Eukaryotic biodiversity of Arctic and Antarctic sea ice – a molecular approach

Diplomarbeit

zur Erlangung des Grades
einer Diplom-Biologin

vorgelegt von

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Braunschweig, den 28. September 2007

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Abbreviations

A	Adenin
ACC	Antarctic Circumpolar Currents
BLAST	Basic Local Alignment Search Tool
bp	base pairs
C	Cytosin
CPG	combined phylogenetic group
DIP	dissolved inorganic phospate
DOC	dissolved organic carbon
DON	dissolved organic nitrogen
DNA	desoxyribonucleic acid
ds	double stranded
dNTP	desoxy ribonukleosid triphosphate
G	Guanin
kb	kilo base pair
NADW	North Atlantic Deep Water
OD	optical density
OTU	operational taxonomic unit
PCR	polymerase chain reaction
PSU	practical salinity unit
rDNA	ribosomal desoxyribonucleic acid
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
rpm	rounds per minute
ss	single stranded
T	Thymin
TAE	Tris.Acetate-EDTA
taq	*Thermus aquaticus*
U	unit: unit for enzyme activity

Abstract

Sea ice covers extensive areas of the polar regions. The conditions within and around the ice are highly variable. This results in many different habitats for organisms, ranging from protozoans to huge animals.
The investigation of sea ice in general is difficult, that is why no spatial or temporal coverage of sampling sites was possible so far. The observation and description of sympagic communities were usually done by various microscopic techniques.
The detectable biodiversity can be increased by methods which base on the identification of characteristic DNA fragments. This approach allows the analysis of organisms, which are too small for light-microscopy, not distinguishable by its morphology or very rare. A well known method for other ecosystems is sequencing of 18S libraries which provides a highly conserved fragment of all eukaryotes in the sample for sequence analysis.
The aim of the present study is to use 18S libraries to analyse the eukaryotic biodiversity of sea ice and to estimate its advantages and boundaries.
Samples from Van Mijenfjorden on Svalbard (Arctic) and from the Weddel Sea (Antarctica) were analysed by sequencing of 18S clone libraries and compared concerning their biodiversity. The samples originated from sea ice which differed in the generic ice class and its age, for instance. Therefore, assumptions about the influence of biotic and abiotic conditions on the biodiversity were possible.
The resulting phylogenetic trees varied significantly, whereas the biodiversity was mainly influenced by the processes during sea-ice formation and ageing. At the Antarctic stations, also biochemical parameters were measured. These data allow the comparison with former investigations which used microscopic methods. The results clearly show similar species compositions, even though the influence of various factors makes significant conclusions difficult.
The Arctic samples were compared with results from expeditions in the southwest of Svalbard. In the present study, surprisingly low numbers of bacillariophyta were found, which is maybe related to the extremely low temperature during this winter or to the influence of the coast nearby.
This study represents a first step into the high-throughput analysis of sea-ice biodiversity by using 18S PCR. For protozoa and metazoa, results agreed in general with those of other identification methods. A quantification of the observed class and kingdoms is not reasonable, especially for metazoa. Descriptions on species-level would require additional primers and sequencing reactions which is difficult for a high-throughput approach but also not necessary for a first large scale monitoring of sea-ice biodiversity.

Chapter 1

Introduction

"Make everything as simple as possible, but not simpler" (Einstein)

In former times, Arctic and Antarctic regions were expected to be almost devoid of life. It was taught that organisms could not tolerate such extreme conditions.
But since 1841, when Ehrenberg found mircroorganisms in Arctic sea ice for the first time, our picture of the sea ice ecosystem has become more and more complex. Organisms, ranging from small bacteria and single-celled eukaryotes up to metazoans, live there and even attain high production rates.
The aim of this thesis is to improve our understanding of eukaryotic biodiversity of sea ice by using molecular methods. Besides being able to detect very small and rare species the molecular approach enables morphologically very similar species to be distinguished or even new ones to be identified. The thesis entails the analysis of sea ice samples from the Weddel Sea during a period of one month and from Van Mijenfjorden during a three month period. Physical processes within the sea ice and in the ocean are analysed in order to understand the mechanisms which control the sea ice biodiversity.

The experimental approach of this work is based on the amplification of 18S rDNA sequences and the generation of 18S clone libraries. The investigation of all abundant species is realized by high-throughput TOPO-TA cloning and sequencing. The gained sequence data are analysed in a comparable way by the construction of phylogenetic trees using the ARB database. All phylogenetic trees will be compared and the influence of abiotic factors will be discussed.

1.1 Polar regions

The polar regions are defined as the areas around the north and the south pole. Most parts of these large areas are covered with snow and ice causing values of incident irradiance of less than 1% under the ice (Kirst and Wiencke, 1995), average winter temperatures being around −30°C and the precipitation as low as 250 mm per year.

Depending on the definition, the Arctic is either the region of the midnight sun, north of the Arctic Circle (66°33') or north of the tree line. Another possibility to define the border of the Arctic region is to use the isotherm of 10°C in July.
The Antarctic region is the area south of 60°S, including the continent Antarctica with 13.2 million square kilometres (Meunier et al., 2007).
The polar regions are covered by sea ice with a great interannual variability in sea ice extent and thickness. The global coverage of sea ice constitutes up to 13 % of the earth's surface and is distributed mainly in the Arctic and Antarctic regions, but also in the Caspian, Baltic and Okhotsk Sea (Thomas and Dieckmann, 2002).
In the Arctic, sea ice is encircled by land which blocks its flow southwards resulting in thicker and often ridged ice. The opposite is the case in the Antarctic where the sea ice around the continent is only bounded by oceans. On average, this results in thinner and younger ice compared to the Arctic.
The maximum sea ice extent in the Arctic is about $14\times10^6\,km^2$ in February and about $20\times10^6\,km^2$ in September in the Antarctic. The difference to the minimum ice extent, which is in the Arctic $7\times10^6\,km^2$ during September and in the Antarctic $4\times10^6\,km^2$ during February (Spindler, 1994), is higher for Antarctic sea ice (see Figure 1.1). Due to the surrounding open oceans, Antarctic sea ice can move freely and reach higher drift speeds leading to higher melting rates in warmer waters further north.

Another consequence of the semi-enclosed Arctic ocean is the formation of multiyear ice which is much more common in the north and can reach a thickness of 4 to 5 m.
Multiyear ice influences the biodiversity and species composition. Sea ice also provides a basis for breeding, resting and hunting and is therefore also important for birds and mammals even during the summer season.
Antarctica and the surrounding oceans are strongly influenced by the Antarctic Circumpolar Currents (ACC), which was first described by Deacon (1937). The ACC flows eastwards due to strong westerly winds around the Antarctic continent (Fahrbach et al., 1992; Smith et al., 2007) and is constrained by land. Between the Antarctic Peninsula and Cape Horn, it flows through the Drake Passage. Deep water from the North Atlantic (NADW) is upwelled at the

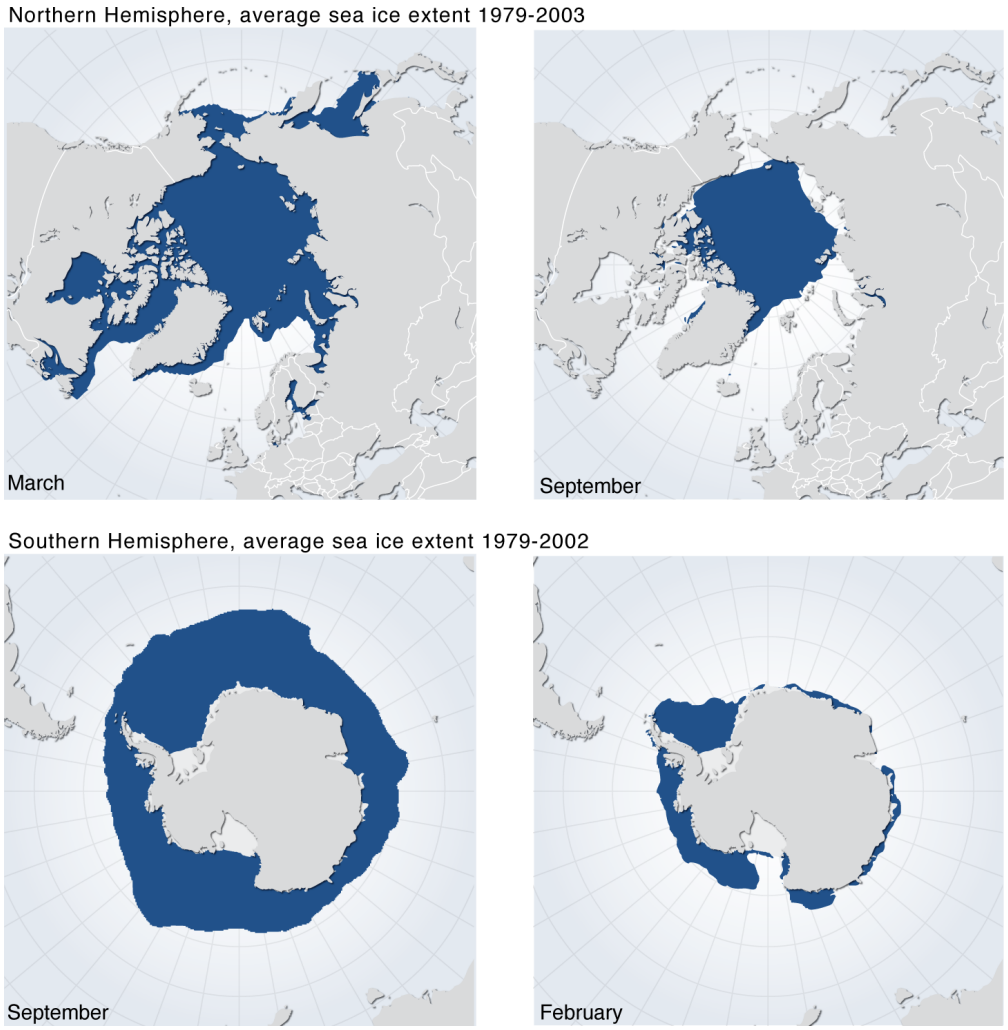


Figure 1.1: Average sea-ice extent in the Arctic and Antarctic (Ahlenius, 2007).

so-called Antarctic Divergence and is mixed with the Antarctic Circumpolar Water. The westerly winds also force a large, near-surface Ekman transport to the north. The direction of the water masses influences the sea ice drift and is therefore important for interpretation of the biodiversity of Antarctic sea ice.
Typical Antarctic sea ice usually does not attain a thickness of solid ice than over 0.7 m (Eicken, 1992), but it is strongly deformed by wind and waves and can therefore reach high concentrations. Especially in the western Weddell Sea, where highly deformed pack-ice occurs and even persists throughout the year.

This causes typical communities within the Antarctic sea ice compared to the dominant type of Arctic sea ice which reaches a thickness of 1 to 4.5 metres.
The composition and distribution of those sympagic communities is strongly dependent on the age and the thickness of the ice due to several processes which influence the physical features within the sea ice such as brine volume, salinity, pH and optical properties. The ice is colonized by viruses, bacteria, protists and fungi, which are partially endemic. These microorganisms are common in both polar regions. They are spread over the whole ice column as shown in Figure 1.3.
Metazoans differ in both regions: Arctic sea ice contains nematodes, rotifers, turbellarians and copepods whereas Antarctic sea ice is dominated by copepods and turbellarians. The so-called krill, which can attain a very high biomass, is a very important component of the food web.

1.2 Structure of sea ice

As described above, the structure of sea ice varies considerably depending on the conditions during formation but also during ageing. These processes clearly influence the biodiversity, as proposed by Günther and Dieckmann (2001), and it is therefore necessary to understand the ongoing mechanism and the ice-history to interpret the resulting species composition.

The formation of sea ice depends on the water turbulence. At temperatures under $-1.8°C$, water forms a dense suspension of ice crystals, called frazil ice or grease ice. Under quiet conditions, the frazil crystals freeze together and form a thin layer of ice on the ocean surface, which is called nilas. On the underside of this layer, ice growth continues to form congelation ice. This congelation growth leads to the typical structure of first-year ice. If the water is moved by wind and waves, the crystals at the surface can not easily stick to each other to form a consolidated layer. Therefore, the cyclic compression of the frazil ice leads to single pancake-like ice floes

with raised rims of ice on the edges. Further in from the ice edge, the water surface is calmed and the pancake freeze together into smaller floes or pancakes of 3-5 metres. The bottom of this ice is very rough because the pancakes were jumbled together during the freezing process. Single pancakes are rafted over one another or even protrude upwards resulting in a much thicker consolidated ice layer. This solid pack ice is typical in the Southern Ocean, but also develops in the Greenland or Bering Seas for example.

The formation of ice also leads to two typical crystal structures: needle like crystals, known as columnar ice, form under calm conditions. On a rough water surface, there will be small, round crystals which form so-called granular ice. Often, a combination of both ice types appears.
During the freezing process of the water molecules, salts are expelled and form the highly saltine brine, with a salinity of 143 parts per thousand at -10°C (Cox and Weeks, 1983). The brine collects in pores and channels ranging in sizes from micrometres to millimetres (Thomas and Dieckmann, 2002) and constitute 1–30% of the ice volume (Weeks and Ackley, 1982; Weissenberger et al., 1992). This semisolid matrix changes during ageing and the brine volume varies depending on air temperature and ice bulk salinity (Frankenstein and Garner, 1967; Leppäranta and Manninen, 1988). At temperatures below -10°C the fraction of this bulk liquid can be less than 5% whereas at lower temperatures it rises up to 20% (Eicken, 1992). As described by Perovich and Gow (1991), this varying porosity of sea ice leads to changes in size and connectivity of pores and hence, to a change in permeability for nutrients and organisms. This interaction is schematically shown in Figure 1.2.

A third characteristic ice type in the Antarctic region is the unconsolidated under-ice layer, called "platelet layer". These thin ice discs are produced when deep water is upwelled underneath ice shelves and supercooled (Grossmann et al., 1996).

