Ecophysiology of Polar Sea Ice Microorganisms in a Changing World

Anders Torstensson

Ph.D. thesis
Department of Biological and Environmental Sciences
University of Gothenburg

Faculty of Science

UNIVERSITY OF GOTHENBURG

Ecophysiology of Polar Sea Ice Microorganisms in a Changing World

Anders Torstensson

Doctoral thesis

2015

Department of Biological and Environmental Sciences

Faculty of Science

University of Gothenburg

UNIVERSITY OF GOTHENBURG

Front cover photograph by Anders Torstensson
Printed by Kompendiet, Aidla Trading AB, Gothenburg, Sweden

ISBN 978-91-85529-86-5

Abstract

Earth's oceans are predominantly cold, with nearly 90% of their volume having temperatures below 5 °C. Microorganisms commonly referred to as psychrophiles have adapted to the temperatures of these cold waters. The most extreme psychrophiles are found inside the sea ice of polar oceans, where bacterial growth can be observed down to -20 °C. Sea ice consists of a matrix of ice and high-saline water (brine) that provide a unique habitat for microbial communities. Microscopic algae and bacteria dominate these extreme environments, which are considered very stressful as they are characterised by large variations in salinity, low temperatures, and low radiation levels. However, the brine-filled channels also provide a platform from which microscopic algae remain in the euphotic zone and refugees from significant grazing, thereby enabling net autotrophic growth. As a result, sea ice hosts some of the highest chlorophyll *a* concentrations on the planet, and is one of the most important factors controlling primary production and bloom dynamics in polar areas.

In this thesis, I focus on the ecophysiology of psychrophiles adapted to the sea ice environment. Physiological acclimation to environmental change needs to be studied in order to address how different stressors may influence organisms' capacity to tolerate both naturally- and climatically-driven changes. Extremophiles growing close to their physiological limits may be especially susceptible to environmental stressors, such as rapid climate change. Therefore, a series of studies has been performed to investigate how environmental stressors, such as increased temperature and elevated CO_2, affect microbial physiology and community structure in polar areas.

The ecophysiology of sea ice microorganisms has been addressed in laboratory experiments (Papers I, II, and IV) and in field measurements (Paper III). In brief, relatively small changes in temperature had considerable effects on the physiology of sea ice diatoms, and indirectly affected the structure of sea ice bacterial communities. Increasing temperature (on both climatic and seasonal scales) positively affected the growth and primary productivity of two sea ice diatom species, and negatively affected the taxonomic richness and diversity of sea ice bacterial communities, probably by the subsequent changes in salinity.

On the other hand, sea ice diatoms seem quite tolerant to changes in pH and partial pressure of CO_2 (pCO_2) in terms of growth, probably due to the fact that they grow in an environment with large seasonal variations in the carbonate system. However, increased pCO_2 resulted in other cellular changes that may have important ecological consequences, such as cellular stoichiometry. This includes changes in fatty acid composition and dissolved organic carbon exudation, which are important components in food webs and biogeochemistry in many marine ecosystems.

Although most studies on marine organisms have focused on short-term responses to increased pCO_2, acclimation and adaptation are key components in order to identify the consequences of climate change in biological systems. In Paper IV, the physiological responses to long-term acclimation to high pCO_2 were investigated in the psychrophilic sea ice diatom *Nitzschia lecointei*. After long-term acclimation (194 days), a small reduction in growth was detected at high pCO_2. Previous short-term experiments have failed to detect altered growth in *N. lecointei* at high pCO_2, which illustrates the importance of experimental duration in ocean acidification studies.

Keywords: Climate change, ocean acidification, ocean warming, CO_2, Arctic, Antarctica, Southern Ocean, algae, bacteria, psychrophiles

Populärvetenskaplig sammanfattning

Omkring 90 % av världshavens volym är kallare än 5 °C. Köldtåliga mikroorganismer (främst bakterier och alger) har anpassat sig till ett liv i dessa kalla vatten. Några av de mest köldtåliga organismerna lever i havsisen runt Arktis och Antarktis, där bakterier kan växa i -20 °C. Havsis består av en blandning av is och mikroskopiska kanaler fyllda med saltlake som bildas när saltvatten fryser. De organismer som lever i dessa kanaler är stressade av stora säsongsvariationer i salthalt, temperatur och ljusförhållanden. Att leva i denna miljö innebär ständiga fysiologiska anpassningar alltifrån att förhindra att cellerna fryser sönder till att cellernas saltinnehåll blir för högt. Men dessa kanaler utgör också en unik miljö där de mikroorganismer som klarat att anpassa sig också kan frodas tack vare att de kan leva kvar i den ljusa delen av havet, samt skyddas från större betare. Detta leder till att höga koncentrationer av mikroskopiska alger, ofta kiselalger, ackumuleras i isen och färgar den brun (se bild på framsidan). Marina alger bidrar med ca hälften av Jordens syrgasproduktion, och utgör basen av polarhavens näringsväv. Havsisen spelar dessutom en viktig roll i polarområden genom att bland annat kontrollera var och när algblomningar bildas.

Syftet med min avhandling är att förstå hur köldtåliga mikroalger och bakterier från havsis kan påverkas av framtida klimatförändringar. Fysiologisk anpassning till olika miljöer (ekofysiologi) är viktigt att studera för att förstå hur en organism kan anpassa sig till nya omgivningar. Extremälskande mikroorganismer, t.ex. isalger, lever ofta nära sin toleransgräns och kan vara extra känsliga för miljöförändringar, såsom snabba klimatförändringar. Därför har vi genomfört en serie studier för att beskriva hur olika miljöfaktorer, t.ex. ökad temperatur och koldioxidhalt, påverkar organismernas fysiologi och artsammansättning i polarområden. Generellt hade relativt små temperaturförändringar ganska kraftiga effekter på fysiologin hos isalger, samt förändrade strukturen av islevande bakteriesamhällen. Vi såg bland annat en ökning i tillväxt och fotosyntetisk produktion hos två arter av islevande kiselalger. När temperaturen ökade, och salthalten indirekt minskade, reducerades också antalet bakteriearter i isen i Antarktiska oceanen. Eftersom dessa mikroorganismer utgör basen av näringsväven, kan även små förändringar hos dem få stora konsekvenser för ekosystemet som helhet.

När koldioxid löser sig i havsvatten bildas kolsyra, vilket leder till att haven också blir surare (lägre pH). Detta kan tänkas ha både positiva och negativa effekter på alger, eftersom de använder koldioxid för fotosyntesen men får svårigheter att växa om vattnet blir för surt. Isalgerna verkade vara ganska tåliga mot framtida förändringar i pH, antagligen för att de är anpassade till en miljö med naturliga pH-variationer. Vi observerade både positiva, negativa och neutrala förändringar i tillväxt, varav de flesta effekterna var relativt små. Men en reducering av pH ledde också till förändringar i fettsyresammansättningen och kolfixeringen hos algerna, förändringar som inte alltid återspeglades i tillväxt. Detta kan potentiellt ge kraftiga konsekvenser högre upp i näringsväven då fettsyresammansättning påverkar födokvalité, och sedan kan utsöndring av organiskt kol kan påverka nedbrytningen i viktiga mikrobiella näringsvävar.

De flesta studier som fokuserats på havsförsurning har utförts under korta perioder. Men för att förstå hur framtida klimatförändringar kommer att påverka olika organismer är det otroligt viktigt att ta hänsyn till anpassning under en lång tid (flera

generationer). I en studie fann vi inga effekter av ökad tillförsel av koldioxid (havsförsurning) förrän vi låtit isalgerna anpassa sig till den nya miljön. Det tog till exempel 147 dagar innan tillväxten minskade jämfört med kontrollbehandlingen. Tidigare korttidsexperiment har inte lyckats upptäcka små förändringar i tillväxt, vilket visar betydelsen av experimentens längd när det handlar om anpassning till kommande klimatförändringar.

List of papers

I. **Torstensson A**, Chierici M, Wulff A (2012). The influence of temperature and carbon dioxide levels on the benthic/sea ice diatom *Navicula directa*. Polar Biology 35: 205-214.

II. **Torstensson A**, Hedblom M, Andersson J, Andersson MX, Wulff A (2013). Synergism between elevated pCO_2 and temperature on the Antarctic sea ice diatom *Nitzschia lecointei*. Biogeosciences 10: 6391-6401.

III. **Torstensson A**, Dinasquet J, Chierici M, Fransson A, Riemann L, Wulff A (2015). Physicochemical control of bacterial and protist community composition and diversity in Antarctic sea ice. Environmental Microbiology 17: 3868-3881.

IV. **Torstensson A**, Hedblom M, Mattsdotter Björk M, Chierici M, Wulff A (2015) Long-term acclimation to elevated pCO_2 alters carbon metabolism and reduces growth and in the Antarctic diatom *Nitzschia lecointei*. Proceedings of the Royal Society of London B: Biological Sciences 282: 20151513.

Related publications not included in this thesis

Granfors A, Andersson M, Chierici M, Fransson A, Gårdfeldt K, **Torstensson A,** Wulff A, Abrahamsson K (2013). Biogenic halocarbons in young Arctic sea ice and frost flowers. Marine Chemistry 155: 124-134.

Garrard SL, Hunter RC, Frommel AY, Lane AC, Phillips JC, Cooper R, Dineshram R, Cardini U, McCoy SJ, Arnberg M, Rodrigues Alves BG, Annane S, de Orte MR, Kumar A, Aguirre-Martínez GV, Maneja RH, Basallote MD, Ape F, **Torstensson A**, Mattsdotter Björk M (2013). Biological impacts of ocean acidification: a postgraduate perspective on research priorities. Marine Biology 160: 1789-1805.

Mattsdotter Björk M, Fransson A, **Torstensson A**, Chierici M (2014). Ocean acidification state in the western Antarctic surface waters: controls and interannual variability. Biogeosciences 11, 57-73.

Fransson A, Chierici M, Abrahamsson K, Andersson M, Granfors A, Gårdfeldt K, **Torstensson A**, Wulff A (2015). CO_2-system development in young sea ice and CO_2-gas exchange at ice/air interface through brine and frost flowers in Kongsfjorden, Spitsbergen. Annals of Glaciology 56: 245-257.

Webster C, Silva T, Ferreria AS, Wiedmann I, Juul-Pedersen T, Varpe Ø, Gislason A, Saiz E, Calbert A, Sainmont J, Dalgaard Agersted M, Helenius L, Tammilehto A, **Torstensson A**, Brierley AS, Engel Arendt K, Gissel Nielsen T. Fate of an Arctic spring bloom (Revised manuscript submitted to Marine Ecology Progress Series).

Table of contents

1. Introduction

1.1. The sea ice ecosystem

The extensive sea ice cover in polar areas provides a unique habitat for microbial assemblages. Sea ice is one of the largest biomes on the planet, as it covers 13% of the Earth's surface at its maximum extent (Lizotte, 2001). When sea ice forms and ages, channels containing highly saline water (brine) establish and create distinct habitats for microbial communities, encompassing members from multiple trophic levels such as small metazoans, unicellular algae, protozoa, bacteria, fungi, and viruses (Horner *et al.*, 1992; Bachy *et al.*, 2011; Bowman *et al.*, 2012). Different algal assemblages generally dominate these communities and play an important role in polar ecology and biogeochemistry (Arrigo & Thomas, 2004; Arrigo *et al.*, 2010; Riaux-Gobin *et al.*, 2011). For instance, sea ice algae are crucial food sources for many species of fish and invertebrates, such as krill (O'Brien, 1987), especially during winter and spring, when the surrounding water column does not support sufficient phytoplankton production (O'Brien, 1987; Marschall, 1988). Microalgal biomass can reach extreme values in sea ice, with volumetric chlorophyll *a* concentrations well above 1,000 μg l^{-1} (Arrigo *et al.*, 2010). Many attempts have been performed to estimate the sea ice algal contribution to primary production in polar areas. Although estimates are variable due to the fact that sea ice is a highly variable and patchy environment, ice algal production is believed to account for 5-25% of seasonally ice covered areas in the Southern Ocean (Arrigo *et al.*, 1997; Lizotte, 2001).

Most sea ice is formed annually, and the presence of ice has a large influence on the location of primary production in polar areas, creating a seasonal succession in algal growth in sea ice-covered oceans (Figure 1). During wintertime, sea ice is primarily regarded as heterotrophic, with high abundances of bacteria and heterotrophic flagellates. In early spring, light conditions are still too low for any significant phytoplankton production to occur. However, sea ice provides a platform from which microscopic algae can remain in the euphotic zone, and refugees from significant grazing (Krembs *et al.*, 2000). A strong low-light adaptation enables net autotrophic production, and as the ice becomes warmer and more permeable to the underlying nutrient-rich water, high sea ice algal biomasses accumulate in the microscopic brine channels. At this point, the ice is coloured a characteristic brown shade primarily due to the antenna pigments of diatoms such as fucoxanthin (Figure 2), and is an important grazing site for sympagic and pelagic fauna. As the ice starts to melt, sea ice algae are dispersed in the water column and seed the pelagic spring bloom, which generally occurs just after the sea ice break-up (Figure 1). Hence, the extent of sea ice is one of the most important factors in controlling primary production in polar areas.

