the Baltic Sea compared to the Arctic Ocean (Kuparinen et al. 2011) and/or the occurrence of minor sea-ice algal ‘blooms’ that may occur in Baltic sea ice before the mass growth of ice algae in spring (Kaartokallio et al. 2008).

Overall, the classes Flavobacteriia (genera *Polaribacter* and *Flavobacterium*), Alphaproteobacteria (genus *Octadecabacter*) and Gammaproteobacteria (genera *Psychromonas*, *Shewanella*, *Glaciecola* and unknown) predominated in the consolidated sea-ice (thick ice) bacterial communities (II, III). In addition to these copiotrophic genera, oligotrophic Alphaproteobacteria (SAR11) were detected in the top ice layer in the Arctic (III), similarly to the midwinter bacterial communities observed in a previous study (Collins et al., 2010). Nevertheless, since the dominant genera observed (III) are commonly present in spring sea ice (Bowman et al. 1997; Brown and Bowman 2001; Junge et al. 2002; Brinkmeyer et al. 2003; Kaartokallio et al. 2008; Bowman et al. 2012), these copiotrophic bacteria are apparently able to outcompete oligotrophic bacteria when ice becomes more permeable and/or when sea-ice algae begin to grow.

The change towards the spring bacterial community composition was also observed in the top ice layer in the Arctic, although

the chl-*a* remained close to the detection limit, indicating that new algal production in the ice did not provide a substrate for the bacterial community. Instead, the increased permeability in sea ice may enable bacteria to use the EPS produced during winter and/or other carbon sources frozen in ice during autumn freeze-up. Another, yet hypothetical, survival strategy under nutrient limitation and salinity stress (Gómez-Consarnau et al., 2007; Feng et al., 2013; Palovaara et al., 2014), may be that bacteria obtain supplementary energy from light via light-harvesting pigments (e.g. proteo- and xanthorhodopsins), which are found in major sea-ice bacterial classes, such as Flavobacteriia, Gammaproteobacteria and Alphaproteobacteria (Koh et al. 2010; Vollmers et al. 2013), also found in this study (III).

5.1.3. Minor members of sea-ice bacterial communities

Both Arctic and Baltic sea ice also had less abundant (< 15% of the total community) bacterial classes, such as Actinobacteria (II, III), Betaproteobacteria (III), Phycisphaerae (II, III) and Opitutae (III). Phycisphaerae was predominant in the Baltic whereas Actinobacteria and Opitutae were predominant in the Arctic. All these classes occur frequently in both polar and Baltic sea ice (Bowman et al. 1997a,b; Junge et al. 1998; Brown and Bowman 2001; Brinkmeyer et al. 2003, 2004; Kaartokallio et al. 2008; Collins et al. 2010; Piiparinen 2011; Bowman et al. 2012; Hatam et al. 2014). The consistent presence of Betaproteobacteria in Baltic sea ice (II; Piiparinen 2011; Kaartokallio et al. 2008) and Arctic melt ponds (Brinkmeyer et al. 2004) indicates that they may play a possible ecological role in sea ice. One

suggestion is that since all these groups are also commonly present in freshwater and soil environments, they may have originated from terrestrial sources (Brinkmeyer et al. 2003; Kaartokallio et al. 2008; Collins et al. 2010). However, their possible ecological roles remain to be elucidated.

5.1.4. Under-ice water bacterial communities

The under-ice water communities were dominated by SAR11 Alphaproteobacteria, both in the Baltic (II) and the Arctic (III), corresponding to the results of previous studies (Collins et al. 2010; Hatam et al. 2014). The under-ice water communities were significantly different from those of both open water (II) and consolidated sea ice (II, III) and formed a separate group in both environments, suggesting specific selection of under-ice water bacterial communities. The similarity among these bacterial communities may relate to their proximity and interaction with the ice cover, e.g. transport of brine and organisms across the ice-water interface, presumably providing more substrate to the organisms than does the open water. In addition, the under-ice water environmental conditions, such as temperature and salinity, are relatively constant throughout the winter, compared with the sea ice, which is exposed to fluctuating salinities and temperatures.

5.2. Meta-analysis (IV): Arctic vs. Baltic sea-ice bacterial communities

Overall, the sea ice bacterial communities in the Arctic differed significantly from those in the Baltic Sea. However, most of