1.3 Biology of sea ice

Primary production of ice algae is 10 to 30% of overall primary production in Arctic and Antarctic ecosystems (Legendre et al., 1992; Arrigo et al., 1997). In Antarctic Waters, 9-25% (Arrigo et al., 1997) of the total primary production is produced by ice algae and in Arctic waters, even 20-25% (Legendre et al., 1992). The biogenic carbon production, is estimated to be 35.7 Tg C year^{-1}, is 1 to 4 % of the annual biogenic carbon production of the Southern Ocean Arrigo et al. (1997). In addition, primary production in sea ice is the main food source for zooplankton during the winter.
The biodiversity of sea ice, as outlined by Günther and Dieckmann (2001), is

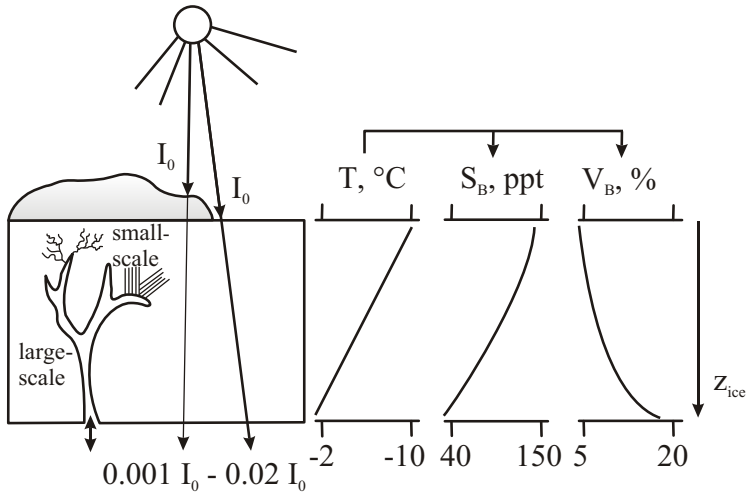


Figure 1.2: Schematic representation of the ice environment depicting attenuation of solar radiation, distribution of small-scale (sub-mm to mm) porosity feeding into large-scale (mm to dm) pores. Salinity-depth and brine volume-depth curves have been computed from the equations given by Cox and Weeks (1983), assuming a homogeneous bulk salinity distribution of 5 parts per thousand (ppt); (modified after Eicken (1992).)

highly variable. A typical distribution of species within the ice is composed of 75% diatoms, 14% autotrophic flagellates and 11% heterotrophs.

Planktonic organisms as well as detrital material from the upper water column are incorporated into the sea ice during its formation. As outlined by Spindler (1994), different enrichment mechanisms are possible.
The various materials such as silt, diatom frustules and living plankton can either rise with frazil ice to accumulate at the surface or by propagating wave fields. This passive "harvesting" process may enhance the introduction of organisms which can adhere to frazil crystals due to their raphe system (Round et al., 1990) or rhizopods (Hemleben et al., 1989) and therefore increase the abundance of raphid pennate diatoms and foraminifers as proposed by Eicken (1992) and Reimnitz et al. (1990). Besides these passive incorporation processes, active colonization has also been reported for metazoans (Tanimura et al., 1984). The species composition of bottom-ice communities is also influenced by platelet-ice scavenging (Gulliksen and Lonne, 1989) and other succession processes .
Depending on the size of the organisms and their ability to adapt to the conditions within the brine channels, so-called sympagic communities develop during the ageing of the sea ice.

The sympagic environment holds a wide range of ecological niches, characterized by different combinations of physico-chemical boundary conditions. Compared to the open ocean, the temporal variability of light conditions is much smaller and the location within space is more fixed. For autotrophic organisms this means more constant irradiance for photosynthesis. On the other hand, they have to sustain lower temperatures and higher salinities than in the water column. At lower temperatures, which leads to a closed or semiclosed pore system, the nutrient supply varies due to lower exchange rates (Thomas and Dieckmann, 2002). Besides, a lack of nutrients can be caused by an intense growth of sympagic communities (Spindler, 1994). These communities include viruses, bacteria, algae, lower fungi, flagellates, protists such as ciliates and foraminifers but also metazoa (Spindler, 1994; Gradinger, 2002).
Archer et al. (1996) outlined the seasonal and spatial variation of sympagic organisms with regard to their species composition, distribution and abundance. Even on a smaller scale, patchy distribution was reported by Spindler and Dieckmann (1986) who discovered a variation of one order of magnitude between ice cores taken 30 cm apart. This indicates the complexity of the sea ice ecosystem. The patchy distribution of organisms is mainly caused by the ice type and therefore by incorporation processes and physico-chemical conditions.

1.4 Ice Algae

The most dominant representatives of sympagic communities are pennate diatoms, which can produce chlorophyll concentrations of up to 1000μg per litre (Thomas and Dieckmann, 2002) and some 100 000 diatom cells per cm^3. The highest biomass of algae can be found in platelet ice and near the bottom of fast ice. Typical ice algae are, depending on the habitat and the season, *Fragilariopsis, Haslea, Thalassiosira, Navicula, Amphiphrora, Melosira, Phaeocystis* and *Nitzschia* species.
A recent study by Werner et al. (2007) outlined, that the diversity of sea-ice algae in Arctic pack-ice is not lower during the winter season, even though the abundance of ice-algal cells is significantly lower. This clearly indicates the potential of surviving the winter, which is possible by several overwintering strategies such as resting spores (Lizotte, 2003), energy reserves (lipids, carbohydrates) but also facultative heterotrophy (Werner et al., 2007). The observed distinct difference between autumn surface-water communities and sea-ice communities (Druzhkov et al., 2001) also leads to the assumption, that the incorporation process into the ice is highly selective for diatoms (Gradinger and Ikävalko, 1998).
Algal blooms are also typical at the ice edge due to shallow and stable surface

mixed layers with favourable light conditions (Lizotte, 2001). These enormous blooms in the open water, after the ice has retread, often cover a huge area and therefore play an important role for grazers.
Sympagic communities can be split into different groups, depending on the ice type and their vertical distribution within the ice. An overview is given in Figure 1.3. As described by Horner et al. (1992), the surface communities consist of the infiltration communitiy at the snow-ice interface, which is typical for Antarctic pack ice. Another surface community occurs due to deformation processes and flooding of the ice surface in the Arctic and Antarctic region. Because of the higher irradiance, the cell concentrations can be 10–100 times higher than the underlying water. A very important community in the Arctic, occurs in melt pools, which can cover 50–60% of the Arctic sea ice. Depending on their formation, either by surface thawing or by splashing, they can be freshwater, brackish or saltwater ponds and contain a variety of organisms (Syvertsen, 12-16 May 1990).
In the Antarctic sea ice, melt pools can develop below the surface of consolidated snow. The high production rates there are due to small diatoms and flagellates on the fast ice or to terrestrial and snow algal assemblages in the coastal tide-crack zone.

The interior habitat begins under the surface communities. The uppermost freeboard community develops in rotting ice and can include algae producing high chlorophyll *a* levels. Krill has also been observed in this layer. Underneath the freeboard community, which was only found in Antarctic sea ice, an area with brine channels and band communities exists. These communities living in the brine channels are of varying diversity, depending on the physical conditions which determine the permeability and therefore an exchange between ice and water communities.
Diffuse communities, which have hardly been studied in the Arctic, in Antarctic pack ice comprise bacteria, diatoms, dinoflagellates, autotrophic and heterotrophic flagellates, foraminifers, ciliates and also micrometazoans (Horner et al., 1992). The so-called band communities, which occur in Arctic and Antarctic sea ice, are dominated by diatoms and dinoflagellates.

The bottom ice communities consist of interstitial communities within the congelation ice layer and the platelet ice communities under it. The most abundant species in the Arctic are pennate diatoms, but autotrophic and heterotrophic flagellates, ciliates, dinoflagellates, heliozoans, rotifers, nematodes, copepods, turbellarians and polychaete larvae can also be found. Centric diatoms occur more often in pack ice areas.

The platelet ice layer is very favourable environment because it com-

bines more stable conditions than in the water column, but also more space than within the consolidated ice. It also allows better nutrient exchange and the opportunity for colonization (Grossmann et al., 1996) which can result in very dense microbial assemblages (Ackley and Sullivan, 1994). In platelet layers of several metres thickness, "superblooms" of algae accompanied by nutrient depletion were observed by Smetacek et al. (1992) in the Weddell Sea. Platelet ice communities cover a clearly different habitat which is defined by a different crystal structure and orientation, more space between the crystals, nutrient exchange potential and shading by the interstitial communities. The so-called sub-ice communities are loosely attached to the underside of the sea ice but live in the seawater (Johnsen and Hegseth, 1991).
In the Arctic, these algal mats or filaments can be *Nitschia* spp. (Tremblay et al., 1989) or *Melosira arctica* (Melnikov and Bonderchuk, 1987; Syvertsen, 12-16 May 1990). Besides microalgae, also harpacticoid Copepods, *Pseudocalanus* sp., amphipods and polar cod (Horner et al., 1992) occur.
Dominant species in the Antarctic sub-ice communities are the pennate diatoms *Berkeleya* sp., *Entomoneis* spp. and *Nitzschia stellata* (Medlin and Hasle, 1990). Here, also amphipods and fish can be found (Gulliksen and Lonne, 1989).

The main abiotic factors causing the described distribution of communities are nutrients, especially silicate for diatoms, brine volume and the generic ice class (Gulliksen and Lonne, 1989). Other probably important factors, such as CO^2-concentration, grazing pressure and short-term extremes as well as interspecific competition have not yet been investigated.
In general, ice algae are adapted to the sea ice by several mechanisms. A cold resistance is realized by antifreeze proteins (AFP), which help to depress freezing temperatures and modifying and suppressing ice crystal growth. AFPs also protect cell membranes from cold-induced damage by inhibiting the recrystallization of ice in and around the cell. Also, an extremely high salt stress has to be tolerated, which varies from 35 to 212 g/l (Cox and Weeks, 1983). High salinities can cause osmotic and ionic stress, but also influence the ionic composition of the cell. Salt-tolerant organisms can increase the extrusion of salt by increased usage of ion transporters. The accumulation of osmolytes enables to maintain the cellular water potential constant. Some of the organic osmolytes even have a cryoprotective function, which helps to recover freeze damaged cells by glycine betaine (Chen and Murata, 2002) and to protect membranes and proteins by sugars. Ice algae also have to adapt rapidly to varying light conditions and to develop protective mechanisms against high UV radiation.

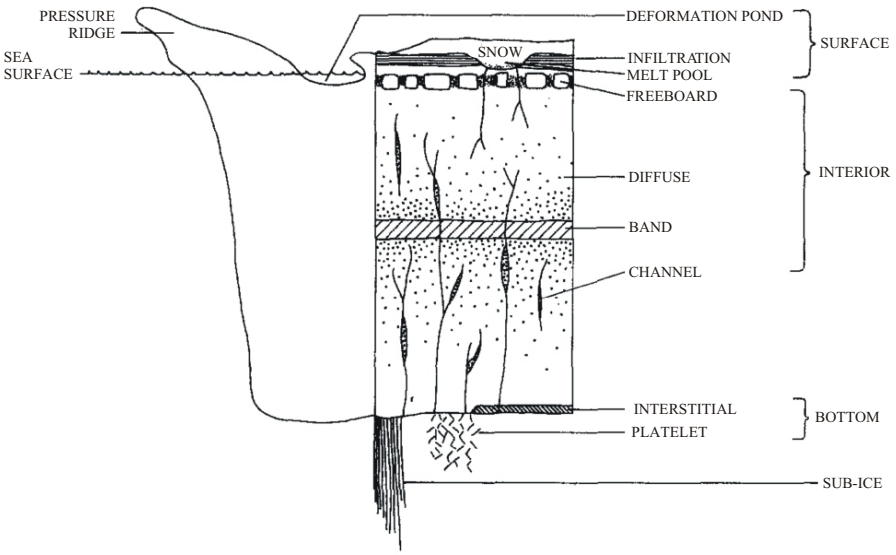


Figure 1.3: Schematic representation of biological communities which can be found in sea ice (Horner et al. (1992); modified from(McConville and Wetherbee, 1983; Horner et al., 1988; Kottmeier and Sullivan, 1990).)

1.5 Molecular biology

1.5.1 Former studies

Previous methods to investigate the biodiversity of sea ice used microscopic techniques to look at the fixed organisms in melted sea ice. But Archaea, Bacteria and microbial Eukarya, which play a very important role in natural ecosytems, often lack distinct morphological characteristics, or can not be prepared for microscopic techniques. Also, as claimed by Lovejoy et al. (2006), extreme and isolated environments have been sources of novel phylotypes. The sequence analysis of the 18S rRNA genes of marine picoeukaryotes, which are "probably the most abundant Eukaryotes on Earth" according to Díez et al. (2001), led to major revisions of eukaryotic phylogeny. To complete our understanding of the species composition in sea ice communities, an increasing number of genetic and immunological methods have been developed. The 18S rRNA gene, which is common for all eukaryotes, is used for 18S PCR, quantitative PCR (Zhu et al., 2005) and fluorescent in situ hybridization experiments (Simon et al., 1995) to compare and describe eukaryotic communities. Analysing cloned and sequenced 18S rDNAs were done with deep-sea samples from the Southern Ocean by López-García et al. (2001) and samples from the Pacific Ocean (Moon-van der Staay et al., 2001). They always found novel lineages and a surprisingly high phylogenetic diversity.

So far, no molecular approach has been used to investigate sea ice samples, neither from the Arctic nor the Antarctic region.