1.2. Physicochemical properties of sea ice

In order to address ecological and physiological dynamics in the sea ice ecosystem, key environmental properties of sea ice must be understood. Sea ice is characterised by large temporal and spatial variations in physicochemical properties. When sea ice forms, salts are excluded from the ice matrix and become concentrated in brine channels and pockets. The channels can be up to a few hundreds of micrometers in diameter, although the majority are considerably smaller (< 40 μm) (Krembs *et al.*, 2000). At the same time, gases, particles, and microorganisms present in the freezing

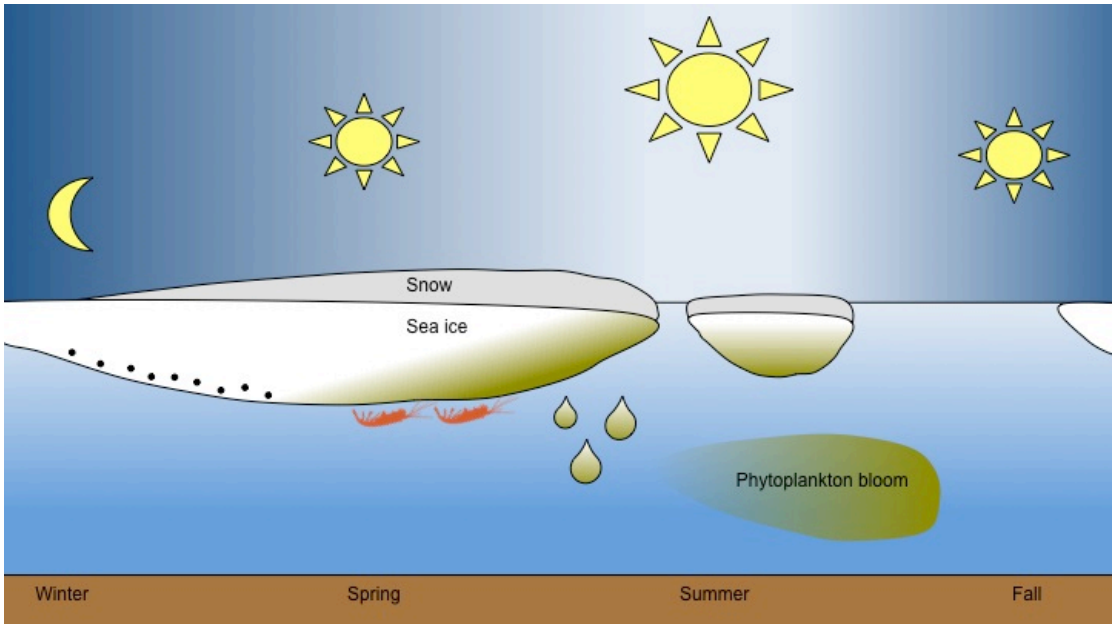

Figure 1. Schematic view of a general succession pattern of microalgal biomass and production in Antarctic pack ice and phytoplankton. Sea ice is considered to be primarily heterotrophic during wintertime (indicated by black points). During spring, sea ice algal biomass begins to accumulate in bottom sea ice, becoming an important food source for grazers, such as krill. As the ice becomes top-flooded and more permeable, biomass accumulates throughout the ice floe. When the ice is melting, algae are dispersed into the water column and seed an emerging phytoplankton bloom close to the sea ice edge.

Figure 2. Turning sea ice floe with an internal brown layer of ice algae. The brine-filled channels provide a unique platform of which microscopic algae can remain in the euphotic zone, and refugees from significant grazing. As a result, high amounts of algal biomass can accumulate in the sea ice.

water become concentrated in the liquid brine, creating enrichment in the brine compared with surrounding water. Temperature controls the enrichment factor and brine salinity, which can exceed 150 under cold conditions (Thomas & Dieckmann, 2002; Granfors *et al.*, 2013). Due to its high density, brine is also excluded from the ice through gravitational drainage, creating a net transport of salts and gases, such as CO_2, to deeper waters – a process referred to as the *Sea ice CO_2 pump* (Fransson *et al.*, 2013). The sea ice permeability to liquids and gases increases as it becomes warmer. When columnar sea ice temperature is above -5 °C, and has a brine volume fraction of > 5% and a bulk salinity of < 5, the ice reaches a tipping point where it becomes permeable to liquids, also known as *the Rule of Fives* (Golden *et al.*, 1998). As long as the ice is permeable, nutrient-rich water can infiltrate the ice and dilute the brine. When the ice is melting, the organisms in sea ice experience hypo-osmotic conditions due to freshwater dilution, where the salinity of brine can be < 10 (Paper III).

Physicochemical properties of sea ice vary spatially and are dependent on ice type and age. For instance, there are fundamental differences between the sea ice of the two polar regions, notably affecting the sea ice community. These differences are mainly consequences of geographical factors due to the fact that the Arctic is an ocean surrounded by land, and Antarctica is a continent surrounded by an ocean. This results in high amounts of precipitation in Antarctica, and sea ice in that region is generally covered with more snow than in the Arctic. The large masses of snow affect the buoyancy of the ice, causing surface flooding and mixed slushy layers on top of the ice. The slushy layers are often characterised by low salinity and generally contain high abundances of flagellates, such as the colony-forming nanoflagellate *Phaeocystis antarctica*. Surface flooding also enhances the permeability and nutrient transport of the ice when seawater infiltrates from the top. This well-drained ice enables significant ice algal production in the interior sea ice. In the Arctic, on the other hand, less precipitation limits seawater intrusion from the surface. There are also large differences between land fast and pack ice. Since fast ice is anchored to a landmass, surface flooding and permeability are very limited. Therefore, the majority of the ice algal biomass is located in bottom sea ice, i.e. at the intersection between ice and water, where conditions are favourable for net autotrophic growth. Snow cover also affects light penetration significantly, and results in a strong low-light adaptation in ice algae (Palmisano *et al.*, 1985; Thomas & Dieckmann, 2002; Lazzara *et al.*, 2007).

Sea ice organisms are, thus, experiencing large spatial and temporal variations in physicochemical properties, such as temperature, salinity, and pH (Gleitz *et al.*, 1995; Thomas & Dieckmann, 2002). These factors affect the sea ice microbial community significantly, and create spatial patchiness in sea ice ecosystems. Both seasonal and environmental changes are strong drivers for structural and functional variables in sea ice. In order to predict how cold-adapted microbial ecosystems may respond to climate and seasonal change, key environmental drivers need to be identified and responses need to be quantified.

1.3. Adaptation to an extreme environment

Many people associate the sea with warm beaches in tropical paradises and relaxing summer days, although this constitutes only a very small part of the marine environment. In fact, approximately 90% of all marine water masses (by volume) are

permanently colder than 5 °C (Schlegel & Jannasch, 1981). Specialised microorganisms have successfully colonised all of these cold waters, and will from hereon be referred to as *psychrophiles*. As there is no single definition of a psychrophilic organism, in this thesis this term will be used to refer to organisms capable of growing well at temperatures close to the freezing point of water.

Due to the highly dynamic physicochemical properties of sea ice, sympagic organisms have adapted to the extreme environment of sea ice. Sea ice microorganisms must cope with low temperatures, low radiation levels, and large variations in salinity (Figure 3). One of the greatest challenges at low temperatures is to maintain functionality and fluidity of lipid membranes. In order to retain membrane fluidity at low temperatures, psychrophiles alter the composition of their fatty acids and membrane phospholipids. Psychrophilic bacteria and algae are known to produce high amounts of unsaturated and polyunsaturated fatty acids (PUFAs) (Fahl & Kattner, 1993; Bowman, 2008), a production that may be promoted by the low irradiance, low temperature, and high salinities experienced in sea ice (Kaiser *et al.*, 2011). As PUFAs are essential for all organisms, and are especially important in polar areas, sea ice algae are important components in the diets of grazers, such as krill (O'Brien, 1987). Sea ice organisms (algae and bacteria) also produce large amounts of extracellular polymeric substances (EPS), which are a broad group of compounds primarily composed of polysaccharides. EPS are mostly known for the establishment of the structural integrity of biofilms, of which 50 to 90% of the organic matter can consist of EPS (Flemming *et al.*, 2000). On a cellular level, EPS are believed to have an important role relevant to sea ice environments in terms of motility (Lind *et al.*, 1997), adhesion (Mora *et al.*, 2008), cryoprotection (Marx *et al.*, 2009; Aslam *et al.*, 2012), and osmoprotection (Ozturk & Aslim, 2010; Aslam *et al.*, 2012). It is also believed that EPS derived from diatoms can be used for manipulating the cells physicochemical environment and sea ice microstructure by release of anti-freeze proteins and pore clogging (Krembs *et al.*, 2011). Hence, EPS are essential compounds in sea ice biology. In addition, enzymes in psychrophiles have a high catalytic efficiency at low temperatures (Huston *et al.*, 2000), and key enzymes, such as ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO), are synthesised in higher concentrations at low temperature (Devos *et al.*, 1998). Enzymes in sea ice microorganisms are also well-adapted for large salinity variations, as changes in

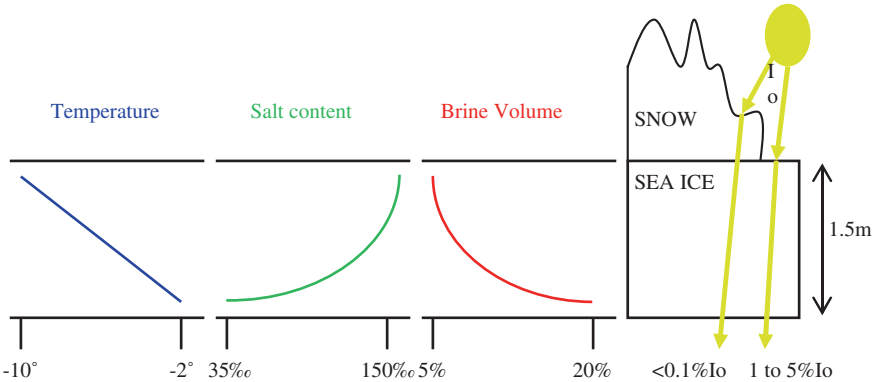

Figure 3. Simplified vertical gradients of temperature, salinity, and brine volume experienced in ~1.5 m thick sea ice. As temperature decreases in the vertical profile of the ice, salinity increases and brine volume decreases. Snow coverage significantly reduces the incident radiation (Io). Reprinted from Mock and Junge (2007), with permission from Springer Science and Business Media.

temperature are often accompanied by changes in salinity (Helmke & Weyland, 1995). An efficient osmolyte regulation is used to respond to this effect. For instance, dimethylsulfoniopropionate (DMSP) is a cryoprotectant and osmolyte found in high concentrations in sea ice algae (Kirst *et al.*, 1991), especially under stressful conditions (Lyon *et al.*, 2011). DMSP is the precursor of the climate-active gas dimethyl sulfide (DMS), and the biogenic production by algae is one of the major sources of sulfur in the atmosphere. Therefore, sea ice algae are believed to play an important role in the sulfuric cycle.

1.4. Climate change

Anthropogenically-driven climate change is one of the largest threats to marine environments on Earth, and will fundamentally alter ocean ecosystems (Hoegh-Guldberg & Bruno, 2010). As changes are occurring more rapidly than in the geological past, there is risk of irreversible ecological transformation in many ecosystems. On an ecological scale, climate change is believed to negatively affect ecosystem functioning and habitat complexity, and to increase the establishment of invasive species (Hoegh-Guldberg & Bruno, 2010). Increasing temperature and CO_2 levels are two climate change variables that are believed to strongly affect various marine organisms, and will be the main factors discussed in this thesis.

Since the beginning of the industrial period, atmospheric levels of CO_2 have increased by approximately 30%, and the world's oceans have absorbed similar proportions of the total anthropogenic CO_2 emissions (Sabine *et al.*, 2004; IPCC, 2013). This increased oceanic CO_2 concentration has caused a change in the marine carbonate system and resulted in a less alkaline state. As CO_2 dissolves into seawater (Equation 1.1), it takes part of the oceanic carbonate system, which acts to buffer against changes in acid-base equilibria according to the following set of equations:

$$CO_2\,(g) \leftrightarrow CO_2\,(aq) \tag{1.1}$$
$$CO_2\,(aq) + H_2O \leftrightarrow H_2CO_3 \tag{1.2}$$
$$H_2CO_3 \leftrightarrow H^+ + HCO_3^- \tag{1.3}$$
$$HCO_3^- \leftrightarrow H^+ + CO_3^{2-} \tag{1.4}$$

At normal seawater pH (close to 8), the major component of the carbonate system is in the form of bicarbonate, HCO_3^- (90%). The remainder consists of carbonate ions, CO_3^{2-} (9%), and total dissolved CO_2, commonly denoted as the sum of CO_2 and carbonic acid, $H_2CO_3^*$ (1%) (Figure 4). Dissolved CO_2 hydrates to form carbonic acid, H_2CO_3 (Equation 1.2), which quickly dissociates twice and produces hydrogen ions according to Equations 1.3 and 1.4. These reactions act as a buffer to maintain a constant pH according to Figure 4. However, this neutralisation is not complete, and the remaining hydrogen ions reduce the pH of the water, a phenomenon referred to as *ocean acidification*.