the bacterial classes in the phylogenetic tree were intermixed between Arctic and Baltic sea ice with few closely related genera, such as *Polaribacter* (97.7%), *Flavobacterium* (97.7%), *Shewanella* (99.5%), *Candidatus Pelagibacter* (98.6%) and *Acidovorax* (99.8%), based on their 16S rRNA genes (IV). Since most of these genera are commonly found in sea-ice bacterial communities (Bowman et al. 1997; Brown and Bowman 2001; Junge et al. 2002; Brinkmeyer et al. 2003; Kaartokallio et al. 2008; Bowman et al. 2012), sea-ice formation and growth appear to select for similar bacteria despite the differences in ambient conditions and geographical distance. Since the analysis was based on approx. 450 bp of the 16S rRNA gene, the resulting lower phylogenetic resolution must be taken into account, because even bacteria with identical 16S rRNA genes may present as separate species with different metabolic traits (Gosink et al. 1997). One clear metabolic difference is the cold tolerance of bacteria: in polar areas the genus *Shewanella* comprises both psychrotolerant (max. 25 °C, ability to grow at low temperatures; Junge et al. 2011) and psychrophilic (Bowman et al., 1997b; growth min. ≤ 0 °C, optimum ≤ 15 °C, max. 20 °C; Morita 1975) strains, whereas *Shewanella sp.* strains in the Baltic sea ice are psychrotolerant (Luhtanen et al. 2014). The results suggest that sea ice selects for closely related bacteria, although at the genomic level they may have specialized adaptations to ambient conditions, i.e. represent different ecotypes (IV; Fuhrman et al. 2015).

The bacterial communities in Arctic drift ice varied vertically, whereas in Baltic sea ice the various layers were intermixed (III, IV). The increasing vertical variability was likely related to the initiation of ice algal growth, providing substrate to the communities.

Lack of vertical variability in bacterial community structure in Baltic sea ice may be a consequence of the thinner ice cover, which is more susceptible to the dynamic growth (e.g. rafting and ridging) that mixes the communities vertically, analogously to the situation in ice algae (Rintala et al. 2010).

6. CONCLUSIONS

This thesis examined sea-ice formation and the winter/spring transition and is based on experimental and field studies on brackish and marine sea ice. Although the studies covered three different water masses and used two different research approaches, the results of three Articles (I–III) together with the meta-analysis in the thesis introduction (IV) form a complementary and coherent view of the factors shaping the bacterial communities during sea-ice formation and growth.

In conclusion, the bacterial community at the early stages of sea-ice formation is a subset of the parent water community, due to loss of species that are not able to tolerate the changing environmental conditions, such as high salinity and low temperature. Bacteria may rapidly adapt to prevailing conditions, as shown by the common sea ice genera *Colwellia*, *Glaciecola*, *Polaribacter* and *Psychromonas*, which appeared immediately after freezing but could not be detected in seawater with the methods applied here (I and II: cloning and Sanger sequencing). However, the rapid adaptation of common sea-ice bacteria is likely dependent on the DOM concentration in the parent water that serves as a substrate for the bacteria, since based on a previous study, Arctic winter ice communities may persist nearly unchanged throughout the winter. The bacterial

community composition may already begin to change before the mass algal growth of the sea-ice algae, the when ice becomes more permeable, presumably enabling bacteria to use the EPS produced during winter and/or substrates frozen in sea ice during autumn freeze-up. However, the major changes in the sea-ice bacterial activity are likely related to the mass growth of sea-ice algae, since the bacteria are able to respond very quickly to increasing algal-derived DOM concentrations and follow the increasing chl-*a* concentrations. In early spring, when sea-ice algae begin to grow, copiotrophic Flavobacteria, Alphaproteobacteria and Gammaproteobacteria outcompete the oligotrophic Alpha- and Gammaproteobacteria. The capability of copiotrophic Gammaproteobacteria to grow opportunistically in sea ice may explain their predominance during the mass growth of ice algae in late spring. In all, sea ice provides an example of the enormous capacity of bacteria to adapt to changing environments, since the sea-ice bacterial community is recruited from the minor community members in the seawater and are able to survive over winter in sea ice. Overall, bacterial community formation and dynamics in sea ice are a combination of prevailing environmental conditions, substrate supply and resource competition.

In general, the Arctic and Baltic sea ice communities are different. However, the most common Arctic and Baltic sea ice bacteria seem to be closely related, based on analysis of partial 16S rRNA genes, indicating that sea-ice formation and growth selects for similar bacteria by physical properties and sea-ice algal growth. However, due to the differing nature of these sea areas, these bacteria may present as different ecotypes or

have variable metabolic adaptations, despite their phylogenetic relatedness.

7. FUTURE PROSPECTS

This thesis sheds light on sea-ice bacterial community dynamics during the previously unexplored stages in ice formation and growth and shows that the bacterial community dynamics in sea ice is defined by a combination of selective survival and resource competition. The logical continuation of the work presented here is to determine the role of the various bacterial groups during sea-ice succession. To that end, relevant research questions should include: What are the substrate preferences of each bacterial group and do they have specific niches during sea ice succession? What is the bacterial growth efficiency in ice, i.e. how much of the DOM is respired and how much of it is channelled into new bacterial biomass during sea ice season? Could specific traits such as proteorhodopsin utilization or production of storage polymers contribute to bacterial survival in ice? Are the minor bacterial groups engaged in specialized biogeochemical processes in ice or are they just entrained in ice during freezing? To answer these questions, both molecular (RNA work and omics) and conventional microbiological methods are needed, as well as experimental and field studies. Knowledge of bacterial communities and their functions is crucial to evaluation of the impact of ice cover on marine and atmospheric systems as well as to predict the possible implications of diminishing ice cover.

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