1.5.2 18S ribosomal DNA

Ribosoms are important organelles in every cell because they are crucial for the biosynthesis of proteins. They consist of ribosomal RNA, which is encoded in the so-called ribosomal DNA (rDNA) and proteins. In eukaryotes, the nucleus encloses between 10^5 and 10^7 ribosomes, each composed of four subunits of different sizes. The 18S subunit is usually used for phylogenetic analyses of eukaryotes. Due to its size, it contains sufficient phylogenetic information but is still easier to handle than the large subunit.
The function of the rRNA is a basis for today's living organisms and was developed early in evolution. As assumed nowadays, rRNA evolves in the same speed in all organisms. Therefore, the rRNA is an important tool to analyse the phylogeny. To simplify the method, the more stable rDNA is used instead of the rRNA.
Depending on the intention of the experiment, more or less conserved fragments are used. Some highly conserved fragments are common for all organisms whereas others can be used to define species. The number of mutations is assumed to differ proportionally to the time and is therefore almost independent of the pressure of natural selection.
The alignment of rRNA sequences of different organisms provides information on their relationship to each other. Based on the calculated phylogenetic distance, a so-called phylogenetic tree can be constructed. But the resulting phylogeny is always completed by morphological and physiological information.

1.6 Study area

The study area in the northern hemisphere is part of the western Svalbard region. The norwegian archipelago is located between 77° and 80° North and 10° and 35° East. The islands are surrounded by the Arctic Ocean, the Barents Sea, the Norwegian Sea and the Greenland Sea. The western part of Svalbard is strongly influenced by the warmer and more saline Atlantic water of the gulf stream system. Compared to the canadian and sibirian region, the area of western Svalbard is free of ice for a longer period of the year. As described in Parkinson (1991), the least interannual variability of sea ice in February occurs in the southern Greenland Sea and southwest of Svalbard. Therefore, the abiotic and biotic conditions there correspond to the conditions in lower latitudes of the canadian and sibirian Arctic.
In 2006, the North Greenland Sea did not freeze near the western coast of Svalbard. An ice cover could only form on the shallow semi-closed fjord system "Van Mijenfjord" (see Figure 2.2) during the late winter season and was therefore chosen as the study area. The oceanographic conditions on the fjord, which is about 70 m deep, are comparable to the open ocean, but the influence of wind and currents is much lower. Due to the proximity to the

Figure 1.4: Schematic representation of the ocean currents in the Arctic (Rekacewicz, 1998).

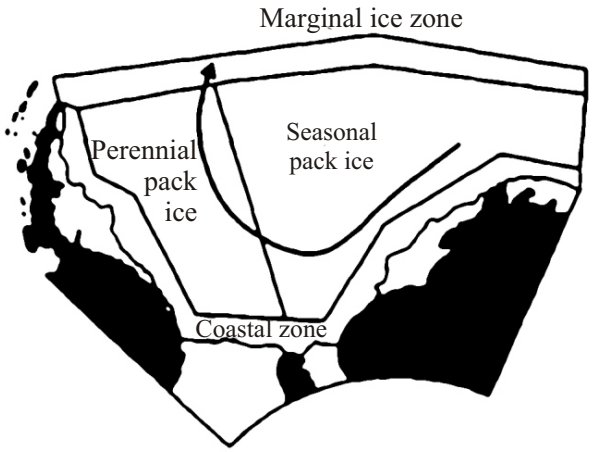


Figure 1.5: Sea-ice regimes and regional structuring of Weddell Sea ecosystem (not drawn to scale). Also shown is the mean path of ice drift as determined by Kottmeier and Hartig (1990); (modified after Eicken (1992).

coast, the biodiversity within the ice can be strongly influenced by the local life-history traits, local topography, glacial runoff as well as local circulation pattern (Renaud et al., 2006).

In Antarctica, all samples where taken in the north-western part of the Weddell Sea (see Figure 2.1). In the Weddell Sea, which covers an area of about 2.8 million km^2, a strong clock wise sea-ice drift with the so-called Weddell Gyre exists. The resulting path of ice drift is shown in Figure 1.5. This area is characterized by a perennial ice cover and a convergent flow regime. The southern, eastern and central parts of the Weddel Sea belong to the circum-antarctic seasonal pack-ice belt. The ACC transports solid ice, as described in Chapter 1.2, to the western Weddel. Compared to the thick ice cover in the western area, the southern and eastern Weddel Sea has areas with thin ice or even ice-free polynias. This leads to much higher light intensities penetrating through the ice and into the water column. The perennial sea ice is then transported further north where it reaches the marginal ice zone. This geographical distribution changes, both the temporal evolution and the structure of the sea ice and therefore has important consequences for the ecosystem.

Chapter 2

Material and Methods

2.1 Sequencing of 18S clone libaries

2.1.1 Sample preparation

The aim of this work is to investigate sea ice from Arctic and Antarctic regions to compare biodiversity under spring conditions. During ANT XXIII/7, RV in autumn 2006 Polarstern collected ice cores from stations between 60°38 and 65°6 south near the Antarctic peninsula. At station 060923, the ice was taken from ice floes broken by the research vessel whereas all other ice cores were drilled (see Figure 2.1). The lowest 30 cm layers of the ice cores were used to extract sea ice communities. Immediately after drilling the ice was crushed and melted in the double amount of pre-filtered (GF/F 0,7μm, Whatman, Dassel, Germany) sea water at 4°C. Samples were fixed to a Polycarbonate filter (Isopore mebrane filter, 1,2 μm pore size, Millipore, Schwalbach, Germany) using a filter apparatus.
In spring 2006 sea ice samples were taken from the Arctic fjord "Van Mijenfjorden" (see Figure 2.2) during 6 different time points between 2nd of March and the 15th of May. To separate the core from the ice cover on the fjord several holes were drilled to form a circle. The loose ice block was lifted out of the water using ice screws. The lowest 30 cm were separated and packed for transport in light and mechanically safe plastic bags and aluminium boxes (Zarges). Duration of transport was between 3 and 48 hours at +4 and −25°C. To fix sea ice organisms by filtering (filter apparatus), sea ice was melted in pre-filtered (GF/F 0,7μm, Whatman, Dassel, Germany) sea water. To estimate the influence of abiotic factors, CTD measurements were done using the SAIV A/S model SD 204.From both investigation sites, filter were frozen in 2 ml Eppendorf cups or Apex-Tubes at −80°C for further analyses in the lab.

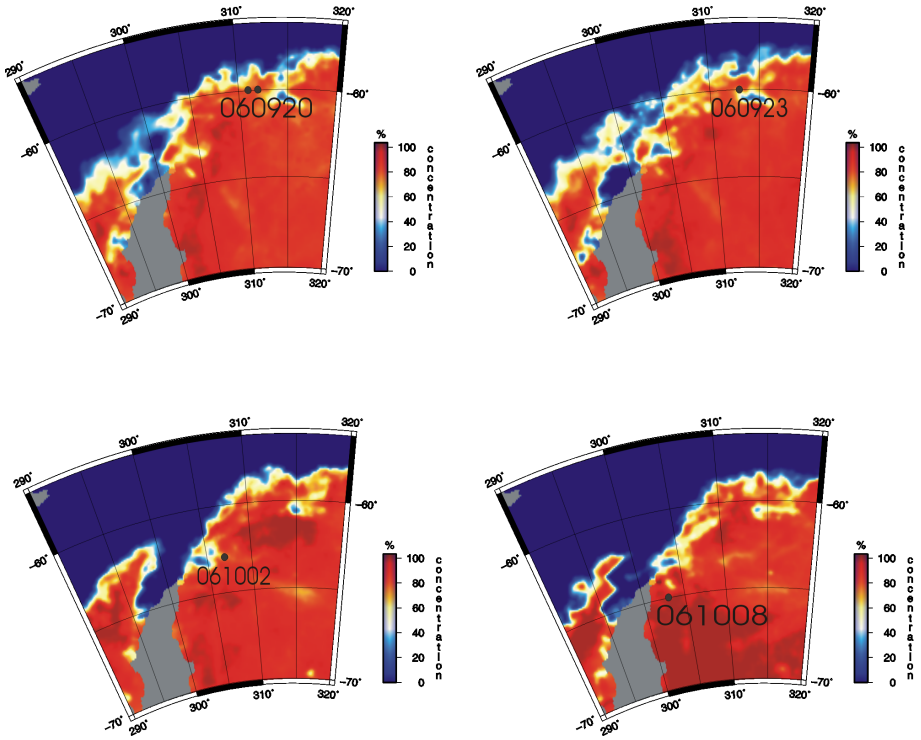


Figure 2.1: Sea-ice concentration maps of the sampling sites at Weddel Sea at the sampling day. On September 20^{th}, 2006, the two samples 060920D (west) and 060920F (east) were taken.

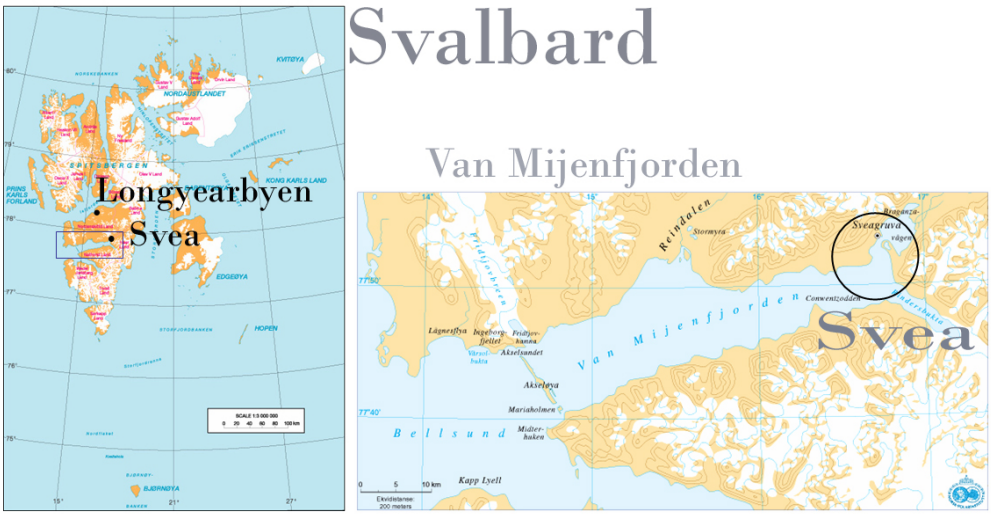


Figure 2.2: Topographic maps of Svalbard and Van Mijenfjorden on Svalbard. The encircled area in the map of Van Mijenfjorden indicates the sampling area of the stations 060331 and 060421.

2.1.2 DNA extraction

Plant DNeasy-Kit (Qiagen)

DNA extraction from Arctic and Antarctic sea ice samples was processed in the same way for all collected samples. The protocol follows instructions of the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and was optimized for samples which are fixed on filters. In this work the principle of the single steps in the protocol will be explained. Detailed informations about the reagents in the protocol are not in hand. All informations offered by Qiagen are listed in the appendix (ref.!)
Organic material was removed from the filter chemically by adding the lysis buffer and mechanically by vortexing. If the filter was not colored anymore after this treatment it was removed from the tube.
The following steps of the protocol aimed at the complete cell lysis without breaking DNA fragments. This was realized by the addition of 2 to 3 spoons glass beads (Sigma-Aldrich, Munich, Germany) to the lysate. In the mini-beadbeater (biospec products, Bartlesville, USA) these glass beads hit for 60 s at 5000 rpm and ground the cell material. Immediately after this treatment the tubes need to be cooled on ice for a view minutes to avoid damage to the DNA because of warming.
For the following work only DNA was needed. Hence the enzyme RNase was added to the lysate to destroy RNA in the sample during incubation at 65°C for 10 min.
The lysate includes several types of cell fragments that need to be removed from the sample. To proceed this separation a second buffer was added which increases the pH to 8. The buffer precipitates proteins, polysaccharides and detergent. The whole lysate was transfered to a Spin Column (QIAshredder Mini Spin Column) with a membrane to collect precipitates. After a centrifugation step all cell fragments except DNA were removed from the flow-through. Centrifugation during the whole DNA extraction was done with Eppendorf centrifuge 5417R.
The flow-through still includes small particles and molecules that can disturb further analyses. The third buffer, consisting of ethanol and salts among other ingredients, is necessary to precipitate DNA and to bind it to the silica-gel-based membrane of a second spin column. Based on positive charged groups of the anion exchanger column, the negative charged backbone of the DNA binds to the membrane. The process depends on the pH of the buffer. Therefore, a defined amount of salts are included in the buffer. After another centrifugation step, only the DNA is bound to the membrane. Proteins and other molecules without strong negative charged groups can pass the membrane.
The DNA was washed by addition of two times wash buffer including ethanol. Afterwards, the ethanol need to be removed completely by centrifugation be-

cause it influences subsequent reactions.
The washed DNA was eluted from the membrane in two steps, in contrast to the protocol. The Arctic samples were eluted first in 40 μL buffer, incubated for 5 min on ice and then centrifuged for 1 min at 8000 rpm. Afterwards, 20 μL buffer were added and the samples were centrifuged for 2 min at 8000 rpm to increase the yield. Samples taken during ANT XXIII/7 were already extracted on board, except sample G which was carried out later in Bremerhaven. The DNA extraction for the Antarctic samples was done following the qiagen protocol except the elution step. Here, a total volume of elution buffer was used in two steps. Centrifugation was done at 8000 rpm. The elution of sample G was done with only 40 μL to increase the yield.

NanoDrop

The concentration and the quality of the extracted DNA had to be quantified to calculate the composition of further experiments. The principle of DNA concentration measurements based on the photometric analysis of liquids. In this work I used the NanoDrop NS-1000 (Peqlab, Wilmington, USA). This spectrophotometer uses a monochromator to fractionise the light before entering the sample. The light is diffracted by molecules while passing the solution. The deflection results in changes of the wavelength and can be measured by a detector. NanoDrop NS-1000 analyses the light spectrum between 220 and 750 nm. For each measurement only 1.5 μL of sample were necessary.
The results of the NanoDrop measurements are shown in Table 3.4.