The increase of atmospheric CO_2 levels since society's industrialisation corresponds to an average global sea surface pH reduction of ~0.1 units, and with the current increase of atmospheric CO_2 levels by ~0.4% per year, models have projected a further decrease of approximately 0.3–0.4 pH units by the year 2100 (Caldeira & Wickett, 2003). Additional models have predicted that atmospheric partial pressure of

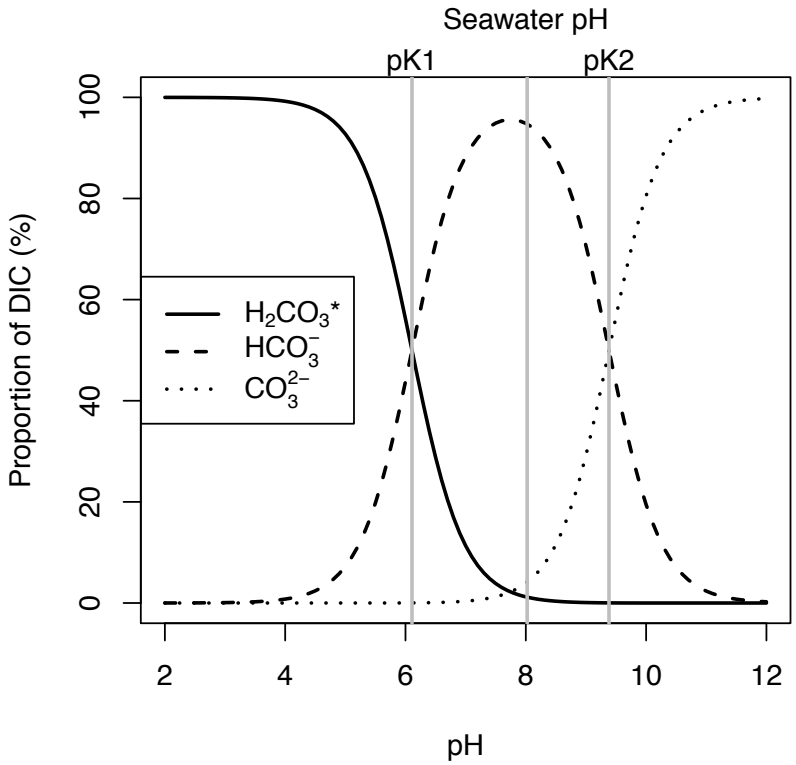

Figure 4. Dissolved inorganic carbon speciation for different seawater pH values (total scale) at 0 °C and salinity 35. Normal seawater pH of 8.022 (at 0 °C, salinity 35, 403 µatm pCO_2 and A_T of 2300 µmol kg^{-1}) is indicated in the graph as "Seawater pH". As ocean acidification progresses, the carbonate system changes towards a state with lower pH, lower CO_3^{2-} concentration, and higher HCO_3^- concentration. The dissociation constants for SO_4^- were determined by Dickson (1990), and *K1* and *K2* were determined by Roy *et al.* (1993) and are indicated as "pK1" and "pK2" in the graph. H_2CO_3* denotes the sum of dissolved CO_2 and H_2CO_3. The CO_2 speciation was calculated in R (R Core Team, 2014) using the seacarb package (Gattuso *et al.*, 2015).

CO_2 (pCO_2) may reach well above 900 ppm by the end of this century (IPCC, 2013). As the world's oceans absorb atmospheric CO_2, causing major impacts on marine biogeochemistry, effects may be profound for several marine organisms. High-latitude marine environments are particularly vulnerable to ocean acidification, primarily due to the already-low carbonate ion concentration and high solubility of CO_2 in cold waters (Orr *et al.*, 2005). For instance, the surface water of the polar Southern Ocean has a naturally low carbonate saturation state and, together with the Arctic Ocean, is believed to be one the first oceans to become persistently undersaturated with respect to the carbonate mineral aragonite (Orr *et al.*, 2005; Steinacher *et al.*, 2009; Mattsdotter Björk *et al.*, 2014), which is often used as a proxy for ocean acidification.

Along with elevated levels of greenhouse gases in the atmosphere, an increase in average sea surface temperature (SST) of 0.74 °C has been recorded from the years 1906 to 2005 (IPCC, 2013). Mean SST is predicted to rise 1–4 °C by the year 2100, and the largest impacts are predicted to occur in polar areas (IPCC, 2013). For instance, the summer sea ice extent in the Arctic has declined since the late 1970s, and recent models have predicted a sea ice-free Arctic Ocean during the summer, within the next 30 years (Wang & Overland, 2009). During the last 100 years, average Arctic temperatures have increased at almost twice the global average rate (IPCC,

2013). Hence, as the most rapid environmental changes are occurring in polar areas, they are believed to be particularly susceptible to climate change.

Climate change has been identified as a potential threat to marine ecosystems, and substantial scientific efforts have been expended on experimentally determining its effects on the physiology and ecology of many marine species (Hoegh-Guldberg & Bruno, 2010; Riebesell & Gattuso, 2015). The effects of global warming and ocean acidification are relatively well studied in microscopic algae. For instance, increased temperature influences microorganisms both directly by altered physiology, and indirectly by changes in ocean stratification in many areas. This process will in turn affect light intensities and nutrient levels. Due to species-specific responses to these factors, changes in species composition are expected (Beardall & Stojkovic, 2006). Such changes in species composition may have impacts on biogeochemistry and ecology through bottom-up effects. In the western shelf of Antarctica, for instance, recent climate change has reduced primary production, as derived from satellite chlorophyll *a* measurements, and is believed to affect krill and penguin populations (Montes-Hugo *et al.*, 2009). Hence, in order to predict future changes in polar ecosystems, it is crucial to understand physiological and ecological responses in the base of the marine food chain.

1.5. Study organisms

In this thesis, I focus on algae and bacteria from Arctic and Antarctic sea ice, using both laboratory cultures of ice algae and natural community samples. The two main cultured species in this thesis, *Navicula directa* (W. Smith) Ralfs 1861 and *Nitzschia lecointei* van Heurck 1909 (Figure 5A and B, respectively), are both free-living pennate diatoms frequently occurring in sea ice (Melnikov, 1997; Riaux-Gobin & Poulin, 2004; Ralph *et al.*, 2005; Riaux-Gobin *et al.*, 2011). *N. lecointei* can be found in high densities in Southern Ocean sea ice, and are often associated with platelet ice and the bottom ice horizons (Ralph *et al.*, 2005; Riaux-Gobin *et al.*, 2011). Upon seasonal sea ice melting, high abundances of *N. lecointei* can also be observed in the under-ice waters, and is believed to play an important role in planktonic ecology and particle flux (Riaux-Gobin *et al.*, 2011). *N. directa* is a somewhat larger but also more

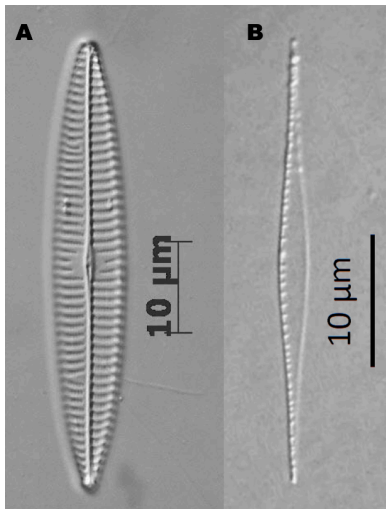

Figure 5. Silica frustules of the cultured sea ice diatom species *Navicula directa* (A) studied in Paper I, and *Nitzschia lecointei* (B) studied in Papers II and IV. Photos taken by Professor Adil Al-Handal.

variable in size, reputedly benthic diatom occurring from polar to temperate areas (Al-Handal & Wulff, 2008; Poulin *et al.*, 2011). Although primarily benthic, it is commonly observed in sea ice environments (Melnikov, 1997; Poulin *et al.*, 2011). Due to their ecological relevance and importance to the sea ice ecosystem, these species were chosen as model organisms for studying sea ice algal ecophysiology under climate change (Papers I, II, and IV).

1.6. Summary

Sea ice is the one of the most important factors controlling primary production and bloom dynamics in polar areas. The sea ice ecosystem is mainly comprised of a unique microbial community with extreme adaptations to low temperature, high salinity, and low-light conditions. This ecosystem is dominated (by biomass) by microscopic algae, which are important primary producers in polar oceans, sustaining the grazer community with food when phytoplankton biomass is scarce. When the ice is breaking up, sea ice algae are dispersed in the water column and seed a subsequent pelagic bloom. The high amounts of unsaturated fatty acids they contain make ice algae an important component of the diets of grazers, and play an important role in the ecology of polar oceans. However, future climate change will affect the physiological and ecological dynamics in polar areas. Both experimental and field samplings are needed to understand to what extent environmental changes will affect microbial communities.

2. Aim of the thesis

The main aim of this thesis is to study the ecophysiological responses of sea ice microorganisms (microalgae and bacteria) exposed to environmental changes. It mainly focuses on two factors (both independently and in combination) that will likely have the largest impact on marine primary producers, namely increased temperature and CO_2. As anthropogenic stressors rarely occur individually, the combined stressors are also addressed, and may strengthen or weaken the effects of the individual stressors. These issues have primarily been addressed experimentally using algal cultures, but also with the sampling of natural microbial communities in the field. More specifically, the aims and approaches of this thesis are:

Paper I: To investigate the short-term (7 days) ecophysiological response of the Arctic benthic/sea ice diatom *N. directa* to the combination of elevated temperature and pCO_2 in terms of growth, photosynthetic activity, and photosynthetic pigment concentration. Hence, we were interested to see if ocean warming and acidification affected the photosynthetic compartments in *N. directa*, and tested the interactions and main effects in a laboratory full-factorial experimental design (n = 4). Statistical testing was performed using two-factor analysis of variance (two-factor ANOVA). As the coupling of several global change aspects is significant, it is necessary to study the stressors in combinations. Increased temperature and pCO_2 are two consequences of global change that can have major effects on photosynthesis and the physiology of primary producers, and are preferably addressed simultaneously.

Paper II: To study the potential synergism between increased temperature and pCO_2, and to estimate optimal growth temperature on a newly isolated strain of the Antarctic sea ice diatom *N. lecointei*. The efficiency of carbon concentrating mechanisms (CCMs) will play a key role in algal responses to high CO_2. One of the most studied CCMs is the enzyme carbonic anhydrase (CA). CA catalyses the reversible interconversion of CO_2 and HCO_3^-, and thereby facilitate many physiological functions, such as photosynthesis and respiration. It has also been suggested that microalgal fatty acid stoichiometry may change in a high-CO_2 world (Rossoll *et al.*, 2012), which could have major consequences in cold waters. This study emphasises growth, photosynthetic activity, primary productivity, external CA activity, and fatty acid composition. We tested the hypothesis that potential effects of elevated CO_2 were temperature-dependent in an orthogonal laboratory experimental design (n = 4), using two-factor ANOVA, with two temperature treatments (-1.8 and 2.5 °C) and two pCO_2 treatments (390 and 960 µatm).

Paper III: To understand how the sea ice microbial community responds to environmental and seasonal changes, natural communities' sensitivity to changes in the physicochemical environment is crucial to address. In this study, we investigated environmental control of microbial diversity and community structure (with respect to 16S and 18S rRNA genes) in bottom sea ice from the Amundsen and Ross Seas during the Oden Southern Ocean 2010/2011 expedition. More specifically, we tested the correlation between bacterial taxonomic richness and sea ice temperature and brine salinity. We also described microbial diversity and the relationships between eukaryotes and environmental variables, and explored potential linkages between the most common taxa of eukaryotes and bacteria using canonical correspondence analysis (CCA).

Paper IV: Most ecophysiological studies on marine algae are performed on a short-term time scale, normally ranging between 7 and 15 days. However, in order to address how climate change will affect marine organisms within the next century, acclimation and adaptation to environmental stressors need to be studied. Due to long-term acclimation to experimental conditions, we hypothesized that the effect of increased pCO_2 was dependent on experimental duration. To test this hypothesis, we investigated growth and carbon metabolism during long-term acclimation (194 days, ~60 asexual generations) to elevated pCO_2 in the sea ice diatom *N. lecointei* (n = 5). Individual and combined effects of CO_2 treatment and sampling day were analysed with linear mixed effects (LME) models, which are useful tools that can provide a better fit than traditional generalized linear models (e.g. repeated-measure ANOVA).

3. Methodological considerations

3.1. Sea ice sampling

Measurement of biological processes and diversity in sea ice samples can be challenging, not only due to the remote locations, but also due to the complex structure of sea ice and the high degree of spatial patchiness. Ideally, the sea ice microbial community is studied *in situ* to fully understand the ecophysiology of sea ice organisms. However, most traditional sampling and concentration methods used by marine scientists are developed for measurements via liquid samples, e.g. filtration and centrifugation. Hence, in order to quantify many functional biological variables, sea ice samples are preferably processed as liquid samples. Several techniques are available to acquire a liquid sample from sea ice, including melting, and brine drainage using centrifugation or sack holes (produced by partially drilling through the ice). All techniques have different pros and cons. The major concern when processing biological samples is to avoid osmotic and thermal shock when organisms are extracted from the brine-filled ice channels. The chosen technique may vary depending on the measured variable and the physicochemical properties of the sea ice. For instance, if the ice is very cold and saline, melting will cause significant shock unless salinity is controlled during thawing, e.g. by the addition of highly saline water. Therefore, brine drainage by centrifugation (Granfors *et al.*, 2013) or collection of brine from sack holes (Becquevort *et al.*, 2009; Granfors *et al.*, 2013) may be preferred. However, brine drainage has been shown to remove only approximately 80% of the brine, probably due to entrapment in pockets not connected to the channels (Weissenberger *et al.*, 1992). Hence, it is likely that larger organisms are trapped in the channels when being extracted. When the ice becomes warmer, brine salinities can be < 35 (Paper III), and is thus technically not brine any longer. At this point, melting together with filtered seawater is a suitable choice for both functional and structural variables (Paper III), optimally reaching a final salinity of > 28 for experimental work (Ryan *et al.*, 2004). However, it should be noted that fast responses of some variables, such as photophysiology (e.g. xanthophyll cycle activity, variable fluorescence), may be lost during *ex situ* melting, and alternative methods should therefore be considered.

Spatial patchiness is high in sea ice, and can be explained by high spatial variability in sea ice physicochemical properties. Consequently, patchiness has major implications on sampling design. In order to reduce spatial variability between sea ice cores in Paper III, homogenous sampling plots were selected based on Light Detection And Ranging (LiDAR) scanning (Weissling & Ackley, 2015). LiDAR scanning thereby amended the comparison between sea ice cores from the sampling plot. This scanning was crucial, since the processing of physicochemical and microbiologic parameters differ considerably, and could not be sampled from the same ice cores.

A more realistic, but also more complicated, method that is an alternative to transferring sea ice communities to liquid samples (e.g. melting) is to measure directly in the ice. This method is preferred for many functional variables such as community primary productivity. For instance, microelectrodes can be used under the ice to estimate *in situ* oxygen production and export (McMinn *et al.*, 2010). However, the latter method is only effective for measuring primary productivity in bottom ice communities, and might not be appropriate if the biomass in interstitial layers is high.

In addition, metabolic rates can be estimated by *in situ* incubations with isotopic labelling (Mock, 2002). This technique inevitably provides more realistic estimates of *in situ* production rates, but is still a rather invasive method. Hence, sampling and processing techniques need to be chosen carefully depending on the measured variable.

3.2. Experimental CO_2 manipulation

One of the most significant challenges in simulating ocean acidification projections is that the seawater carbonate system must be appropriately manipulated and measured in order to simulate realistic changes in the carbonate system projected in a high-CO_2 world. Many previous studies have used inappropriate manipulation techniques, but the publication of *The Guide to Best Practices for Ocean Acidification Research and Data Reporting* has significantly improved the choice of manipulation and measuring techniques (Gattuso *et al.*, 2010; Cornwall & Hurd, 2015). Several procedures are available for manipulating the carbonate system in seawater with different pros and cons depending on the studied system (Gattuso *et al.*, 2010). The most common methods to manipulate CO_2 levels in seawater are by aeration of the targeted pCO_2, addition of CO_2-rich water, and addition of acid, CO_3^{2-}, and HCO_3^-. By definition, CO_2 is not parameterised in seawater total alkalinity (A_T) (Dickson, 1981), and remains unaffected by CO_2 changes. Hence, it is favourable if all of the manipulated carbonate system species resemble the projected values of the climate scenario we are simulating. For instance, several studies have implemented the addition of strong acids (i.e. adding hydrogen ions) in systems closed from the atmosphere to achieve a simple and relatively precise control of pCO_2 (Riebesell *et al.*, 2000; Langer *et al.*, 2006; Czerny *et al.*, 2009). However, unless the addition of acid is counterbalanced by the addition of CO_3^{2-}/HCO_3^-, A_T will decrease and consequently result in lower DIC concentrations than what could be expected during the climate scenarios. This finding is especially important when working with primary producers capable of utilising HCO_3^- for photosynthesis. Therefore, it has been suggested that gas bubbling is superior to acid addition when experimenting with algae (Iglesias-Rodriguez *et al.*, 2008). In Papers I, II, and IV, constant bubbling of pre-mixed gases of different pCO_2 controlled the carbonate system. In systems capable of substantial CO_2 perturbation (e.g. photosynthesis, respiration), constant bubbling is preferred over an initial equilibration of the water, as CO_2 uptake will significantly affect the manipulations during algal growth.

When manipulating pCO_2 levels in algal cultures, it is crucial that the biomass is kept sufficiently low so that the drawdown of CO_2 due to primary production does not affect the carbonate system substantially (Gattuso *et al.*, 2010). This observation is especially important to consider when CO_2 levels are manipulated by constantly bubbling the growth medium with pre-mixed gases (as in Papers I, II, and IV), since the equilibration rate of CO_2 must not exceed primary productivity in the system. For example, high primary productivity resulted in slightly lower CO_2 levels compared with the pre-mixed gases in the latter papers, although values were within the expected ranges of pCO_2 changes within the next century (IPCC, 2013, Paper II and IV). However, this was a more significant issue in high-temperature treatments when *N. directa* reached cell densities of approximately 10^5 cells ml^{-1}, which resulted in depletion of CO_2 (Paper I). On the contrary, one of the main advantages of constant bubbling compared with maintaining a fixed pH is that the natural diurnal cycle of pH

levels is sustained (Figure 6A). Microorganisms, and especially sea ice algae that accumulate high densities in a medium with restricted gas transport, never experience a fixed pH in the field. Therefore, it could be ambiguous to expose the organism to a static pH. In addition, the gradual equilibration of the carbonate system that follows constant bubbling with pre-mixed gases (Figure 6B) gives the organisms time to slowly acclimate to the experimental conditions, which may take a few days depending on manipulation technique.

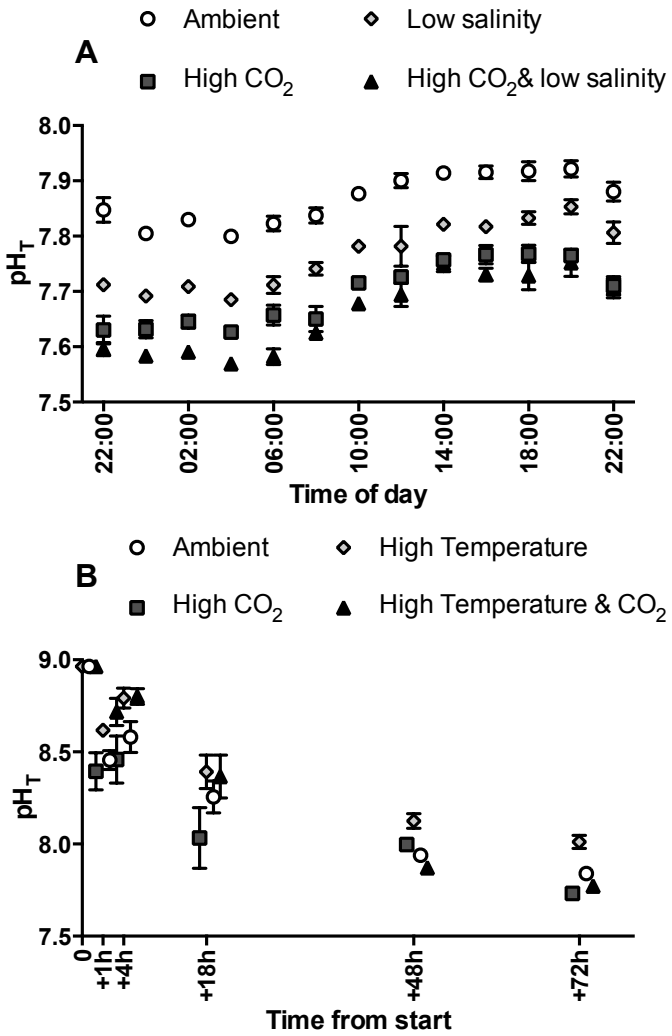

Figure 6. pH_T data from two CO_2 enrichment experiments in which the carbonate system was manipulated by continuous bubbling of pre-mixed air with pCO_2 of 390 (Ambient CO_2) or 960 ppm (High CO_2). A) Diurnal variation of pH_T (at 25 °C) in a mesocosm experiment with a blooming Baltic Sea phytoplankton community. The continuous bubbling enables a natural fluctuation of pH from the diurnal cycle of photosynthesis. B) pH_T (at 15 °C) equilibration during the first 72 h of a laboratory experiment with *Navicula directa* (Paper I). Equilibration of the carbonate system may take several days depending on manipulation technique, and enables the organisms to gradually adjust to altered pH. Error bars in indicate standard error (n = 3).

Although CO_2 levels were controlled by the aeration of pre-mixed gases (i.e. no change in A_T), a relatively high A_T was present in Papers I, II, and VI. The increase of A_T was caused by two reasons, the addition of H_2SiO_4 in the form of $Na_2SiO_3 \times 9H_2O$, and organic alkalinity. When $Na_2SiO_3 \times 9H_2O$ is dissolved in water, SiO_3^{2-} combines with H_2O and forms $H_2SiO_4^{2-}$. This form quickly converts into $H_3SiO_4^-$ and thereby consumes one proton (Equation 2.1). Most of the $H_3SiO_4^-$ is further converted into H_4SiO_4 at the normal seawater pH range, consuming yet another proton (Equation 2.2).

$$H_3SiO_4^- \leftrightarrow H^+ + H_2SiO_4^{2-}, \qquad k_A = 10^{-12.6} \tag{2.1}$$

$$H_4SiO_4 \leftrightarrow H^+ + H_3SiO_4^-, \qquad k_A = 10^{-9.5} \tag{2.2}$$

These equations show that for each mole of $Na_2SiO_3 \times 9H_2O$ that is added, A_T increases by two moles. In the f/2 medium (Guillard, 1975) that was used in Papers I, II, and IV, we added 108 µmol kg^{-1} $Na_2SiO_3 \times 9H_2O$ and thereby increased the alkalinity by approximately 217 µmol kg^{-1} in seawater. Although CO_2 (aq) and pCO_2 will not be affected, it inevitably results in higher CO_3^{2-} and HCO_3^- levels than what would be expected in a high-CO_2 world. Hence, if the utilisation of HCO_3^- is significant in the algae, this artefact may result in an overestimation of the response. The addition of two moles of HCl per mole of $Na_2SiO_3 \times 9H_2O$ will counterbalance the problem, and should be considered for future experiments with high silicate concentrations.

As cells are growing, the A_T changes in a complex manner due to a combination of nutrient transformations and from the production and exudation of organic bases. For instance, nitrogen assimilation affects A_T depending on the nitrogen source and may be one of the major contributors to A_T in algal cultures (Brewer & Goldman, 1976). If nitrate (NO_3^-) is assimilated, A_T will increase by the equimolar amount of removed NO_3^- from the medium, due to the production of hydroxide ions (OH^-). On the other hand, if ammonia (NH_4^+) is the nitrogen source, A_T will decrease due to the release of hydrogen ions (H^+) during protein synthesis. We observed increased A_T values during algal growth in Papers I, II, and IV, which correlates well with cell density (Figure 7).

Figure 7. Unpublished data. Not available electronically.

Figure 8. Unpublished data. Not available electronically.

This increase is probably due to a combination of nitrate uptake and organic alkalinity, and may result in an overestimation of A_T derived from potentiometric titration. When over-determining the carbonate system with data from three parameters (pH, A_T, DIC) in one experiment, the error in A_T measurements results in an underestimation of pCO_2 (Figure 8). Hence, biologically mediated changes in A_T are an additional explanation of why pCO_2 is lower than expected in all experiments included in this thesis. Therefore, it is recommended to use additional parameters beyond A_T and pH, such as DIC, for a higher accuracy when describing the carbonate system in samples with high levels of organic alkalinity, such as algal cultures. By overestimating the carbonate system, we can estimate the error from organic A_T, and could potentially predict its influence via cell number and growth rate.

3.3. Sea ice diatom culturing

Due to the complex microstructure of brine channels that was described above, the ecophysiology of sea ice algae is difficult to study in their natural environment. Experiments on community responses are often performed directly in the field (Ryan *et al.*, 2004; McMinn *et al.*, 2014), but in order to isolate mechanistic physiological responses to environmental changes, single-species experiments are more desirable. Therefore, sea ice algae are often cultured in seawater at low temperature (Mitchell & Beardall, 1996; Mock & Hoch, 2005; Lyon *et al.*, 2011, Paper I, II and IV). Strains of psychrophilic sea ice diatoms in Papers I, II, and IV were isolated from ice cores that were thawed with 0.2 µm filtered seawater for 12-20 h to avoid osmotic stress. Cultures were subsequently established by single cell isolation and maintained at -1.8 °C and salinity of 34 until the start of experiments (from 18 to 24 months, i.e. relatively fresh cultures). Hence, it should be highlighted that the experimental conditions in this thesis (Papers I, II, and IV) do not completely mimic the extreme

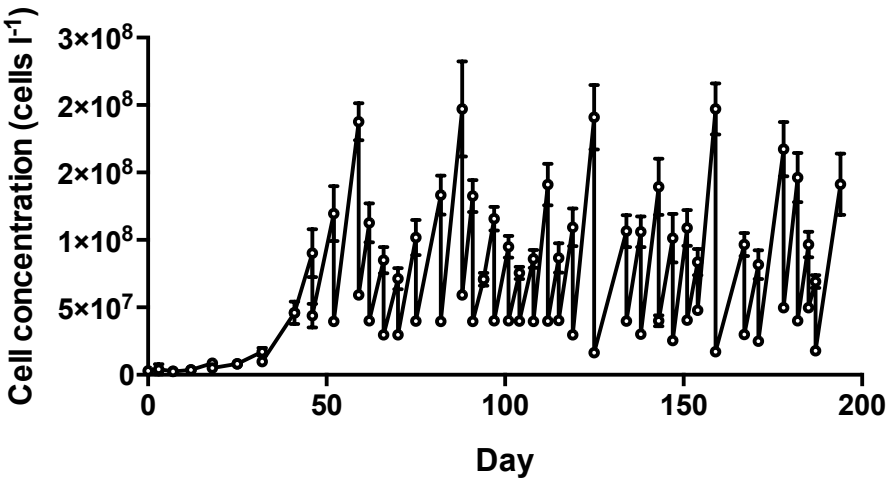

Figure 9. Cell concentration in semi-continuous dilution cultures during 194 days of experiment (Paper IV). Cultures were diluted with fresh f/2 medium twice a week for 194 days, thereby maintaining active growth at cell concentration between 3×10^7 to 2×10^8 cells L^{-1}. Error bars indicate standard error ($n = 15$).

conditions that can occur in sea ice. However, the conditions in Papers II and IV closely resemble the temperatures and salinities found in Antarctic summer pack ice (Paper III), or conditions similar to what occurs when sea ice algae are dispersed into the water column after ice breakup. It should also be noted that these diatom species do not exclusively grow in sea ice, but are also abundant in shallow benthic ecosystems (Horner & Schrader, 1982; Al-Handal & Wulff, 2008; Wulff *et al.*, 2008) and as phytoplankton upon ice breakup (Horner & Schrader, 1982; Riaux-Gobin *et al.*, 2011).