2.1.3 PCR amplification of 18S rDNA fragments

The aim of this work is to identify eucaryotic organisms by means of 18S rDNA sequencing. Therefore highly conservative DNA fragments were amplified by Polymerase Chain Reaction (PCR) and analysed by sequencing defined parts of these fragments.
The DNA encoding for ribosomal proteins is very conserved between all organisms. The 18S rDNA only occurs in eukaryotic organisms and makes it therefore possible to exclude bacterial DNA from further investigations. Some regions of the 18S rDNA are more suitable for phylogenetic analyses than others. In this work fragments were choosed following results of Medlin et al. (1988).
The PCR reaction is based on the elongation of nuclein acids analogous to the transcription process in living cells. Hence the enzyme polymerase as well as all four nucleotids and suitable conditions are essential for the process. An overview about the reaction steps can be seen at 2.3 The utilized amount of DNA template aimed to be 50 ng/ μL. In cases with very low concentrated DNA the amount of used template was much lower but at least 10 ng/ μL. The HotMasterMix (Eppendorf, Wesseling-Berzdorf, Germany) includes Taq DNA

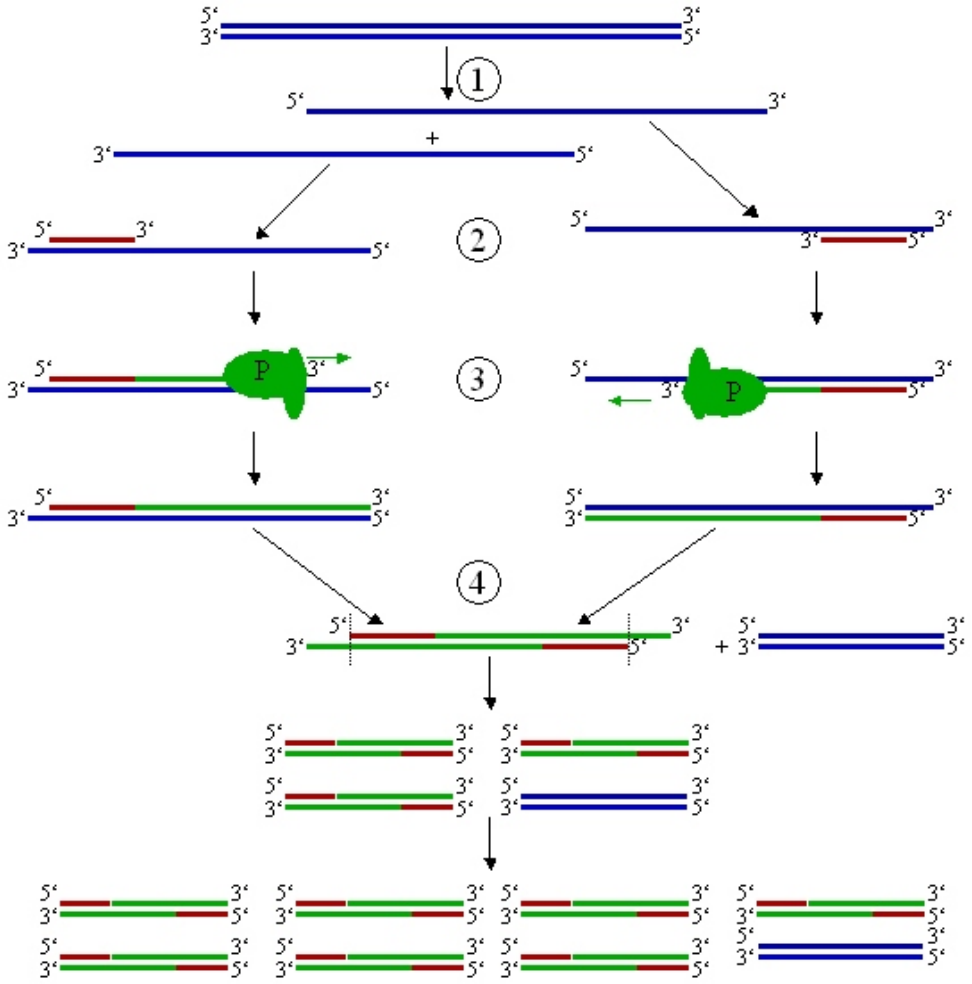


Figure 2.3: Schematic representation of the PCR cycle (Price Ball, 2006): (1) denaturation at 94°C, (2) Hybridisation of template DNA and primers at 52°C, (3) Elongation of the new strand from 5' to 3' by the DNA Polymerase and (4) first cycle of DNA amplification is finished.

Table 2.1: Thermocycler program for 18S PCR showing the temperature and the duration of each cycle as well as the number of repetitions.

temperature	duration	repetition
94°C	3 min	1 cycle
94°C	1 min	30 cycles
52°C	3 min	30 cycles
68°C	2.5 min	30 cycles
72°C	7 min	1 cycle
4°C		hold

Polymerase, 200 μM of each dNTP and 1.5mM $MgCl_2$. For amplification of eukaryotic 18S rDNA fragments the forward primer 1F and the reverse primer 1528R were used, following Medlin et al. (1988). Primer were synthesized by Operon Biotechnologies GmbH (Germany). Thermal cycling was carried out on the PCR Mastercycler (Eppendorf, Wesseling-Berzdorf, Germany). This ensures the denaturation (94°C) of double-stranded DNA, the annealing (52°C) of the primers to the complementary DNA and the extension of the primer (68°C to 72°C) to a new DNA strand. The used program is shown in Table 2.1.
After PCR amplification the 18S rDNA fragments were transformed into bacterias to built up clone libraries. Hence, DNA fragments of the appropriate size were selected by gel electrophoresis using the Mini-sub cell GT and the PowerPac basic power supply (BioRad). The PCR products were marked with SybrGreenI nucleic acid gel stain (Invitrogen, Karlsruhe, Germany) and mixed with loading buffer (Qiagen, Hilden, Germany). An incubation for 5 min at room temperature under low light conditions allows the cyanine dye to bind to double-stranded DNA. To reduce the decomposition of the light sensitive SybrGreenI the whole electrophoresis process needs to be protected against strong light. According to the size of the DNA fragments, agarose gels (Agarose high resolution, Sigma-Aldrich, Munich, Germany) with 1.2 % agarose in TAE buffer (Tris Acetate-EDTA buffer, Sigma-Aldrich, Munich, Germany) were prepared. By using a 1kb ladder (Sigma-Aldrich, Munich, Germany), the size of the DNA fragments can be defined. To view the stained DNA a Safe Imager blue light transilluminator (Invitrogen, Karlsruhe, Germany) was used. The DNA-dye-complex can absorb blue light at 498 nm and emit it at 522 nm. Hence, the green parts of the gel indicate included DNA. An example for the result of the gel electrophoresis is shown in Fig.
DNA bands of appropriate size were cut out of the gel precise and purified by means of the MinElute Gel Extraction Kit (Qiagen, Hilden, Germany). Following the protocol the gel was dissolved in a high-salt buffer during the incubation step. The high-salt conditions allow the selective binding of DNA on the silica membrane. Impurities can be washed from the DNA by use of an ethanol con-

taining wash buffer. The elution was processes with a low-salt buffer releasing the DNA from the silica membrane. To concentrate the eluate only 10 μL elution buffer were used for all Antarctic samples. In all cases of Arctic samples, which yielded very low DNA concentrations, the elution step was repeated to guarantee the maximum yield.
Again, the concentration of the purified DNA was measured using the NanoDrop. The results are presented in Table 3.4.

2.1.4 Clone library generation

The construction of clone libraries from 18S rDNA fragments was done using the TOPO TA Cloning Kit (Invitrogen, Karlsruhe, Germany). This method is based on the following steps: ligation into the plasmid vector, transformation into bacteria cells, separation and amplification of the cells and extraction of the plasmids from bacteria cells. The 18S rDNA PCR fragments were ligated with the pCR2.1-TOPO plasmid vector (see Figure 2.5), proceed by the enzyme Topoisomerase I. Compared to other ligation protocols, neither the digestion of DNA fragments nor the linearisation, dephosporylation or digestion of the vector is necessary. The supplied vector pCR2.1-TOPO is already linearised and bound to the Topoisomerase I. The formation of a covalent bond between the tyrosyl residue of the enzyme and the 3'phosphate of the cleaved vector allows to keep the vector in the activated state. The single 3'-thymidine overhang of the vector binds efficiently over hydrogen bonds to the single 3'-adenosine overhang of the PCR-product, which is always synthesised by the *Taq* polymerase. The unbound 5'hydroxyl group of the PCR fragment then attacks the phospho-tyrosyl bond between the vector and the enzyme. The energy saved in the phospho-tyrosyl bond is used by the enzyme to catalyse the ligation process between vector and 18S rDNA fragment. The ligation was done following the protocol in the TOPO TA Cloning Kit using at least 50ng/μL of PCR product. Transformation of plasmids into bacteria cells can be done in two different ways. The chemical transformation uses heat shock to allow insertion of PCR products into the chemically competent *E.coli* (One Shot TOP10 Chemically Competent Cells, Invitrogen, Karlsruhe, Germany). The heat shock is performed at 42°C in a waterbath (Thermomixer comfort, Eppendorf, Wesseling-Berzdorf, Germany) following the TOPO TA Cloning Kit (Invitrogen, Karlsruhe, Germany) which includes besides cells and vector also S.O.C.medium (Invitrogen, Karlsruhe, Germany)for the incubation of cells. The transformation rate for the chemical transformation is lower than for electroporation. Hence, all samples except sample 061008 were transformed using electroporation. The electroporation bases on the increase of the permeability of the cell membrane caused by an external electrical field. Pores in the semipermeable membrane are formed when the electrical field is applied in the appropriate strength and duration. Substances, for instance plasmids, can pass the pores and enter the cell nu-

cleus.
The cell suspension (25 to 50 μL), gently mixed with 2 μL ligation product, needs to be protected from arcing processes. A higher salt concentration increases the electrical conductivity which may kill the bacteria cells. Hence, the ligation product was desalted by pipetting it on a nitrocellulos filter (0,025 μm; 13 mm; Millipore, Schwalbach, Germany)sitting on deionized water in cell culture plates (Nunc, Germany). An incubation of 5 min allows the diffusion of salt through the membrane from the higher concentrated solution to the lower concentrated one. The desalted ligation product was directly used for the electroporation process.
The cell suspension, mixed with the ligation product, was added to a precooled quartz cuvettes (BioRad, Hercules, USA). Immediately, the cuvette was put into the electroporation chamber of the GenePulser Xcell (BioRad, Germany). The set voltage was 1.8 kV and the pulse length 5 ms. The actual time and voltage always differed little from the set one and is shown in Directly after the pulse, 250 μL room-tempered S.O.C. medium was added to the cells. The whole suspension was transfered into a 15ṁL Falcon tube (BD Biosciences, San Jose, USA)and incubated at 37°C (incubator 1000, Heidolph Instruments, Schwabach, Germany) and mixed at 250 rpm (Unimax 1010; Heidolph Instruments, Schwabach, Germany) for one hour to allow gene expression of antibiotics resistance genes.
After the incubation step, transformed cells were plated on cell culture plates (Nunc, Wiesbaden, Germany) containing LB medium (see Table A)with 50 μg/mL Ampicillin. To make blue-white selection possible, 40 μL of X-Gal (promega, Mannheim, Germany) with a concentration of 40 μg/ μL were added to each plate. The cell suspension was plated in different concentrations, depending on the transformation method and the resulting transformation rate The dilution of the cells was proceed in S.O.C. medium to a final volume of 50 to 80 μL to allow an equal dispersion and separation of the cells. Plating was done with a Trigalski-spattle under a clean bench (Hera safe, Heraeus, Hanau, Germany). During the incubation at 37°C the formation of colonies of different clones was possible. Each of them was containing a variation of the 18S-fragment, depending on the DNA pool in the origin sample.
Working with clone libraries makes an sufficient amount of cells necessary. Therefore, the cells were amplified by picking some cells from each colony and transferring them to LB medium. For all clones of station 061008 and partial for station 060923 , the common LB medium (see Table A)was used whereas all other cells were grown in LB PlasmidGRO^{TM} (Genetix, Munich, Germany) to maximize the yield of cells.
For each sample, the first clones were grown in 3 mL medium in 15 mL Falcon tubes and processed separately until sequencing. If the results were successfully, all steps were done at 96 scale. The LB medium was distributed to the tubes of

the Deep Well-Plates 96 (Assay Block 2 mL, Costar, Bodenheim, Germany) by using multi-channel pipettes (Eppendorf, Wesseling-Berzdorf, Germany) during working process under the clean bench. For 96 scale, only 1,2 mL medium were used leading to a smaller yield of cells. The cells were allowed to grow for 20 to 24 hours at 37°C while mixing at 250 rpm in the incubator (Heidolph unimax, Heidolph instruments GmbH & Co.KG, Schwabach, Germany). The duration was found to be optimal for LB PlasmidGRO^{TM} being 21 hours. This was the shortest incubation time leading to a sufficient yield of cells. Longer growth periods may cause an increasing number of dead cells.
From each sample a backup was made by freezing 140 μL of cell suspension (in 96-well Mikrotiter plates, Biozym, Germany), mixed with 60 μL of 50% glycerol at −80°C. The rest of the suspension was centrifuged for 10 min at 4000 rpm (centrifuge 5810R, Eppendorf, Wesseling-Berzdorf, Germany)to harvest the cells and remove the medium.
For analyzing only the 18S fragments the plasmids needed to be extracted from the bacteria cells. This is done, in general, by breaking the cell walls by acidification, precipitating the genomic DNA and pelleting the cell fragments and genomic DNA by centrifugation. The supernatants, containing the plasmids in high-salt buffer, are selective absorbed by silica membranes in filter columns. This selective binding process allows, after a washing step using ethanol, the elution of pure plasmid DNA in low-salt buffer. Proteins, RNAs, salts and other metabolites flow through the membrane. Binding, washing and elution processes were proceed by either centrifugation or using vacuum manifolds depending on the used protocol and throughput.
Each station was first proceed in a low throughput to testify the method and the quality of the 18S fragment. This was done with the QIAprep Spin Miniprep Kit (Qiagen, Hilden, Germany) using centrifugation (centrifuge 5417R, Eppendorf, Wesseling-Berzdorf, Germany) as discribed in the protocol. The washing step was proceed only once, using PE buffer but the second centrifugation step was enlarged to 3 min to completely remove ethanol.
The higher throughput was done for station 061008 with R.E.A.L. Prep 96 Kit (Qiagen, Hilden, Germany) allowing plasmid preparation for 96 samples at once. The performance followed the protocol and used the vacuum manifold (Qiagen, Hilden, Germany). All other 96 plates were processed by the robot freedom evo (Tecan, Germany) using the software EVOware Standard. The program corresponds to the preparation steps of the R.E.A.L. Prep 96 Kit but the elution volume was set to 75 μL.
A few results of the plasmid preparation were measured by using the NanoDrop. The mean concentration of plasmid DNA, as shown in Table 3.4,were used to define the necessary volume for sequencing reaction.

2.2 Sequencing of clone libraries

Sequence Analysis (DyeEx-Kit)

Sequencing of DNA and RNA is the only way to view nucleotides base by base. Other methods compare sizes of fragments or describe binding with known probes. New sequencing methods such as the primer-extension-method allow the analysis of up to 1000 nucleotides. Although you get signals for each single nucleotide there are still mistakes possible in the sequence.
Sequencing reactions bases on a PCR step using dideoxynucleotides (ddNTPs) carrying base-specific fluorescent dyes and working as terminators. Randomly, these ddNTPs are incorporated at each single position of the whole sequence. The missing hydroxide group at the third as well as at the fifth position of the nucleotide avoid further elongation. The separately labeled ddNTPs, which fluorescent at different wavelengths, can be analyzed using fluorescent-based electrophoresis. The gel has to be capable to separate between molecules which differ in size by only one nucleotid.
In this work, sequencing PCR was proceed using 50 to 100 ng DNA, the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Hercules, USA) and the universal primer for eukaryotic organisms 528R. The BigDye Sequencing Kit contains 5x Sequencing buffer and Premix hence supplying enzyme, dNTPs, ddNTPs, salts and water. The used Primer, positioned 500 bp behind the 3'end of the 18S fragment, was developed by L.Medlin Medlin et al. (1988). To process the incorporation of dNTPs and labeled ddNTPs accordingly to the DNA sequence a thermocycler is necessary to realize sequencing specific heating and cooling steps. The program used for this work is shown in Table 2.2. It is optimized for a wide range of organisms by using only 52°C for the annealing step. The lower the annealing temperature is set, the less specific do the primers bind. The sequencing PCR was proceed with the PCR Mastercycler (Eppendorf, Wesseling-Berzdorf, Germany).
High-throughput sequencing at 96-scale was done for all stations. For sequencing twin.tec PCR Plates 96 (Eppendorf, Wesseling-Berzdorf, Germany) were used. Products of the sequencing reaction need to be purified by residual primers, unincorporated dye terminators and free salts. For a small number of samples this was done with the DyeEx 2.0 Spin Kit (Qiagen, Hilden, Germany). First, 96-scaled samples were done with the aid of the DyeEx 96 Kit (Qiagen, Hilden, Germany) using the vakuum manifold. Most sequencing reactions proceed at 96 scale were purified by the robot freedom evo (Tecan, Germany) using the software EVOware Standard and the sequencing reaction cleanup kit "Montage SEQ_{96}" (Millipore GmbH, Schwalbach, Germany). As shown in Table 2.2 samples were first eluted in 20 μL but later in 30 μL elution buffer into twin.tec PCR Plates 96 (Eppendorf, Wesseling-Berzdorf, Germany).

Table 2.2: Thermocycler program for sequencing reaction showing the temperature, duration and number of repetitions of several cycles.

heating up to	1°C/min	96°C		1 cycle
hold		96°C	1 min	28 cycles
heating up to		96°C		28 cycles
hold		96°C	10 s	28 cycles
heating up to		50°C		28 cycles
hold		50°C	5 s	28 cycles
heating up to		60°C		28 cycles
hold		60°C	4 min	28 cycles
hold		4°C		

samples were first eluted in 20 μL but later in 30 μL elution buffer into twin.tec PCR Plates 96 (Eppendorf, Wesseling-Berzdorf, Germany). The set volume was dependent on the ensuing preparation proceedings. One possibility is to add 10 μL of 20 μL eluate to 10 μL HiDi Formamide (Applied Biosystems, Lincoln, USA) and denatured at 95°C for 3 min (PCR Mastercycler, Eppendorf, Wesseling-Berzdorf, Germany). Another way is to vaporise 30 μL eluate using a SpeedVac (Savant SPD131DDA SpeedVac Concentrator, Thermo Scientific, Dreieich, Germany), combined with a Refrigerated Vapor Trap (RVT400, Thermo Scientific, Dreieich, Germany) and a Oil-Free Vacuum Pump (OFP400, Thermo Scientific, Dreieich, Germany). and dissolve it in 10 μL HiDi Formamide (Applied Biosystems, USA). Again, the denaturing was proceed. The twin.tec PCR Plates 96 (Eppendorf, Wesseling-Berzdorf, Germany) were closed with 96-Well Plate Septa (Applied Biosystems, Lincoln, USA).
Automated sequencing was done using a 4-capillary sequencer (3130 Genetic Analyzer, Applied Biosystems, Lincoln, USA). Viewing chromatograms can help to estimate the quality of the sequencing reaction. High and clearly separated amplitudes are necessary to gain reliable sequences. In this work chromatograms were viewed using chromas 2.31 (technelysium).

2.3 Phylogenetic Analysis

2.3.1 Alignement

Sequencing only gives informations about the succession of nucleotides and therefore about the number of differences. To fit it into a phylogenetic tree by comparing with known sequences, different procedures are possible. The first and very common way to get a rough idea of the position of the sample in the phylogenetic tree is the usage of online databases such as BLAST (Basic

Local Alignment Search Tool). A phylogenetic tree can be obtained by computational phylogenetic methods using special programs, for instance Staden package (SourceForge, version 1.5). The genetic distance between sequences can be calculated by the neighbor-joining tool. For the estimation of phylogenie the tree needs to have the least number of evolutionary changes. Hence, the method of Maximum Parsimony (Eck and Dayhoff, 1966; Fitch and Margoliash, 1967) was used here to fit the DNA sequences, which were isolated from sea ice, to a phylogenetic tree.
The quality of all sequences was controlled by quality clipping and Phred which are included in the Staden Package. The quality clipping uses confidence values and a minimum sequence length. Additional, the Phred software can calculate quality values to each base. These quality values are log-transformed error probabilities. The Staden Package provides also the alignment tool Gap4 shotgun assembly. This tool compares sequences and defines regions of high similarity to interpret the relation between these sequences. In this work, the minimum exact match was set to 200bp, the maximum number of pads to 10 and the maximum consensus length to 10000bp. The maximum percentage mismatch, which was 2.0 in this case, defines the minimal percentage of consensus which groups aligned sequences to one OTU (Operational Taxonomic Unit). The OTUs are compaired with the sequences of an existing phylogenetic tree and added to the appropriate position. In this work the OTUs were put into ARB (Ludwig et al., 2004) using the fast aligner and filters which were calculated by base frequency. The filter was programmed to exclude the whole column if unknown residues appeared at any position and if real gaps were maximal. Ambiguity codes and lower case letters were ignored.
The treeing method of the ARB-package is based on the maximum parsimony. Since the resulting tree does not always correspond to the NCBI BLAST (Altschul et al., 1990), single OTUs were deleted from the tree and added again. This treatment often leads to a completely different position of the OTU in the tree which is now more similar to the sequence with the highest score in BLAST search.
The species diversity of the phylogenetic tree only take the OTUs in account. Therefore, also the number of sequences which form on OTU need to be included into the results to compare quantitative.

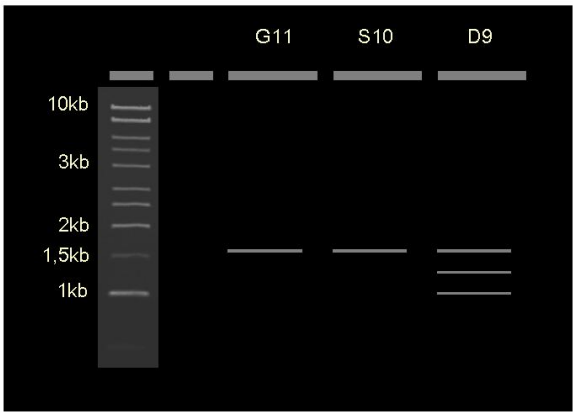


Figure 2.4: Illustration of an agarose gel; marker (left column) and Antarctic samples were stained with SybrGreenI.

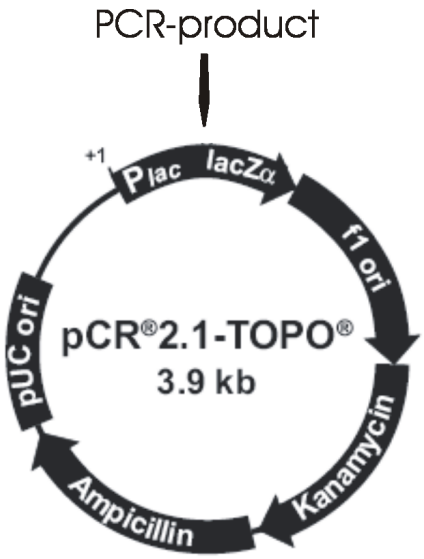


Figure 2.5: Map of the utilized vector pCR 2.1-TOPO; the PCR product is inserted at the position of the arrow (Invitrogen, 2006).

Chapter 3

Results

3.1 Sampling of ice cores

In the Arctic, ice cores were taken at 5 dates and 2 different locations, respectively. For the preparation of 18S libraries the samples originating from the fjord were chosen, because their salinity was comparable to those of the Antarctic sea ice samples. The water at the second location is only seasonally connected to the ocean but always to a freshwater inflow and is therefore less saline. To determine the abiotic conditions in the sea ice, temperature measurements were done in the air over the ice and in the water column. Salinity, density, and fluorescence were also determined in the water. The structure of the lowest ice layer was observed and ice and snow thickness were measured. All data of the surface water layer of the selected stations are shown in Table 3.1.
Abiotic parameters in the Antarctic sea ice were analysed by David Thomas, Stathis Papadimitriou (School of Ocean Science at the University of Wales, Bangor) and Marcel Nikolaus (Norsk Polar Institute, Tromsø). Because these data are more suitable for the interpretation of a relationship between the sea ice biodiversity and abiotic conditions, no additional information about the water column are given here. In Table 3.1, temperature, bulk, and brine salinity and chlorophyll concentration are shown besides snow- and ice thickness data. The given salinities and bottom-ice temperatures for the samples 060920D, 060920F and 060923 were not determined at the same ice core but only at 060919 and 060924 and therefore must be interpreted with care. The mentioned physical measurements were only done once a day. Hence, in Table 3.1, 060920D and 060920F are given as 060920. Because only the lowest 10 cm were used for further experiments only the appropriate abiotic data are shown in Table 3.1. The nutrient supply within the Antarctic samples is shown in Table 3.2. These data were also provided by David Thomas and Stathis Papadimitriou (School of Ocean Science at the University of Wales, Bangor).

For the Antarctic samples, 5 dates were investigated which are spread over the whole sampling period and sampling area. Because the Arctic sea ice developed very late in 2006, samples were selected which date later in the season but also spread over almost one month.
The position of the sampling sites in the Weddel Sea is shown in Figure 2.1. The map is modelled using ice concentration data collected during ANT XXIII/7 and plotted by Lasse Rabenstein (AWI, Bremerhaven).
Due to the varying facilities at the Arctic and Antarctic sampling sites, only measurements of the air temperature and ice and snow thickness are comparable. The temperature-dependent stratigraphy of the Antarctic samples was plotted by Marcel Nikolaus (Norsk Polarinstitutt, Tromsø), as shown in Figure 3.1.