To exclude the interference of growth limitation in experimental set-ups, algal cultures were maintained in nutrient-replete growth media, i.e. f/2 medium (Guillard, 1975). These nutrient levels are substantially higher than what algae experience in summer sea ice (Fransson *et al.*, 2011). Different culturing techniques are available for allowing the cultures to continue growing in the exponential phase. Batch culture techniques were used in Papers I and II, and are easy to use and reliable for short-term experiments. However, when performing longer experiments (Paper IV), continuous or semi-continuous cultures are preferred to maintain the cultures in the exponential phase over a longer period. Semi-continuous cultures were established in Paper IV by diluting the cultures with fresh medium roughly twice a week (Figure 9).

4. Results and discussion

4.1. Approaches to study the ecophysiology of sea ice microorganisms

Physiological measurements under climate change scenarios and community composition of natural sea ice microbial ecosystems are required to address how different stressors may influence organisms' capacity to tolerate both naturally- and climatically-driven changes. The responses of sea ice microorganisms to environmental changes are complex and include influences from interacting factors, acclimation, and adaptation. In laboratory experiments (Papers I, II, and IV) and field measurements (Paper III), the ecophysiological responses of sea ice microorganisms were addressed and are discussed in detail below.

4.2. Combined effects of increased temperature and pCO_2 on sea ice diatoms

Growth of both of the sea ice diatoms *N. directa* (from Svalbard) and *N. lecointei* (from Ross Sea) responded positively to temperature increased by approximately 4 °C (Papers I and II). In addition, *N. lecointei* had an optimal growth around 5 °C (Paper II), which illustrates the psychrophilic character of this species. Increased temperature also stimulated primary productivity, as well as maximum and effective quantum yield of photosystem II (PSII) (Papers I and II). Measurements of chlorophyll fluorescence provide information about the efficiency of PSII and thereby the photosynthetic capacity, which was assessed through different quantum yields depending on the cells' light adaptation state. In addition, concentration of polyunsaturated fatty acids (PUFAs) was four times higher at low temperature (-1.8 °C) compared with 2.5 °C (Paper II), which illustrated the requirement of unsaturated fatty acids at low temperature to maintain membrane fluidity (Bowman, 2008).

On the other hand, increased pCO_2 resulted in various responses in the two diatom species on a short time scale. First of all, growth rates in *N. directa* were significantly reduced by 5% when cultures were bubbled with an air mixture of 960 µatm pCO_2 (Paper I). Although CO_2 was drawn down in the high-temperature treatment (~ 20% of the DIC), this effect persisted, suggesting that differences in the carbonate system were apparent earlier in the experiment. The effect could potentially be an artefact resulting from improper acclimation (as discussed under Section 3.2. Experimental CO_2 manipulation). However, due to the relatively slow equilibrium of the carbonate system (Figure 6B), we exposed the cells to a gradual change in pH instead of a rapid change that could trigger shock responses. We also showed that the effect of increased pCO_2 could depend on experimental temperature and may affect organisms in an antagonistic manner (Paper II). At low temperature (-1.8 °C), elevated pCO_2 did not have an effect on growth in *N. lecointei*. On the other hand, there was a positive effect (6% increase) at high temperature (2.5 °C) and elevated CO_2. In addition, synergism between the two treatments was also observed in fatty acid (FA) content (Paper II); when grown at -1.8 °C, cellular FA content was reduced by 37% at elevated CO_2. Nevertheless, at 2.5 °C there was no effect of CO_2 level on FA content. Changes in the environment seldom occur individually, and this study illustrates the importance of synergism in physiological responses to climate change. As many physiological responses in polar areas are limited by narrow thermal windows (e.g. Pörtner, 2002), synergism with temperature may be especially important for psychrophiles.

Therefore, it is important to select relevant experimental temperatures when designing and interpreting culture experiments, as the response of elevated pCO_2 may depend on it. Temperature should be chosen from ecologically relevant scales (e.g. carefully considering the site of origin for the strain) to correctly mimic changes that may occur in the future. Hence, growing psychrophiles at optimal growth temperature may thereby yield little ecological relevance when combined with additional stressors.

4.3. Perspective of time

In paper IV, we addressed the long-term acclimation of *N. lecointei* to three pCO_2 levels (280, 390, and 960 µatm) during an experiment lasting 194 days. These results revealed new insights in acclimation rates to ocean acidification. We showed that when integrated over a longer time, accumulated generations were reduced by 3–4% after long-term cultivation at 960 µatm pCO_2 compared with 280 and 390 µatm pCO_2. Accumulation of generations was performed to acquire a proxy for integrated growth rate over time. This observation is complementary to the study in Paper II, where no change in growth rate was observed at the same temperature after 14 days of experiment, although changes were in a similar range as in *N. directa* (Paper I). This small reduction did not appear until > 140 days, which suggests that it was a result of long-term acclimation. Meanwhile, increased pCO_2 resulted in *carbon overconsumption*, a process that may occur when the assimilation of carbon relative to nitrogen and phosphorus increases as compared with the Redfield ratio of 106C:16N:1P (Toggweiler, 1993). In this study, DOC release was interpreted as a stress response to increased pCO_2. In turn, DOC release from *N. lecointei* promoted bacterial productivity rates, probably due to the release of labile dissolved organic carbon. Similar observations have recently been described in Arctic planktonic communities during a mesocosm experiment (Engel *et al.*, 2013). Elevated CO_2 levels may lead to a shift in the ocean's elemental ratios, which could control the fate of key biogeochemical cycles such as primary and bacterial productivity.

The majority of all ocean acidification studies are designed to mimic climate change scenarios projected to occur within the next century (e.g. ~1,000 µatm pCO_2, IPCC (2013). Still, experiments addressing such issues are generally performed during relatively short experimental durations that do not account for proper acclimation (< 1 month) (e.g. Yang & Gao, 2012; Trimborn *et al.*, 2013; McMinn *et al.*, 2014, Paper I and II). Although short-term changes in physiology give us an idea of how organisms may respond initially to environmental change, acclimation and adaptation to climate change will play an important role within the next century (Garrard *et al.*, 2013).

The rate of climate change will affect organisms differently depending on life strategy, and time is only subjective to its observer. Considering mutation rates, polar organisms with slow generation times will probably have less potential to adapt to a changing world. Ominously, the rate of climate change is also faster in polar regions compared with the rest of the planet. Therefore, polar organisms are particularly susceptible to environmental change. However, to date, only a few experimental studies have addressed acclimation and adaptation in psychrophiles (Novitsky & Morita, 1977; Mock & Hoch, 2005; McKeown *et al.*, 2009). Addressing long-term acclimation in psychrophilic organisms is especially important due to their slow growth rates. Considering enzymatic and mutational rates at low temperatures, acclimation and adaptation will probably occur at a slower rate in psychrophiles

compared with temperate and tropical species. However, most previous long-term experiments have been performed on fast-growing phytoplankton capable of generating high numbers of generations in a relatively short time (Lohbeck *et al.*, 2012; Low-Décarie *et al.*, 2013; Tatters *et al.*, 2013a; Tatters *et al.*, 2013b; Bermúdez *et al.*, 2015). Long-term studies on marine and freshwater phytoplankton have shown varying growth responses to high pCO_2. For instance, Schaum and Collins (2014) grew the chlorophyte *Ostreococcus* lineages for 400 generations and observed high growth rates, although the growth rate at high pCO_2 was not reduced until 100 cell cycles had elapsed. In contrast, Low-Décarie *et al.* (2013) reported increasing growth rates after < 340 generations (184 days) of high pCO_2 conditioning in various freshwater species of diatoms, chlorophytes, and cyanobacteria. In addition, no difference in growth rate was reported after 1,000 generations in the chlorophyte *Chlamydomonas reinhardtii* grown at ambient and high pCO_2 (Collins & Bell, 2004). In contrast to other long-term studies, there were considerably fewer cell cycles in our experiment (~60 generations) due to the naturally slow growth rate of ice algae. Hence, the potential for biological adaptation is less plausible in our experiment. In contrast, slow-growing organisms will also accumulate fewer cell cycles within the next century, and acclimation to climate change will be an important factor in determining polar species' responses to climate change. In addition, polar organisms already living at the extremes will have less room for mitigation. Many temperate species have the potential to change spatial distribution when conditions are unfavourable, e.g. shifting pole-wards during ocean warming. As this re-distribution is impossible for polar organisms, psychrophiles may be the organism group most affected by climate change.

It has previously been noted that responses to elevated pCO_2 are highly species- and strain-specific (Kremp *et al.*, 2012; Tatters *et al.*, 2013a; Pančić *et al.*, 2015). These findings limit the interpretability of single-species laboratory experiments, and extrapolations should be performed with care. Although single-species experiments provide useful information about mechanistic behaviours related to climate change, they do not account for the many indirect ecological effects that may influence biological interpretation. In order to understand how environmental and biological changes combined with indirect ecological effects (e.g. DOC release from carbon overconsumption) will influence ecological processes, there is a need to study multiple stressors and multiple species in combination.

4.4. Small temperature changes affect physiology and diversity of sea ice algae and bacteria

We showed that temperature plays an important role in controlling the physiology and diversity of psychrophilic organisms. For instance, an increase by approximately 4 °C resulted in 43 and 50% higher growth rates in *N. directa* and *N. lecointei*, respectively (Papers I and II). The species 'temperature window' for growth is much narrower in psychrophiles such as polar algae, compared with species isolated from temperate and tropical oceans (Eppley, 1972; Boyd *et al.*, 2013). For instance, *Phaeodactylum tricornutum* is known to grow quickly (> 0.5 day^{-1}) between 5 and 25 °C (Kudo *et al.*, 2000). These findings mean that the impact of small temperature changes can have more severe consequences in cold-adapted species. On the contrary, it might seem ambiguous that optimal growth temperature is higher than what the algae would experience in the field (e.g. 5 °C in Paper II). This observation is also true for many

mesophillic species, i.e. species that grow best in moderate temperatures. For example, the average soil temperature is 12 °C in temperate climate, although the optimum growth rate of mesophilic species usually occurs between 20 and 45 °C (Schlegel & Jannasch, 1981). Nevertheless, this observation can be explained by the large variation in temperature that temperate species are required to cope with. Sea ice microorganisms, on the other hand, rarely reach their optimum growth temperature in nature. However, it should be noted that the optimal temperature only reflects kinetic effects and that optimal growth temperature is not necessarily a sign of optimal adaptation. Microorganisms' growth responses to temperature normally follow the bell-shaped relationship between enzyme-catalysed reactions and temperature, i.e. an initially exponential response until optimal temperature is reached, followed by more a rapid decrease in activity beyond the optimum due to denaturation (e.g. Nichols *et al.*, 2000). In addition, it should be noted that the optimal temperatures for growth and photosynthesis do not always coincide (Mackey *et al.*, 2013), since growth is also a combined result of other metabolic processes such as nutrient assimilation. Therefore, cells need to constantly balance photosynthetic rates with other cellular metabolic demands (Mackey *et al.*, 2013). From an ecological perspective, it may be disadvantageous for an organism to grow at its optimum, as the maximum temperature is generally close to the optimum, and a sudden increase may result in the denaturation of proteins. In addition, the ability to grow slowly may be advantageous in oligotrophic environments, to avoid nutrient exhaustion and starvation. Hence, temperature responses may be different in the field compared with the laboratory, where cells are grown under optimal conditions. Therefore, data from laboratory studies should be treated with care, and the species' natural temperature environment should always be considered.

Temperature also plays an important role in structuring natural sea ice microbial communities. In Paper III, we describe how diversity of sea ice bacteria is correlated negatively with increased *in situ* temperatures and positively with theoretical brine salinity (Figure 10). As salinity is traditionally described as a function of temperature with a series of phase equations (Frankenstein & Garner, 1967), it is impossible to tell if this was a direct effect of temperature or an indirect effect from decreased salinity.

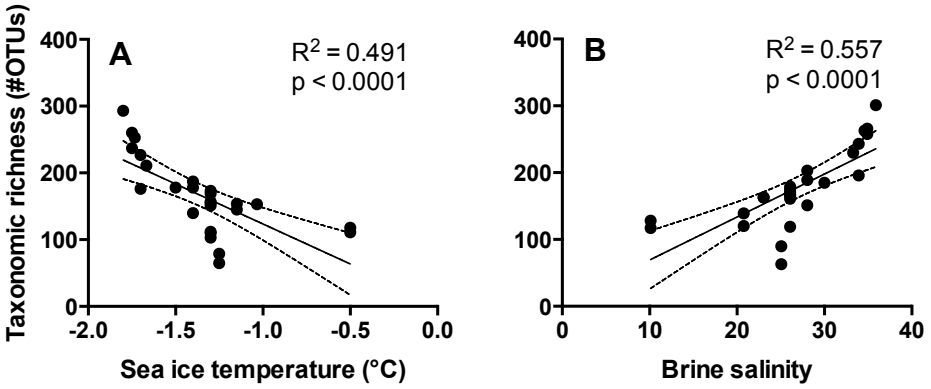

Figure 10. Environmental control of 16S rRNA gene richness and diversity (from Paper III). A) Temperature dependency of taxonomic richness (expressed as number of operational taxonomic units, OTUs). Increasing temperature (from -1.8 to -0.5) significantly decreases taxonomic richness ($p < 0.0001$, Pearson's correlation). B) Temperature dependency of calculated brine salinity. Increasing brine salinity significantly increases taxonomic richness ($p < 0.0001$, Pearson's correlation). Dashed lines show ± 95% confidence interval of the fitted line.

On the other hand, considering the ranges of salinity (10 to 35) and temperature (-1.8 to -0.5 °C), it is more likely that salinity exerts a greater physiological stress on the sea ice microorganisms. However, it should be noted that these are theoretical salinities directly derived from temperature, bulk salinity, and brine volume calculated the equations in Frankenstein and Garner (1967). These relationships assume no significant contribution of biomass in the brine, and EPS excreted from microorganisms may cause the brine salinity to deviate significantly from this relationship (Krembs *et al.*, 2011).

4.5. Dominance of kleptoplastic dinoflagellates in Antarctic sea ice

In Paper III, we described protist diversity in bottom sea ice of the Amundsen and Ross Seas (Figure 11) using 454-sequencing of the 18S ribosomal RNA gene. The pack ice was in a break-up stage, with relatively warm ice temperatures (-1.8 to -0.5 °C), and was exclusively dominated by a dinoflagellate closely related to the kleptoplastic phylotype described in Gast *et al.* (2006). It is generally believed that diatoms dominate the polar oceans, including the sea ice ecosystem (Arrigo *et al.*, 2010). Diatoms are the most studied sea ice organisms, although a vast diversity of flagellated species are associated with sea ice. For instance, the upper and slushy layers of sea ice are often dominated by dinoflagellates, such as the unique sea ice species *Polarella glacialis* (Montresor *et al.*, 1999; McMinn *et al.*, 2014) and a

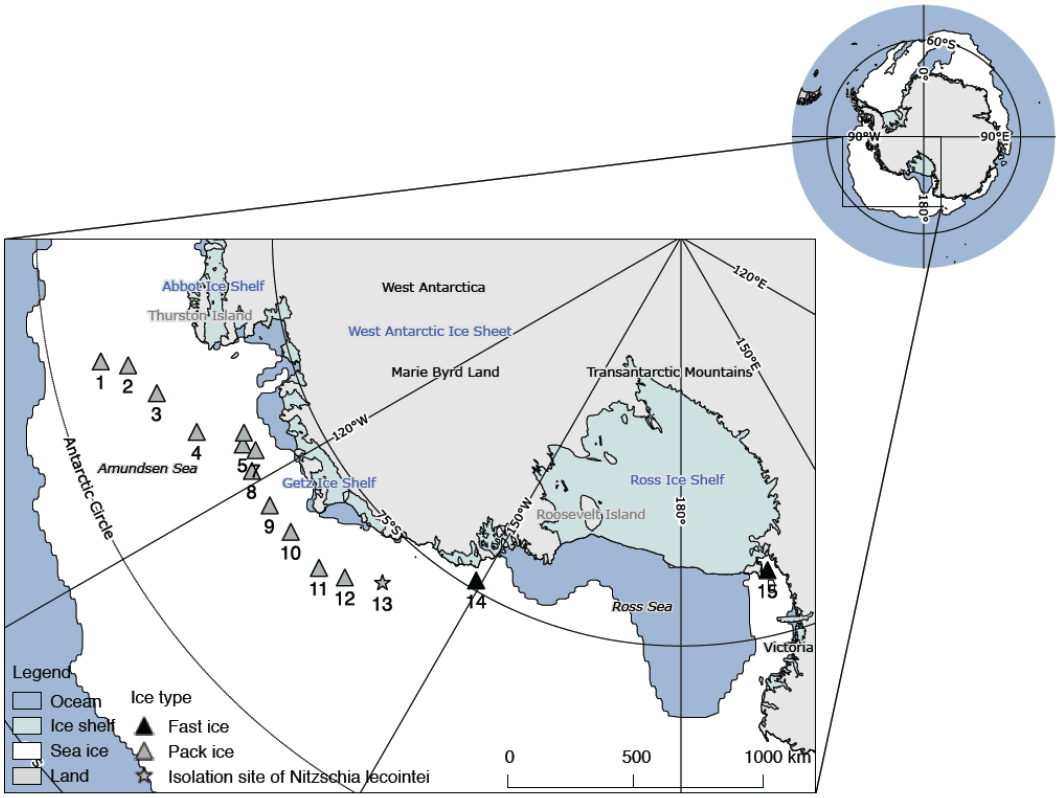

Figure 11. Sea ice sampling stations (station numbers 1-15) during the Oden Southern Ocean 2010/11 cruise. Bottom sea ice was sampled for microbial diversity at all stations (16S and 18S rRNA genes, Paper III). *Nitzschia lecointei* was isolated from sea ice at station 13 and used for experimental studies (Papers II and IV). Data of average sea ice extent from December 2010 were provided by the National Snow and Ice Data Center (www.nsidc.org). Map was created using the Quantarctica QGIS package, developed by the Norwegian Polar Institute (www.quantarctica.org).

kleptoplastic dinoflagellate phylotype (Gast *et al.*, 2006; Gast *et al.*, 2007). Dominance of heterotrophic dinoflagellates has also been recorded in Arctic sea ice during the polar night (Bachy *et al.*, 2011), and of small unidentified flagellates (< 6 μm) in the Beaufort Sea (Horner & Schrader, 1982). However, interior and sea ice are generally reported to be exclusively dominated by diatoms in the growing season of ice algae. Although cyanobacteria are abundant and play an important role in many freshwater polar ecosystems, they are often rare in sea ice (Koh *et al.*, 2012, Paper III).

As discussed in Paper III, naked dinoflagellates have previously been observed in high abundances in the slush layer of sea ice (Garrison & Buck, 1989; Gast *et al.*, 2006). From 18S rRNA gene clone library data, Gast et al. (2006) reported the dominance of a novel dinoflagellate phylotype, closely related to *Karenia* and *Karlodinium* species, in the slush layer of sea ice in the Ross Sea. Previously, this phylotype had been believed to be rare in interior and bottom Antarctic sea ice (Gast *et al.*, 2006). Other dinoflagellates such as *Polarella glacialis* have also been observed in high abundances, but have been primarily restricted to the upper section of land-fast ice (Montresor *et al.*, 1999; McMinn *et al.*, 2014). It is still unknown why the prevalence of dinoflagellates in sea ice that has been reported using molecular techniques (Gast *et al.*, 2006; Bachy *et al.*, 2011) is not always reflected in traditional microscopy approaches. One possible explanation could be that dinoflagellates are under-sampled due to their fragile cell structures, and may not remain structurally intact during the stressful processing techniques of sea ice samples (e.g. thawing or centrifuging; see Section 3.1. Sea ice sampling) (Garrison & Buck, 1986). In addition, the lack of distinctive morphologies of gymnodinoid dinoflagellates makes them very difficult to identify using light microscopy (Gast *et al.*, 2006). The presence of dinoflagellates and their ecological importance in Southern Ocean sea ice may be underestimated and need to be further addressed, especially in summer pack ice.

5. Conclusions and future perspectives

In this thesis, I focus on the ecophysiology of sea ice-associated microorganisms (primarily algae, but also bacteria). On the basis of laboratory and field studies, conclusions can be drawn about the acclimation to environmental stressors affecting the physiology and community composition of sea ice organisms. First of all, increasing temperature (on both climatic and seasonal scales) positively affected the physiology of two sea ice diatom species (Papers I and II), but negatively affected the taxonomic richness and diversity of sea ice bacterial communities (Paper III), likely by the subsequent change in salinity. On the other hand, increased pCO_2 had only minor effects (positive and negative) on growth, ranging from 3–6% (Papers I, II and IV). In addition, pH did not explain much of the variability in the microbial community composition of Antarctic sea ice (Paper III). In terms of growth, sea ice algae seem quite tolerant to changes in pH and pCO_2 (Paper I, II and IV), probably due to the fact that they grow in an environment with large seasonal variations in the carbonate system. Other climate-related problems, such as ice thinning and increased temperature, probably play a greater role than ocean acidification in sea ice algal growth. However, increased pCO_2 also resulted in other physiological changes that may have important ecological consequences, such as cellular stoichiometry. For instance, we observed changes in carbon metabolism (Paper IV), and fatty acid content and composition (Paper II), that did not affect the growth rate. As sea ice algae are an important food source for many grazers, changes in their fatty acid profile may have large ecological consequences through bottom-up effects. Bottom-up effects have already been observed in western Antarctica, where phytoplankton primary production has been reduced by rapid climate change, and is believed to have affected krill and penguin populations (Montes-Hugo *et al.*, 2009). In addition, the reduction of unsaturated fatty acids may reduce their thermo-tolerance, which remains to be tested. Changes in carbon metabolism, such as carbon overconsumption, may have significant effects on bacterial mineralisation of primary-produced organic matter and could indirectly affect the microbial loop. Although growth rate is a principal variable in this thesis, it does not always reflect the large number of cellular changes that occur in diatoms at reduced pH. Therefore, we might underestimate the ecological consequences of ocean acidification by only interpreting the growth rate data of algae.

When interpreting results from experiments involving simulated climate change, it is also important to consider the targeted organisms' natural environment. For instance, many studies use the Mauna Loa Observatory pCO_2 measurements (today 403 µatm) as a reference for the control group. As pCO_2 varies greatly globally, it is important to consider the natural levels and variation of the targeted species when interpreting climate change experiments. Since pCO_2 is highly variable in sea ice, a larger span of different pCO_2 values could provide more insight into tolerance levels and reveal physiological tipping points of the cells. For instance, pCO_2 may be much higher than 960 µatm in the brine when the ice is forming and CO_2 is being enriched (e.g. Fransson *et al.*, 2015). We also showed that the choice of temperature may influence the outcome of the experiment (Paper II). Therefore, it is crucial to select realistic and ecologically relevant temperatures in laboratory experiments, based on the temperature range at the origin of collection of the organism. In addition, we also showed that experimental duration is crucial when performing laboratory-based ocean acidification experiments (Paper IV). Acclimation and adaptation to global change are

therefore key components in understanding how organisms will respond to a future ocean, and cannot be neglected when interpreting short-term experiments.

Data from sequencing of the 18S and 16S rRNA genes from natural communities revealed that both the diversity and the complexity of sea ice microbial communities might be greater than previously believed in the Southern Ocean sea ice (Paper III). However, sequencing of DNA only reflected the standing stock community of microorganisms in sea ice. Due to the fact that sea ice is also an important medium for many protist resting stages (Buck *et al.*, 1992) and can contain high amounts of metabolically inactive material (Melnikov, 1997; Granfors *et al.*, 2013), DNA sequencing may be biased towards the inactive ecosystem. Therefore, sequencing of RNA could supply more information about the active ecosystem, and RNA sequence diversity would provide an important dimension in the understanding of microbial sea ice ecosystems.

In summary, my studies of the ecophysiology of sea ice microorganisms provide new insights into how psychrophilic species may respond and acclimate to a changing environment. Since sea ice microbial communities also seed the pelagic production (Riaux-Gobin *et al.*, 2011) and provide a direct link to higher trophic levels through grazing (O'Brien, 1987), changes in these communities may have significant consequences for the biogeochemistry (e.g. primary production) of polar ecosystems as such (Montes-Hugo *et al.*, 2009). Global warming is a key factor that is affecting many polar microorganisms, and will significantly change the polar environment. However, sea ice diatoms appear rather tolerant to increased CO_2 levels and decreased pH (Papers I, II and IV), although processes that could have important ecological consequences may be affected (Papers II and IV). To fully understand the consequences of ocean acidification in polar areas, the ecological and biogeochemical importance of carbon overconsumption and fatty acid stoichiometry in a high-CO_2 world should be further addressed and quantified. This may be accomplished by multi-trophic experimental setups capable of tracing fatty acids, carbon trough food webs, and biogeochemical cycles.

Acknowledgements

First and foremost I would like to thank my supervisor Angela. You are a true source of inspiration and the best supervisor that anyone could ask for! You make up for your lack of snowmobile-driving skills in pedagogics and with a good humor (except before lunch, of course). Thank you Melissa and Agneta for teaching me about marine carbonate chemistry, taking me on various adventures and introducing me to the polar fever. Also thank you Kristina for sticking with me as my examiner, even though your retirement in Portugal may be very temping by now!