Table 3.1: Abiotic conditions at the sampling sites; water temperatures, salinities, densities and fluorescence is measured at 1.25 m depth at station 060331 and at 1.01 m depth at station 060421.

sample	60331	60421	060920	60923	61002	61008
ice thickness [cm]	50	65	75	110	123	124
snow thickness [cm]	15	30	6	34	3	2
air temp. [°C]	−15	4	−10.9	−16	−6	−7.6
bottom-ice temp. [°C]			−2.0	−2.0	−2.1	−2.8
water temp. [°C]	−10.61	−1,73				
ice structure			granular	columnar	columnar	columnar
brine salinity S_b [psu]			35.6	34.8	37.4	49.2
bulk salinity S [psu]			2.3	6.3	8.5	9.0

3.2 DNA extraction and amplification

The DNA of the selected ice cores was extracted as described in Chapter 4.2.1. Concentration and absorption ratio of the template DNA can be found in Table 3.3. The 18S rDNA fragments were amplified using 18S PCR (see Chapter 2.1.3). After the ligation between the 18S rDNA fragments and the vector pCR 2.1-TOPO, the transformation of the plasmids into *E.coli* was done as described in Chapter 2.1.4. From all clone libraries, the plasmids were extracted and

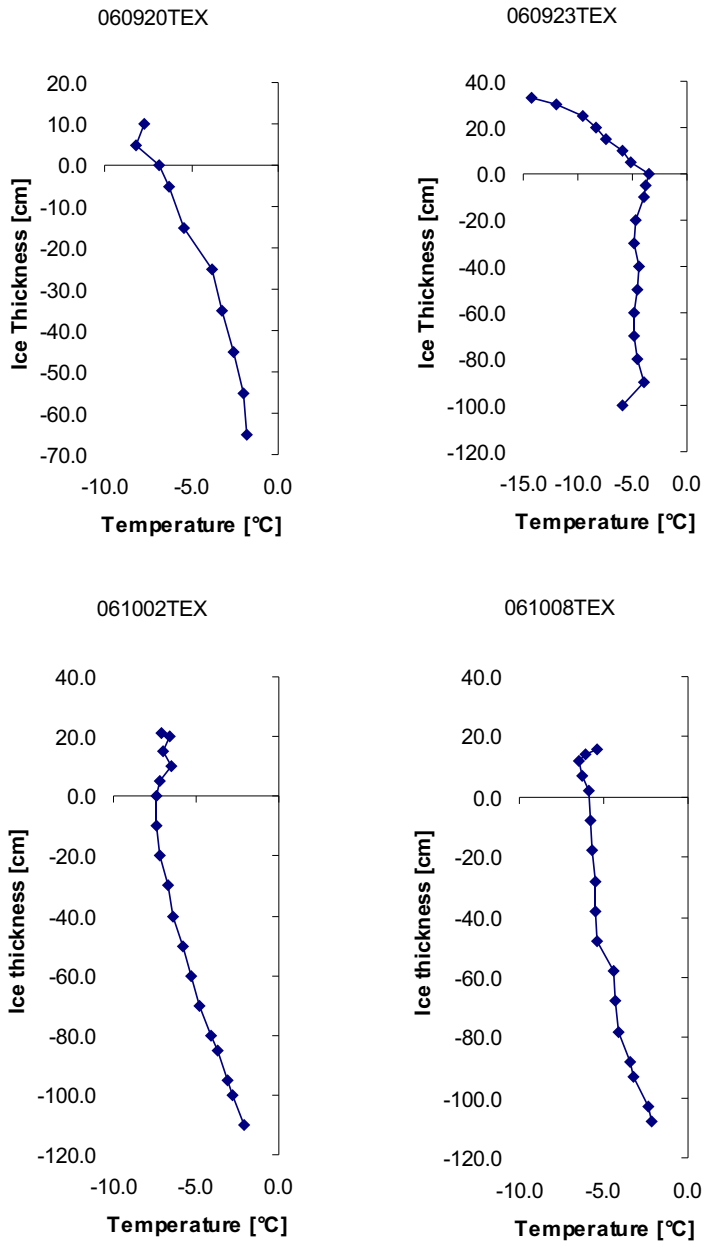


Figure 3.1: Temperature-dependent stratigraphy of Antarctic sea-ice samples (modified after Nikolaus (2006)).

Table 3.2: Nutrient supply of the Antarctic stations.

sample	060920	60923	61002	61008
DOC [μmol l^{-1}]	28	17	159	169
DON [μmol l^{-1}]	8.1	2.5 15.3	22.5	
DIP [μmol l^{-1}]	0.9	0.4	1.55	2.23
NO_3+ NO_2 [μmol l^{-1}]	1.8	4.9	2.8	7.8
Si [μmol l^{-1}]	4.5	10.3	3.4	6.1
N:P ratio	2	12.25	1.8	3.5
Si:N:P ratio	2.78	5.25	0.78	0.35
Si:N:P ratio	5 : 2 : 1	25.7 : 12.3 : 1	2.2 : 1.8 : 1	2.7 : 3.5 : 1
Chl a [μg l^{-1}]	32.71	2.7	99.7	93.22

sequenced (see 2.2). The number of sequences of each station, which passed the quality clipping and which were longer than 500 bp are listed in Table 3.3.

Table 3.3: Results of the generation of the 18S libraries and analyses in ARB for Arctic and Antarctic stations

sample	60331	60421	060920D	060920F	60923	61002	61008
extracted DNA [ng/μl]	2.52	3.39	41.66	29.95	83.09	24.49	20.09
A260/A280			1.78	1.97	1,662	1.65	1.83
A260/A230			4.19	5.65	0,893	1.33	3.84
number of seq. >500bp	174	91	196	80	273	69	245
number of OTUs	28	18	50	14	75	38	46
number of CPGs	7	8	8	5	6	6	7

3.3 Phylogenetic analysis

The gained sequences were grouped to OTUs using staden package (SourceForge, version 1.5). All OTUs were compared to the ARB database. The corresponding trees can be found in the appendix (Figure B.1 until B.7). By including the results of the BLAST searches (Altschul et al., 1990) into

Table 3.4: Results of NanoDrop measurements: DNA concentration and OD ratios are given after DNA extraction from environmental samples, purification after gel extraction and plasmid preparation (mean values).

sample	60331	60421	060920D	060920F	60923	61002	61008
extracted DNA [ng/μl]	2.25	3.39	41.66	29.95	83.09	24.49	20.09
A260/A280	1.74	1.68	1.78	1.97	1.662	1.65	1.83
A260/A230	1.45	1.31	4.19	5.65	0.893	1.33	3.84
eluted DNA [ng/μl]	14.61	15.13	16.5	8.46	28.2	35.78	94.84
A260/A280	2.03	2.1	4.82	2.3	0.563	0.716	1.937
A260/A230	0.03	0.02	0.04	0.02	0.276	0.369	1.058
plasmid preparation [ng/μl]	64.6	86.6	45.12	41.89	53	83.8	43.1
A260/A280	1.89	1.9	2.45	1.94	1.79	1.9	1.77
A260/A230	0.95	1.1	0.94	0.87	0.66	1.26	2.4

the interpretation, new groups of species could be identified. These so-called "Combined Phylogenetic Groups" (CPG) pool all sequences with the same closest relative in ARB and the same BLAST search result. In Table B.1 examples of the BLAST search are listed, showing the overlap between query and best hit ("query coverage") the and maximal identity.
The CPGs are displayed in Figure 3.2 and the relative contribution of the defined CPGs were calculated as part of the total number of OTUs (see Figure 3.2a) and sequences (see Figure 3.2b).
The most abundant groups (bacillariophyta (see Figure 3.3a), dinophyceae (see Figure 3.3b) and metazoa (see Figure 3.4)) are plotted in separate Figures. The total number of sequences or OTUs of each taxonomic group can be found on top of each column. The given CPGs are calculated relatively to this total number.

The columns in Figure 3.2 show differences in species composition between all stations which are more clear in the sequence-based bar chart (see Figure 3.2b). In Figure 3.3 and 3.4, the relative distribution of the CPGs within the appropriate kingdom is shown. The charts are calculated for the kingdoms which contain the highest numbers of sequences and several different classes or phyla. In Figure 3.3a, the distribution of centric and pennate diatoms is shown. The fraction of the genus Fragilariopsis can be plotted, because this well observed algae class was safely identified by ARB and BLAST. The species names are not given because different Polar Fragilariopsis species produced the same bootstrap values in ARB and comparable scores by using the BLAST search.
The bacillariophyta are represented by Fragilariopsis in all stations, but in 3

stations, this class is not dominant. In (b) of Figure 3.3, different orders of the family dinophyceae are plotted. Except at station 061002, which is only represented by 2 sequences, all sequence-based values are dominated by one order of the dinophyceae family.
In Figure 3.4, the distribution within the kingdom metazoa is presented in the same way. Here, no significant difference between the sequence- and OTU-based fractions can be observed and each station contains only one or two CPGs.

3.4 Calculation of species diversity

The number of sequenced clones from the different stations varies as shown in Table 3.3 and Figure 3.3. Therefore, the probability to find new species with increasing sequencing effort has to be estimated. This so-called saturation level of a library can be visualized by rarefraction as described by Colwell et al. (2004). Using EstimateS version 8.0 (Colwell, 2006), the phylotype diversity can be calculated. Rarefraction curves of S_{obs}(Mao Tau) were plotted for the two stations with high numbers of sequences clones and also OTUs and CPGs (see Figure 3.5). The rarefraction curve would approach an asymptote, if the saturation of the sequencing effort would be reached.

The phylogenetic trees of the different stations need to be compared to analyze the results from an ecological point of view. A common method to characterize species diversity in a community is the calculation of the Shannon index (Shannon, 1948), which is described by the following equation:

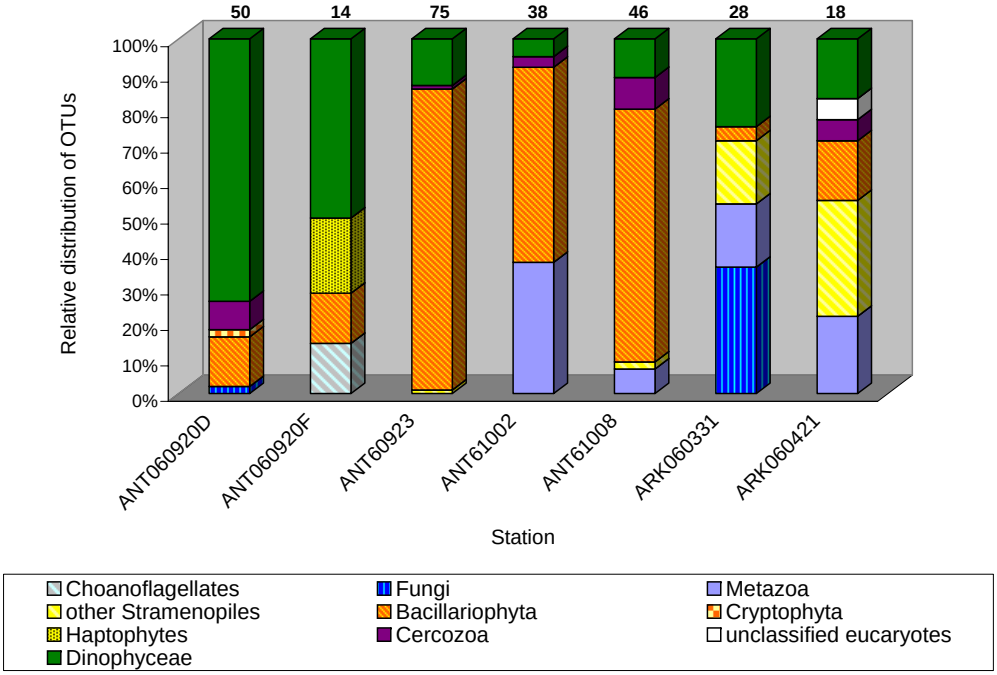
$$H = -\sum_{i=1}^{s} p_i \, ln \, p_i$$

The equation bases on the proportion of each species (i) relative to the total number of species (p_i). It includes the species abundance and the evenness of the present species. The value of H can rise ad infinitum, depending on the species richness and the equability. The evenness, which approaches 1 if all species are equally abundant, can be calculated as following:

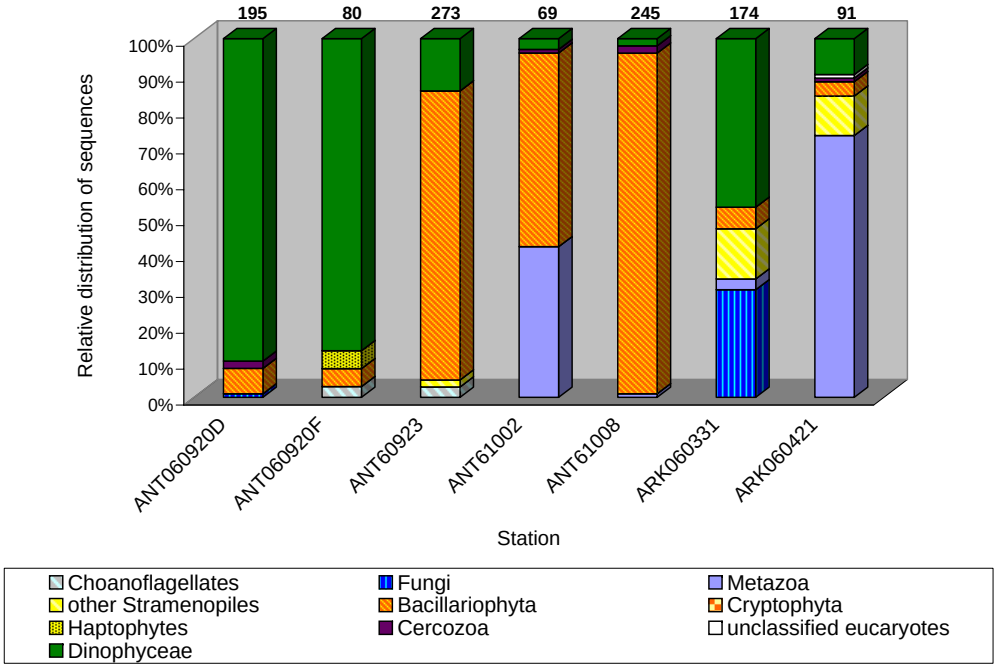
$$E = H/ln \, s$$

The Shannon indices and the evenness of all stations are shown in Table 3.5. The influence of the sequencing effort on both indices is analysed by plotting the Shannon diversity indices in relation to the sample size (see Figure 3.6). The calculated values for the eveness is related to the sample size in Figure 3.7.

The biodiversity indices were used to estimate the influence of abiotic parameters on the species diversity. In Figure 3.8, biodiversity is plotted in relation to the total thickness, including the ice layer and the snow layer on the

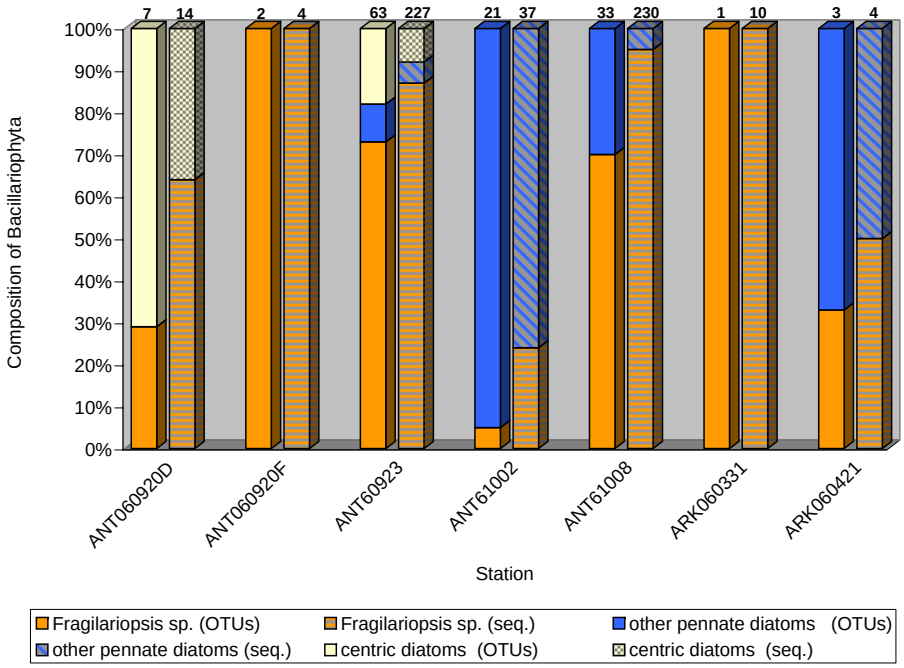


(a) Composition of CPGs of Arctic and Antarctic stations; calculation based on relative distribution of OTUs.