I would like to thank all fantastic expeditions buddies - the GreMeCA-lites, Carlini-mates, Disco-team and OSO10/11 fellows. No polar adventure would be the same without you! I would also like to thank the captain and crew of *IB Oden* for their support, and the Swedish Polar Research Secretariat, US National Science Foundation, Alfred Wegener Institute for logistical support before and during various expeditions. Thanks to all the present and former members of the Wulff-Pack. I will be ever grateful to Mikael for all your your assistance in the field, lab and by the computer. I look forward to show you how horrible penguin smell! Thank you all wonderful colleagues on the fifth floor who turn the labs, offices, teachings halls, corridors and the lunchroom into great places! I am sorry for turning the common (used to be) thermoconstant room into a constant polar room. Also, thank you Findus for offering frozen delicacies straight from Morocco (probably).

I would like to thank Adil for your help with species identification and photographing of my algal cultures. Also, thank you Monica for all your assistance in the lab and with various instruments. I would like to give a special thanks to Carlos for being an excellent host in Malaga, and to all fantastic friends and colleagues at the UMA. I am now convinced that it is possible to do polar research in southern Spain!

Marie, thank you for all your patience and support, even when I have been delayed in some remote location far away. I could never have finished this this thesis without your support and your excellent door-to-door seawater-delivery service. Soon you will see more of me, I promise!

I would like to give a special thanks to my family for all your support during my studies, and for convincing me to go to the university instead of working in the Kumlakorv factory. I look forward to our soon-to-be traditional Christmas lunch in March! Thank you all my friends who knows that number 41 is the best, and to everyone who have helped me to escape reality and thesis writing by various underwater activities.

Finally I would like to acknowledge the financial support from the Adlerbertska Research foundation, Campus de Exelencia Internacional del Mar (CEIMAR), Helge Ax:son Johnsons foundation, Herbert and Karin Jacobssons foundation, Johan and Jakob Söderbergs foundation, Lars Hierta Memorial foundation, Längmanska Cultural foundation, Royal Society of Arts and Sciences in Gothenburg (KVVS), Wilmer and Martina Lundgren foundation and the Ymer –80 foundation.

References

Al-Handal A, Y., Wulff A (2008) Marine benthic diatoms from Potter Cove, King George Island, Antarctica. Botanica Marina, **51**, 51-68.

Arrigo KR, Mock T, Lizotte MP (2010) Primary producers and sea ice. In: *Sea Ice.* (ed Thomas DN, Dieckmann, G.S.) pp 283-325. Oxford, Wiley-Blackwell.

Arrigo KR, Thomas DN (2004) Large scale importance of sea ice biology in the Southern Ocean. Antarctic Science, **16**, 471-486.

Arrigo KR, Worthen DL, Lizotte MP, Dixon P, Dieckmann G (1997) Primary production in Antarctic sea ice. Science, **276**, 394-397.

Aslam SN, Cresswell-Maynard T, Thomas DN, Underwood GJC (2012) Production and characterization of the intra- and extracellular carbohydrates and polymeric substances (EPS) of three sea-ice diatom species, and evidence for a cryoprotective role for EPS. Journal of Phycology, **48**, 1494-1509.

Bachy C, López-Garcia P, Vereshchaka A, Moreira D (2011) Diversity and vertical distribution of microbial eukaryotes in the snow, sea ice and seawater near the north pole at the end of the polar night. Frontiers in Microbiology, **2**, 106.

Beardall J, Stojkovic S (2006) Microalgae under global environmental change: Implications for growth and productivity, populations and trophic flow. Science Asia, **32**, 1-10.

Becquevort S, Dumont I, Tison JL, Lannuzel D, Sauvée ML, Chou L, Schoemann V (2009) Biogeochemistry and microbial community composition in sea ice and underlying seawater off East Antarctica during early spring. Polar Biology, **32**, 879-895.

Bermúdez R, Feng Y, Roleda MY, Tatters AO, Hutchins DA, Larsen T, Boyd PW, Hurd CL, Riebesell U, Winder M (2015) Long-term conditioning to elevated pCO_2 and warming influences the fatty and amino acid composition of the diatom *Cylindrotheca fusiformis*. PLoS ONE, **10**, e0123945.

Bowman JP (2008) Genomic analysis of psychrophilic prokaryotes. In: *Psychrophiles: from Biodiversity to Biotechnology.* (eds Margesin R, Schinner F, Marx J-C, Gerday C) pp 265-284. Springer Berlin Heidelberg.

Bowman JS, Rasmussen S, Blom N, Deming JW, Rysgaard S, Sicheritz-Ponten T (2012) Microbial community structure of Arctic multiyear sea ice and surface seawater by 454 sequencing of the 16S RNA gene. ISME Journal, **6**, 11-20.

Boyd PW, Rynearson TA, Armstrong EA, Fu F, Hayashi K, Hu Z, Hutchins DA, Kudela RM, Litchman E, Mulholland MR, Passow U, Strzepek RF, Whittaker KA, Yu E, Thomas MK (2013) Marine phytoplankton temperature versus growth responses from polar to tropical waters – outcome of a scientific community-wide study. PLoS ONE, **8**, e63091.

Brewer PG, Goldman JC (1976) Alkalinity changes generated by phytoplankton growth. Limnology and Oceanography, **21**, 108-117.

Buck KR, Bolt PA, Bentham WN, Garrison DL (1992) A dinoflagellate cyst from Antarctic sea ice. Journal of Phycology, **28**, 15-18.

Caldeira K, Wickett ME (2003) Oceanography: Anthropogenic carbon and ocean pH. Nature, **425**, 365.

Collins S, Bell G (2004) Phenotypic consequences of 1,000 generations of selection at elevated CO_2 in a green alga. Nature, **431**, 566-569.

Cornwall CE, Hurd CL (2015) Experimental design in ocean acidification research: Problems and solutions. ICES Journal of Marine Science: Journal du Conseil, doi:10.1093/icesjms/fsv118, *in press*.

Czerny J, Barcelos e Ramos J, Riebesell U (2009) Influence of elevated CO_2 concentrations on cell division and nitrogen fixation rates in the bloom-forming cyanobacterium *Nodularia spumigena*. Biogeosciences, **6**, 1865-1875.

Devos N, Ingouff M, Loppes R, Matagne RF (1998) RUBISCO adaptation to low temperatures: A comparative study in psychrophilic and mesophilic unicellular algae. Journal of Phycology, **34**, 655-660.

Dickson AG (1981) An exact definition of total alkalinity and a procedure for the estimation of alkalinity and total inorganic carbon from titration data. Deep Sea Research (Part I, Oceanographic Research Papers), **28**, 609-623.

Dickson AG (1990) Standard potential of the reaction: $AgCl(s) + OH_2(g) = Ag(s) + HCl(aq)$, and the standard acidity constant of the ion HSO_4 in synthetic seawater from 273.15 to 318.15 K. Journal of Chemical Thermodynamics, **22**, 113-127.

Dickson AG, Millero FJ (1987) A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep Sea Research (Part I, Oceanographic Research Papers), **34**, 1733-1743.

Engel A, Borchard C, Piontek J, Schulz K, Riebesell U, Bellerby R (2013) CO_2 increases ^{14}C-primary production in an Arctic plankton community. Biogeosciences, **10**, 1291-1308.

Eppley RW (1972) Temperature and phytoplankton growth in the sea. Fishery Bulletin, **70**, 1063-1085.

Fahl K, Kattner G (1993) Lipid Content and fatty acid composition of algal communities in sea-ice and water from the Weddell Sea (Antarctica). Polar Biology, **13**, 405-409.

Flemming H-C, Wingender J, Griegbe T, Mayer C (2000) Physico-chemical properties of biofilms. In: *Biofilms: recent advances in their study and control.* (ed Evans LV) pp 19–34. Amsterdam, Harwood Academic Publishers.

Frankenstein G, Garner R (1967) Equations for determining the brine volume of sea ice from -0.5 °C to -22.9 °C. Journal of Glaciology, **6**, 943-944.

Fransson A, Chierici M, Abrahamsson K, Andersson M, Granfors A, Gårdfeldt K, Torstensson A, Wulff A (2015) CO_2-system development in young sea ice and CO_2 gas exchange at the ice/air interface mediated by brine and frost flowers in Kongsfjorden, Spitsbergen. Annals of Glaciology, **56**, 245-257.

Fransson A, Chierici M, Miller LA, Carnat G, Shadwick E, Thomas H, Pineault S, Papakyriakou TN (2013) Impact of sea-ice processes on the carbonate system and ocean acidification at the ice-water interface of the Amundsen Gulf, Arctic Ocean. Journal of Geophysical Research: Oceans, **118**, 7001-7023.

Fransson A, Chierici M, Yager PL, Smith WO (2011) Antarctic sea ice carbon dioxide system and controls. Journal of Geophysical Research: Oceans, **116**, C12035, doi:12010.11029/12010JC006844.

Garrard S, Hunter RC, Frommel AY, Lane AC, Phillips JC, Cooper R, Dineshram R, Cardini U, McCoy SJ, Arnberg M, Rodrigues Alves BG, Annane S, de Orte MR, Kumar A, Aguirre-Martínez GV, Maneja RH, Basallote MD, Ape F, Torstensson A, Bjoerk MM (2013) Biological impacts of ocean acidification: A postgraduate perspective on research priorities. Marine Biology, **160**, 1789-1805.

Garrison D, Buck K (1986) Organism losses during ice melting: A serious bias in sea ice community studies. Polar Biology, **6**, 237-239.

Garrison D, Buck K (1989) The biota of Antarctic pack ice in the Weddell sea and Antarctic Peninsula regions. Polar Biology, **10**, 211-219.

Gast RJ, Moran DM, Beaudoin DJ, Blythe JN, Dennett MR, Caron DA (2006) Abundance of a novel dinoflagellate phylotype in the Ross Sea, Antarctica. Journal of Phycology, **42**, 233-242.

Gast RJ, Moran DM, Dennett MR, Caron DA (2007) Kleptoplasty in an Antarctic dinoflagellate: Caught in evolutionary transition? Environmental Microbiology, **9**, 39-45.

Gattuso J-P, Epitalon J-M, Lavigne H (2015) seacarb: Seawater Carbonate Chemistry. R package version 3.0.8. http://CRAN.R-project.org/package=seacarb

Gattuso JP, Gao K, Lee K, Rost B, Schulz KG (2010) Approaches and tools to manipulate the carbonate chemistry. In: *Guide to best practices for ocean acidification research and data reporting*. (eds Riebesell U, Fabry VJ, Hansson L, Gattuso JP). Luxembourg, Publications Office of the European Union.

Gleitz M, Rutgers v.d. Loeff M, Thomas DN, Dieckmann GS, Millero FJ (1995) Comparison of summer and winter inorganic carbon, oxygen and nutrient concentrations in Antarctic sea ice brine. Marine Chemistry, **51**, 81-91.

Golden KM, Ackley SF, Lytle VI (1998) The percolation phase transition in sea ice. Science, **282**, 2238-2241.

Granfors A, Andersson M, Chierici M, Fransson A, Gårdfeldt K, Torstensson A, Wulff A, Abrahamsson K (2013) Biogenic halocarbons in young Arctic sea ice and frost flowers. Marine Chemistry, **155**, 124-134.

Guillard RRL (1975) Culture of phytoplankton for feeding marine invertebrates. In: *Culture of marine invertebrate animals*. (eds Smith WL, Chanley MH) pp 29-60. New York, Plenum.

Helmke E, Weyland H (1995) Bacteria in sea ice and underlying water of the eastern Weddell Sea in midwinter. Marine Ecology Progress Series, **117**, 269-287.

Hoegh-Guldberg O, Bruno JF (2010) The impact of climate change on the world's marine ecosystems. Science, **328**, 1523-1528.

Horner R, Ackley S, Dieckmann G, Gulliksen B, Hoshiai T, Legendre L, Melnikov I, Reeburgh W, Spindler M, Sullivan C (1992) Ecology of sea ice biota. Polar Biology, **12**, 417-427.

Horner R, Schrader GC (1982) Relative contributions of ice algae, phytoplankton, and benthic microalgae to primary production in nearshore regions of the Beaufort Sea. Arctic, **35**, 485-503.

Huston AL, Krieger-Brockett BB, Deming JW (2000) Remarkably low temperature optima for extracellular enzyme activity from Arctic bacteria and sea ice. Environmental Microbiology, **2**, 383-388.

Iglesias-Rodriguez MD, Halloran PR, Rickaby REM, Hall IR, Colmenero-Hidalgo E, Gittins JR, Green DRH, Tyrrell T, Gibbs SJ, von Dassow P, Rehm E, Armbrust EV, Boessenkool KP (2008) Phytoplankton calcification in a high-CO_2 world. Science, **320**, 336-340.

IPCC (2013) Climate change 2013: The physical science basis. Contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change. (eds Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Y. X, Bex V, Midgley PM). 1535 pp. Cambridge, United Kingdom and New York, NY, USA, Cambridge University Press.

Kaiser MJ, Attrill MJ, Jennings S, Thomas DN, Barnes DKA, Brierley AS, Hiddink JG, Kaartokallio H, Polunin NVC, Raffaelli DG (2011) Polar Regions. In: *Marine Ecology – Processes, Systems, and Impacts*. (eds Kaiser MJ, Attrill MJ, Jennings S, Thomas DN, Barnes DKA, Brierley AS, Hiddink JG, Kaartokallio

H, Polunin NVC, Raffaelli DG) pp 325-357. New York, USA, Oxford University Press.

Kirst GO, Thiel C, Wolff H, Nothnagel J, Wanzek M, Ulmke R (1991) Dimethylsulfoniopropionate (DMSP) in ice algae and its possible biological role. Marine Chemistry, **35**, 381-388.