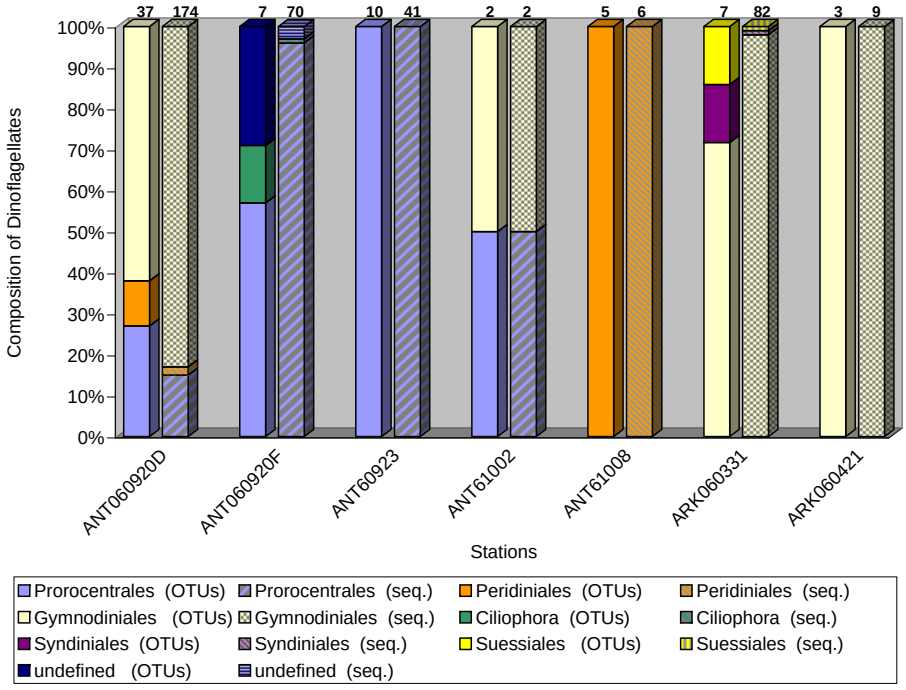


(b) Relative composition of CPGs of Arctic and Antarctic stations; calculation based on relative distribution of sequences.

Figure 3.2: Relative composition of "Combined phylogenetic groups" using OTUs (a) and all sequences (with >500bp and after quality clipping) of the clone library (b). The number on top of each column indicates the total number of OTUs (a) or sequences (b), which were used for the calculation.



(a) Composition of CPGs of Arctic and Antarctic stations; calculation based on relative distribution of OTUs and sequences (shaded) of all Bacillariophyta.



(b) Composition of CPGs of Arctic and Antarctic stations; calculation based on relative distribution of OTUs and sequences (shaded) of all Dinophyceae.

Figure 3.3: Relative composition of "Combined phylogenetic groups" of all Bacillariophyta (a) and Dinophyceae (b). The number on top of each column indicates the total number of OTUs or sequences, which were grouped to Bacillariophyta (a) and Dinophyceae (b). The left column of each station shows OTU-based numbers, sequence-based numbers are shaded.

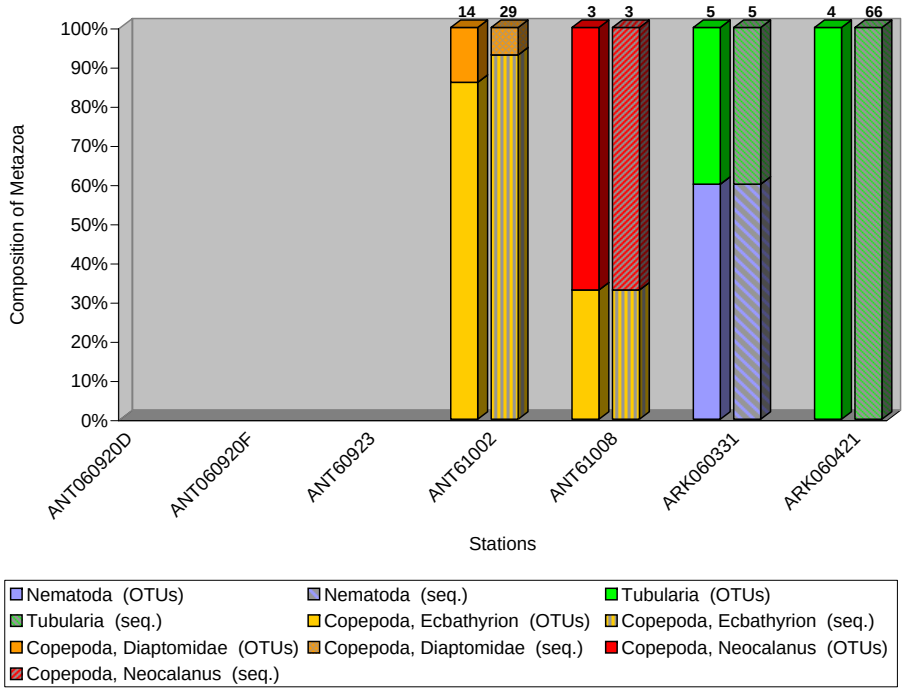
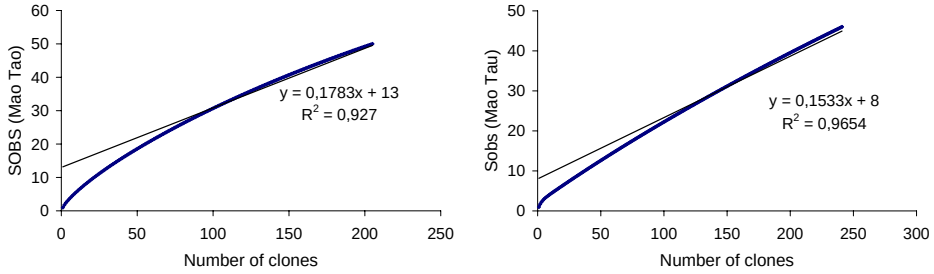


Figure 3.4: Relative distribution of "Combined phylogenetic groups"; calculation based on OTUs and sequences (shaded) of all Metazoa. The number on top of each column indicates the total number of OTUs or sequences, which were grouped to Metazoa



(a) Rarefraction curve analysis of the Antarctic station 060920D.

(b) Rarefraction curve analysis of the Antarctic station 061008.

Figure 3.5: Rarefraction curve analyses of S_{obs}(Mao Tau) vs. the sequencing effort of Antarctic stations (blue line) using EstimateS version 8.0 (Colwell, 2006); calculated trend line (black line) with a clearly rising slope.

Table 3.5: Shannon- diversity indices of Arctic and Antarctic stations.

sample	60331	60421	060920D	060920F	60923	61002	61008
Shannon index H	1.337	0.971	0.962	0.663	0.909	1.18	0.494
Eveness	0.644	0.499	0.462	0.41	0.508	0.659	0.252

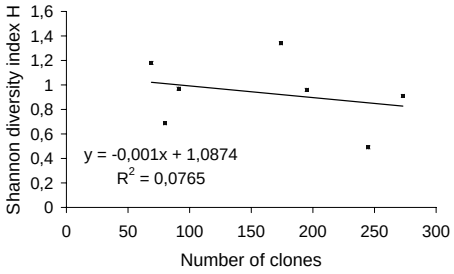


Figure 3.6: Correlation between Shannon diversity index and the number of sequenced clones of all Arctic and Antarctic stations.

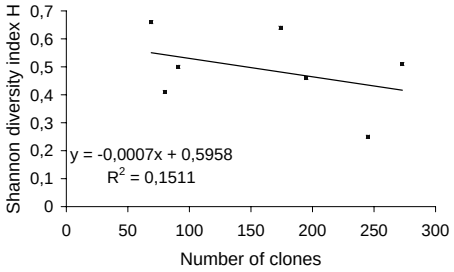
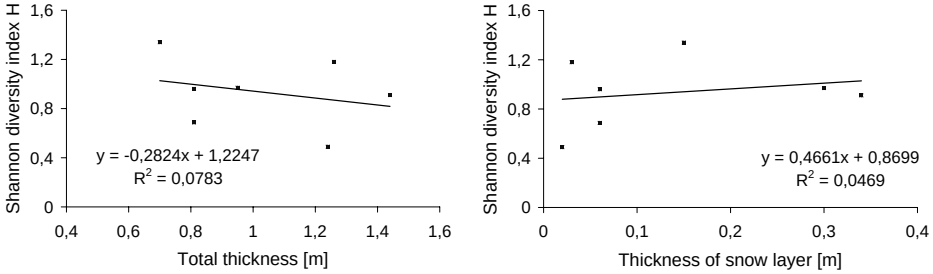


Figure 3.7: Correlation between Eveness and the number of sequenced clones of all Arctic and Antarctic stations.



(a) Distribution of the Shannon diversity index of Arctic and Antarctic stations depending on the total thickness of the snow and ice layer

(b) Distribution of the Shannon diversity index of Arctic and Antarctic stations depending on the thickness of the snow layer

Figure 3.8: Influence of the total thickness of the snow and ice layer (a) and only the snow layer (B) of Arctic and Antarctic stations on the sea ice biodiversity; the line indicates the slope of the calculated regression.

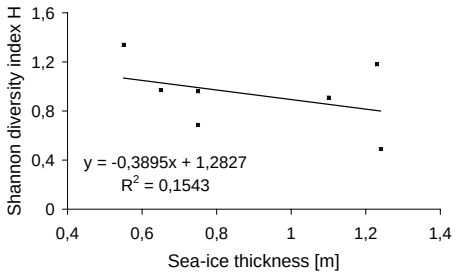
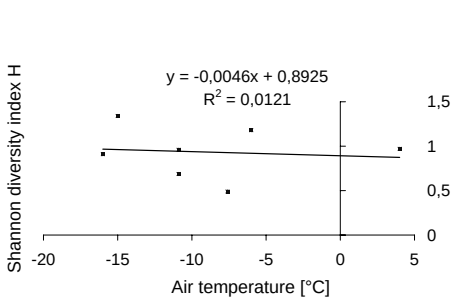


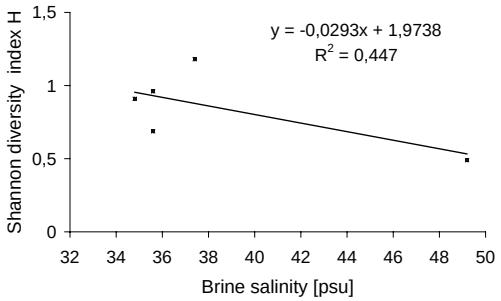
Figure 3.9: Influence of the thickness of the ice layer of Arctic and Antarctic stations on the sea-ice biodiversity; the line indicates the slope of the calculated regression.

ice (a) as well as the thickness of the snow layer (b). The influence of only the ice-thickness is shown in Figure 3.9.

The biodiversity index was also related to the air temperature in Figure 3.10. Because the air temperature of station Ark060421 rose up to +4°C within a short time, the change can not be proportional to the change in biodiversity. Hence, this station was excluded from Figure 3.10.



(a) Distribution of the Shannon diversity index of Arctic and Antarctic stations depending on the air temperature during sampling.



(b) Distribution of the Shannon diversity index of Antarctic stations depending on the brine salinity.

Figure 3.10: Influence of the air temperature (a) and the brine salinity (b) of Arctic and Antarctic stations on the biodiversity of sea ice; the line indicates the slope of the calculated regression.

Chapter 4

Discussion

4.1 Sampling

4.1.1 Sampling site

The explanation for the differences between the observed sea-ice samples requires the consideration of several factors. In the Arctic, the sampling site is a shallow, semi-enclosed fjord which hence, is influenced by the nearby coast. Both Arctic stations were taken from the same location, that is why no spatial variation was expected. Hence, differing results can be explained by temporal variations leading to changing light and temperature conditions.
In contrast, the Antarctic samples were all taken at different positions. Therefore, the results need to be interpreted relating to spatial and temporal variations. Also, the distance to the coast differs a lot as shown in Figure 2.1. For these reasons, a conclusion about a distinct community composition being present due to defined parameters is difficult. The observed species compositions at the different sampling sites can not be clearly related to the location, the abiotic conditions or the time of sampling.
The sampling conditions in Antarctica were much better because the drilled ice cores could be directly transported to the lab to be filtered. The DNA was extracted on board of RV Polarstern or immediately frozen at −80°C.
In the Arctic, the sampling took one or two days, meanwhile the ice cores stayed at the air temperature. The filtration and freezing of the filters was done after even longer periods. But a clear influence on the discovered biodiversity is not expected since the number of organisms would not change distinctly at these low temperatures. After filtering the melted sea ice, the filters were frozen at −80°C and further analyzed at AWI Bremerhaven. The transport of the samples back to Germany was, due to logistic problems, only possible in cooled, isolated containers, which could not avoid a warming up to 0°C for maximal a few hours. Because DNA is comparatively stable, no significant degradation of the DNA is assumed.