Koh EY, Cowie RO, Simpson AM, O'Toole R, Ryan KG (2012) The origin of cyanobacteria in Antarctic sea ice: marine or freshwater? Environmental Microbiology Reports, **4**, 479-483.

Krembs C, Eicken H, Deming JW (2011) Exopolymer alteration of physical properties of sea ice and implications for ice habitability and biogeochemistry in a warmer Arctic. Proceedings of the National Academy of Sciences, **108**, 3653-3658.

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. Journal of Experimental Marine Biology and Ecology, **243**, 55-80.

Kremp A, Godhe A, Egardt J, Dupont S, Suikkanen S, Casablanca S, Penna A (2012) Intraspecific variability in the response of bloom-forming marine microalgae to changed climate conditions. Ecology and Evolution, **2**, 1195–1207.

Kudo I, Miyamoto M, Noiri Y, Maita Y (2000) Combined effects of temperature and iron on the growth and physiology of the marine diatom *Phaeodactylum tricornutum* (Bacillariophyceae). Journal of Phycology, **36**, 1096-1102.

Langer G, Geisen M, Baumann K-H, Kläs J, Riebesell U, Thoms S, Young JR (2006) Species-specific responses of calcifying algae to changing seawater carbonate chemistry. Geochemistry, Geophysics, Geosystems, **7**, Q09006.

Lazzara L, Nardello I, Ermanni C, Mangoni O, Saggiomo V (2007) Light environment and seasonal dynamics of microalgae in the annual sea ice at Terra Nova Bay, Ross Sea, Antarctica. Antarctic Science, **19**, 83-92.

Lind JL, Heimann K, Miller EA, van Vliet C, Hoogenraad NJ, Wetherbee R (1997) Substratum adhesion and gliding in a diatom are mediated by extracellular proteoglycans. Planta, **203**, 213-221.

Lizotte MP (2001) The contributions of sea ice algae to Antarctic marine primary production. American Zoologist, **41**, 57-73.

Lohbeck KT, Riebesell U, Reusch TBH (2012) Adaptive evolution of a key phytoplankton species to ocean acidification. Nature Geoscience, **5**, 346-351.

Low-Décarie E, Jewell MD, Fussmann GF, Bell G (2013) Long-term culture at elevated atmospheric CO_2 fails to evoke specific adaptation in seven freshwater phytoplankton species. Proceedings of the Royal Society of London B Biological Sciences, **280**, 20122598.

Lyon BR, Lee PA, Bennett JM, DiTullio GR, Janech MG (2011) Proteomic analysis of a sea-ice diatom: Salinity acclimation provides new insight into the dimethylsulfoniopropionate production pathway. Plant Physiology, **157**, 1926-1941.

Mackey KRM, Paytan A, Caldeira K, Grossman AR, Moran D, McIlvin M, Saito MA (2013) Effect of temperature on photosynthesis and growth in marine *Synechococcus* spp. Plant Physiology, **163**, 815-829.

Marschall H-P (1988) The overwintering strategy of Antarctic krill under the pack-ice of the Weddell Sea. Polar Biology, **9**, 129-135.

Marx JG, Carpenter SD, Deming JW (2009) Production of cryoprotectant extracellular polysaccharide substances (EPS) by the marine psychrophilic

bacterium *Colwellia psychrerythraea* strain 34H under extreme conditions. Canadian Journal of Microbiology, **55**, 63-72.

Mattsdotter Björk M, Fransson A, Torstensson A, Chierici M (2014) Ocean acidification state in western Antarctic surface waters: controls and interannual variability. Biogeosciences, **11**, 57-73.

McKeown RM, Scully C, Enright A-M, Chinalia FA, Lee C, Mahony T, Collins G, O'Flaherty V (2009) Psychrophilic methanogenic community development during long-term cultivation of anaerobic granular biofilms. ISME Journal, **3**, 1231-1242.

McMinn A, Müller MN, Martin A, Ryan KG (2014) The response of Antarctic sea ice algae to changes in pH and CO_2. PLoS ONE, **9**, e86984.

McMinn A, Pankowskii A, Ashworth C, Bhagooli R, Ralph P, Ryan K (2010) *In situ* net primary productivity and photosynthesis of Antarctic sea ice algal, phytoplankton and benthic algal communities. Marine Biology, **157**, 1345-1356.

Mehrbach C, Culberson CH, Hawley JE, Pytkowicz RM (1973) Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnology and Oceanography, **18**, 897-907.

Melnikov IA (1997) *The Arctic Sea Ice ecosystem,* Amsterdam, Gordon & Breach Science Publishers.

Mitchell C, Beardall J (1996) Inorganic carbon uptake by an Antarctic sea-ice diatom, *Nitzschia frigida*. Polar Biology, **16**, 95-99.

Mock T (2002) In situ primary production in young Antarctic sea ice. Hydrobiologia, **470**, 127-132.

Mock T, Hoch N (2005) Long-term temperature acclimation of photosynthesis in steady-state cultures of the polar diatom *Fragilariopsis cylindrus*. Photosynthesis Research, **85**, 307-317.

Mock T, Junge K (2007) Psychrophilic Diatoms: Mechanisms for Survival in Freeze–Thaw Cycles. In: *Algae and Cyanobacteria in Extreme Environments.* (ed Seckbach J). Dordrecht, The Netherlands, Springer.

Montes-Hugo M, Doney SC, Ducklow HW, Fraser W, Martinson D, Stammerjohn SE, Schofield O (2009) Recent changes in phytoplankton communities associated with rapid regional climate change along the western Antarctic peninsula. Science, **323**, 1470-1473.

Montresor M, Procaccini G, Stoecker DK (1999) *Polarella glacialis*, gen. Nov., sp. Nov. (dinophyceae): suessiaceae are still alive! Journal of Phycology, **35**, 186-197.

Mora P, Rosconi F, Franco Fraguas L, Castro-Sowinski S (2008) *Azospirillum brasilense* Sp7 produces an outer-membrane lectin that specifically binds to surface-exposed extracellular polysaccharide produced by the bacterium. Archives of Microbiology, **189**, 519-524.

Nichols DS, Olley J, Garda H, Brenner RR, McMeekin TA (2000) Effect of temperature and salinity stress on growth and lipid composition of *Shewanella gelidimarina*. Applied and Environmental Microbiology, **66**, 2422-2429.

Novitsky JA, Morita RY (1977) Survival of a psychrophilic marine Vibrio under long-term nutrient starvation. Applied and Environmental Microbiology, **33**, 635-641.

O'Brien DP (1987) Direct observations of the behavior of *Euphausia superba* and *Euphausia crystallorophias* (Crustacea: Euphausiacea) under pack ice during the Antarctic Spring of 1985. Journal of Crustacean Biology, **7**, 437-448.

Orr JC, Fabry VJ, Aumont O, Bopp L, Doney SC, Feely RA, Gnanadesikan A, Gruber N, Ishida A, Joos F, Key RM, Lindsay K, Maier-Reimer E, Matear R, Monfray P, Mouchet A, Najjar RG, Plattner G-K, Rodgers KB, Sabine CL, Sarmiento JL, Schlitzer R, Slater RD, Totterdell IJ, Weirig M-F, Yamanaka Y, Yool A (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature, **437**, 681-686.

Ozturk S, Aslim B (2010) Modification of exopolysaccharide composition and production by three cyanobacterial isolates under salt stress. Environmental Science and Pollution Research, **17**, 595-602.

Palmisano AC, SooHoo JB, Sullivan CW (1985) Photosynthesis-irradiance relationships in sea ice microalgae from McMurdo Sound, Antarctica. Journal of Phycology, **21**, 341-346.

Pančić M, Hansen PJ, Tammilehto A, Lundholm N (2015) Resilience to temperature and pH changes in a future climate change scenario in six strains of the polar diatom *Fragilariopsis cylindrus*. Biogeosciences, **12**, 4235-4244.

Pierrot D, Lewis E, Wallace DWR (2006) MS Excel program developed for CO_2 system calculations. Oak Ridge, Tennessee, ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy.

Pörtner HO (2002) Physiological basis of temperature-dependent biogeography: Trade-offs in muscle design and performance in polar ectotherms. Journal of Experimental Biology, **205**, 2217-2230.

Poulin M, Daugbjerg N, Gradinger R, Ilyash L, Ratkova T, von Quillfeldt C (2011) The pan-Arctic biodiversity of marine pelagic and sea-ice unicellular eukaryotes: A first-attempt assessment. Marine Biodiversity, **41**, 13-28.

R Core Team (2014) R: A language and environment for statistical computing. Vienna, Austria, R Foundation for Statistical Computing.

Ralph PJ, McMinn A, Ryan KG, Ashworth C (2005) Short-term effect on temperature on the photokinetics of microalgae from the surface layers of Antarctic pack ice. Journal of Phycology, **41**, 763-769.

Riaux-Gobin C, Poulin M (2004) Possible symbiosis of *Berkeleya adeliensis* Medlin, *Synedropsis fragilis* (Manguin) Hasle et al. and *Nitzschia lecointei* van Heurck (Bacillariophyta) associated with land-fast ice in Adèlie Land, Antarctica. Diatom Research, **19**, 265-274.

Riaux-Gobin C, Poulin M, Dieckmann GS, Labrune C, Vétion G (2011) Spring phytoplankton onset after the ice break-up and sea-ice signature (Adélie Land, East Antarctica). Polar Research, **30**, 5910.

Riebesell U, Gattuso J-P (2015) Lessons learned from ocean acidification research. Nature Climate Change, **5**, 12-14.

Riebesell U, Zondervan I, Rost B, Tortell PD, Zeebe RE, Morel FMM (2000) Reduced calcification of marine plankton in response to increased atmospheric CO_2. Nature, **407**, 364-367.

Rossoll D, Bermúdez R, Hauss H, Schulz KG, Riebesell U, Sommer U, Winder M (2012) Ocean acidification-induced food quality deterioration constrains trophic transfer. PLoS ONE, **7**, E34737, doi:34710.31371/journal.pone.0034737.

Roy RN, Roy LN, Vogel KM, Porter-Moore C, Pearson T, Good CE, Millero FJ, Campbell DM (1993) The dissociation constants of carbonic acid in seawater at salinities 5 to 45 and temperatures 0 to 45 °C. Marine Chemistry, **44**, 249-267.

Ryan KG, Ralph P, McMinn A (2004) Acclimation of Antarctic bottom-ice algal communities to lowered salinities during melting. Polar Biology, **27**, 679-686.

Sabine CL, Feely RA, Gruber N, Key RM, Lee K, Bullister JL, Wanninkhof R, Wong CS, Wallace DWR, Tilbrook B, Millero FJ, Peng T-H, Kozyr A, Ono T, Rios AF (2004) The oceanic sink for anthropogenic CO_2. Science, **305**, 367-371.

Schaum CE, Collins S (2014) Plasticity predicts evolution in a marine alga. Proceedings of the Royal Society of London B Biological Sciences, **281**, 20141486.

Schlegel HG, Jannasch HW (1981) Prokaryotes and their habitats. In: *The Prokaryotes*. (eds Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG). New York, Springer-Verlag Berlin Heidelberg.

Steinacher M, Joos F, Frölicher TL, Plattner G-K, Doney SC (2009) Imminent ocean acidification in the Arctic projected with the NCAR global coupled carbon cycle-climate model. Biogeosciences, **6**, 1877-1882.

Tatters AO, Roleda MY, Schnetzer A, Fu F, Hurd CL, Boyd PW, Caron DA, Lie AAY, Hoffmann LJ, DA H (2013a) Short- and long-term conditioning of a temperate marine diatom community to acidification and warming. Philosophical Transactions of the Royal Society of London B Biological Sciences, **368**, 20120437.

Tatters AO, Schnetzer A, Fu F, Lie AAY, Caron DA, Hutchins DA (2013b) Short- versus long-term responses to changing CO_2 in a costal dinoflagellate bloom: Implications for interspecific competitive interactions and community structure. Evolution, **67**, 1879-1891.

Thomas DN, Dieckmann GS (2002) Antarctic sea ice – a habitat for extremophiles. Science, **295**, 641-644.

Toggweiler JR (1993) Carbon overconsumption. Nature, **363**, 210-211.

Trimborn S, Brenneis T, Sweet E, Rost B (2013) Sensitivity of Antarctic phytoplankton species to ocean acidification: Growth, carbon acquisition, and species interaction. Limnology and Oceanography, **58**, 997-1007.

Wang M, Overland JE (2009) A sea ice free summer Arctic within 30 years? Geophysical Research Letters, **36**, L07502, doi:07510.01029/02009GL037820.

Weissenberger J, Dieckmann GS, Gradinger R, Spindler M (1992) Sea ice: A cast technique to examine and analyze brine pockets and channel structure Limnology and Oceanography, **37**, 179-183.

Weissling BP, Ackley SF (2015) Spectral analysis of Amundsen Sea pack ice roughness and estimates of air-ice drag coefficient. Annals of Glaciology, **56**, *Accepted manuscript*.

Wulff A, Roleda MY, Zacher K, Wiencke C (2008) Exposure to sudden light burst after prolonged darkness — A case study on benthic diatoms in Antarctica. Diatom Research, **23**, 519-532.

Yang G, Gao K (2012) Physiological responses of the marine diatom *Thalassiosira pseudonana* to increased pCO_2 and seawater acidity. Marine Environmental Research, **79**, 142-151.