4.1.2 Geophysical and oceanographic measurements

During ANT XXIII/7, various geophysical, oceanographic and biological measurements were done. Therefore, data for the calculation of ice-concentration maps could be used and the structure of the sea ice layers could be defined. Also, time-consuming measurements of different nutrients as well as salinity measurements were made by David Thomas and Stathis Papadimitriou (School of Ocean Science at the University of Wales, Bangor) and could be used for this work. These data are more reliable than the Arctic CTD data which only allowed measurements in the water column. The instruments on RV Polarstern were used during the whole cruise and gave comparable data.
For this work, only selected physical data were used depending on their assumed impact on the biodiversity. Oceanographic data colleced in the water column under the ice, such as temperature, salinity and density were not included into the interpretation because the variations are probably too small to influence the highly tolerant sea ice communities.

4.2 Processing

4.2.1 DNA extraction

The melted sea ice was filtered by following the same protocol. Even though Arctic samples were prepared by another person and under different laboratory facilities than the Antarctic ones, no significant impact of the sampling procedure on the quality of the samples is expected. Important details, such as melting in prefiltered seawater at 4°C or mixing the sample bottles to include also fast-sinking organisms, were considered in both cases.
DNA extraction on board of the RV Polarstern was done using the same protocol as for the extractions at AWI. The application of the standard DNA extraction kit "DNeasy Plant Mini Kit" ensured a sufficient quantity of DNA. By disrupting the samples with glass beads and the bead beater, also the DNA of those cells was extracted which are surrounded by frustules or other difficult-to-break cell walls. But in some cases, e.g. if high concentrations of polysaccharides and polyphenolics were present, other extraction methods such as CTAB extraction would be necessary. For high-throughput applications, this method is too time-consuming. Hence, the fast but possibly not exhaustive method was used.
The quality of the extracted DNA was always tested by NanoDrop measurements. The OD ratios of OD260/OD280 of pure DNA is between 1.8 and 2.0 and a smaller value is caused by protein contamination. The OD260/OD230 ration is used to determine the contamination by phenolate ions, thiocyanates and other organic compounds, which is indicated by values smaller than 2.0.
The measured values and ratios are shown in Table 3.4. The DNA concentration after extraction from environmental samples was sufficient except for

Arctic samples. Nevertheless, these two samples showed clear DNA bands at the appropriate size after gel electrophoresis and were used for further analysis. The purity indicated by OD260/OD280 values of 1.6 to 1.8 was good enough for subsequent steps. In contrast, the OD230/OD260 ratios were not satisfactory. Anyway, the extracted DNA was used because this contamination would not affect the following 18S PCR.

4.2.2 18S PCR

The amplification of the 18S fragments was done in exactly the same way for all samples. The results were controlled by gel electrophoresis, as described in Chapter 2.1.3. In case the gel contained a clear band at the expected size of 1528 bp, it was cut out and eluted out of the gel. Otherwise, the 18S PCR was repeated. These steps allow a purification of the desired DNA fragment from other nucleic acids or contaminations. The purity of the eluted DNA was insufficient, which can be explained by the applied chemicals for the gel extraction step. These contaminations were not expected to interfere with further reactions, hence, the extracted DNA was used.
The usage of only one primer set for all samples makes it possible to compare them. On the other hand, an effective amplification of 18S rDNA fragments from all eukaryotic organisms is not assured. As discovered by Katja Metfies (pers. comm.), the used primer does not bind with the appropriate efficiency to 18S rDNA of Cryptophyta. The ratio of Cryptophyta in a sample was lower if 18S PCR was used compared to other methods. Even though the primer sequence is highly conserved within eukaryotic organisms, mismatches can not be excluded because the primer was not tested for all eukaryotic organisms. Mismatches at the primer region would necessitate a lower annealing temperature during the PCR reaction. But since only one annealing temperature was used here, probably not all 18S rDNAs were amplified in the same quantity.

4.2.3 Cloning and sequencing

The extracted 18S rDNA fragments needed to be inserted into plasmids and cloned in *E.coli* to differentiate between the single 18S rDNAs in the sample. Because only a small fraction of the 18S rDNA is finally transformed into the *E.coli* cell, a very high number of clones is necessary to include also rare species into the clone library.
Following (Countway et al., 2005), rarefraction curves were used to estimate the total diversity. The construction of rarefraction curves was done for two stations with a high number of sequenced clones, OTUs and CPGs. The rarefraction curves for the stations 060920D and 061008 (see Figure 3.5) clearly showed a rising slope. This means, that a higher sampling effort would have lead to more OTUs and the phylogenetic diversity would have increased. For

the remaining stations with similar or lower sample sizes it can be assumed, that the saturation point is also not reached yet. Recapitulatory, a higher number of clones needs to be sequenced at all stations to gain a complete image of the biodiversity, i.e. also including rare species.

The ligation and transformation using the TOPO TA Cloning kit (see Chapter 2.1.4) is of the same efficiency for all electroporated samples. The chemical transformation of station 061008 was less efficient and it was necessary to plate 10 times more transformed cells to achieve a sufficient number of clones on the selective cell culture plates. The varying transformation rate and the different transformation method does not effect the results because the success of the transformation process does not depend on the sequence of the vector. Therefore, it is a question of statistics which fragment is included into the *E.coli* cells.
Also, there is no known influence of the inserted DNA on the ability of the cells to grow on the selective plates. Therefore, the colony picking, choosing white, clearly shaped and big colonies, did not influence the resulting biodiversity analyses.
Possible mistakes, such as picking colonies consisting of two clones, did result in overlaying sequences and were therefore excluded at quality clipping step. A contamination of the clone libraries in the liquid LB medium can not occur due to the addition of Ampicillin. If, anyhow, bacteria or yeast would grow, their genomic DNA would be excluded at the plasmid preparation step. Bacterial plasmids could be amplified during the sequencing reaction, if the plasmid contains the sequence for the primer annealing. The resulting sequence could then be identified as non-eukaryotic and hence, be excluded from the final interpretation.
The plasmid preparation step was done for most samples by the robot freedom evo (Tecan, Germany). This made the high throughput analysis possible. At the same time it causes a risk for cross-contaminations. Even though, the robot works more exact than any human being could, a software or program error could cause wrong pipetting steps. A mixing of liquids from different samples can not be ruled out. Cross-contaminations would lead to chromatograms with superposed signals, which are also excluded due to the quality clipping.
The quality and quantity of the isolated plasmids was investigated by using the NanoDrop. Quantities, as shown in Table 3.4, differed between 20 and 280 ng/μL. The OD260/OD280 ratios were in most cases between 1.8 and 2.0 indicating pure enough nucleic acids. In contrast, the OD260/OD230 ratios were too low. This can be explained by the incomplete removal of the ethanol during the plasmid preparation. An inhibition of the following sequencing reaction was not expected due to the residual ethanol. An extra purification step, which would lead to big losses of plasmid DNA, was therefore not carried

out.
The plasmid DNA was used for the sequencing reaction. As described in Chapter 2.2, one special primer was used which ensured only the amplification of the transformed 18S rDNA fragments. Also this PCR reaction uses only one annealing temperature, which maybe prefers the amplification of some of the 18S rDNA fragments. But because the annealing temperature is set low, the specificity of the annealing step is low and therefore, relatively many mismatches are tolerated. No eukaryotic species are known whose 18S rDNA fragments are not amplified by the used primer.
The products of the sequencing reaction were purified. Mistakes or unequal treatments at this step would not influence the results, but the quality of the sequence. If the sequence reactions would stand too long after adding the HiDi Formamide (Applied Biosystems, USA) until sequence analysing, it could lead to week signals. Also, the usage of too old and therefore untight septa may cause a weakening of the labeled ddNTPs, especially of ddGTPs and ddCTPs. This might change the resulting sequence in single positions.

4.3 Phylogenetic trees

4.3.1 Phylogenetic analysis using Staden package and ARB

For the construction of phylogenetic trees the dissimilarity between sequences is used because it is qualitatively related to the evolutionary distance between organisms. That means, at least for highly conserved genes, that the most recent common ancestor has a high sequence identity to the query sequence. For eukaryotes, the similarity between closely related species should be at least 98%.
The sequence information resulting from the sequence analyzer were examined by the quality clipping function of the staden package (SourceForge, version 1.5). This function allows to remove weak and overlying sequences. Though, the program does not mark single "weak" bases, which can be indicators for sequencing errors. Hence, these errors are treated and interpreted as mismatches. If several of these mismatches accumulate, this will result in lower similarity values and therefore in incorrect numbers of OTUs per sample.
The sequences were grouped by staden package on the basis of a 98 % similarity. The gained OTUs had to be compared to each other and to known sequences of the phylogenetic tree of the ARB package, which contains about 26.000 sequences. Nevertheless, many species are missing in the ARB-phylogenetic tree, especially those of rare and not often detected species. The closest relatives to the sequences in this thesis are therefore often species which does not occur in Polar regions. In these cases, only the class or phylum can be defined.
Many of the bootstrap values in the ARB-tree were low, i.e. less than

95%, which can be due to distantly related sequences or due to an incorrect alignment. For this reason, an additional classification method is helpful.
As a second tool for comparative sequence analysis, the NCBI database was used for all OTUs. Exemplary chosen results of the BLAST search in the NCBI database are listed in Table B.1. Information about the fraction of the query sequence, which is aligned are given as query coverage values. The upper limit of identical aligned residues in the alignment is called maximal identity. They were compared with the positions of the OTUs in the ARB-tree. If they were not sorted into the same phylum, the ARB-tree and the appendant filters were calculated again for this OTU. In most cases, the outcome of this was a new position of the sequence in the ARB-tree with higher bootstrap values. The taxonomic position with the highest similarity values in ARB and BLAST were used for further analyses. The resulting trees are attached in the Appendix (see Figure B.1 until B.7). Each phylogenetic tree shows all OTUs with more than 500 bp of one station, together with the closest relatives in the ARB tree. All OTUs which grouped together using the ARB database and which also gave the same BLAST results, were marked with rectangles. Even though more than 2% of all these OTUs did not match each other, they had to be analysed as on group, a so-called CPG. Due to a lack of known 18S rDNA sequences from polar regions, a more detailed phylogenetic analysis is not possible. The lowest taxonomic level which could be defined using the combined results from ARB and BLAST, classifies the CPGs.

A common conclusion for all phylogenetic trees is the dominance of bacillariophyta, dinoflagellates and metazoa. But also, a very high variability between the stations is obvious.
All trees include OTUs with bootstrap values down to 96%. A similarity of less than 98% means that the two compared sequences belong to organisms from different classes or even phyla. If neither in ARB nor in BLAST a sequence from this class can be found, the query sequence belongs to a very rare class or even to an unknown class or phylum. Another reason for the low bootstrap value can be a mistake during the alignment process. Mutations or bad sequence qualities also have to take into account. A possibility to control the phylogenetic position of this OTU can be a second sequence reaction. This can be done either of the same fragment to test the method or the whole 18S rDNA can be investigated.
A comparison of the results of the different stations are visualized in Figure 3.2. This shows the relative abundance of the different CPGs, which were grouped together to the phylum or kingdom level to simplify the analysis. In (a) all OTUs were used for the calculation. To quantify the distribution of the CPGs between all sequenced clones, the columns in (b) are based on all sequences with more than 500 bp. The bar charts show distinct differences between the

stations. Whereas the stations 060920D and 060920F are clearly dominated by dinophyceae (see Figure 3.2b), bacillariophyta are the most abundant group in all other Antarctic stations. Arctic samples include besides dinophyceae, stramenopiles and bacillariophyta with more than 10%each also high fractions of metazoa and fungi and are therefore different from the Antarctic stations. Rare classes and families such as haptophytes, choanoflagellates or cryptophyta only occur at particular stations. This could be either due to a patchy distribution or because of the low number of analysed sequences.
A conclusion about the quantity of each CPG in the environmental sample needs to be made with caution. First, not all abundant organisms are represented by the number of clones in this work, as shown by the rarefraction curves. The second problem is the specificity of the method: it is not clear, whether 18S rDNAs of all eukaryotic organisms are amplified equally well. Additionally, many species contain more than one copy of the 18S rDNA gene. The limitations of the method will be discussed later in this chapter.
In the next Chapter, the bar charts will be compared and interpreted concerning the causes for the variable diversity. Also, the influence of abiotic factors will be discussed using restriction curves.

4.3.2 Ecology

The ecosystem sea ice is highly variable with respect to species composition. An extensive investigation of the present conditions, biotic as well as abiotic, is therefore of particular importance. Sea ice, as described in Chapter 1.1, varies due to its generic ice, brine volume and structure, temperature, brine salinity, nutrient supply, irradiance, grazing and interspecific pressure as well as CO_2-concentration. The complexity of the sea-ice ecosystem combined with a small number of samples which all differ in their spatial and temporal distribution, makes it hard to identify distinctly all factors, which caused the observed species composition. Former studies also consider different factors to be the limiting one, for instance light (Kirst and Wiencke, 1995) or nutrient supply (Gosselin et al., 1990). The definition of ice types with similar physico-chemical conditions was used by Gleitz and Thomas (1993) to simplify the interactions and to characterize the corresponding sea-ice communities.
All possibly important factors will therefore be discussed here, even though the results can only be assumptions. The size of clone-libraries is too small as shown by the rarefraction curves. The unequal number of clones per sample (see Figure 3.3) as well as the number of analyzed stations is too small to allow significant environmental conclusions. The correlation between biodiversity index and sample size, but also between eveness and sample size was plotted in Figure 3.6 and Figure 3.7 to estimate the influence of the sample size. Both trend lines decrease if the sampling size increases. This means, that the dominance of certain species becomes more clear the more clones are

sequenced. For the following discussion, which use the biodiversity index, an equal sample sample size would be meaningful.

The spatial distribution can only be discussed for Antarctic samples. Here, a north to south transect was investigated, and the northernmost samples can be assumed to be the oldest. This is due to the sea ice formation in the south which than, general speaking, drifts to the north. As discussed before, older sea ice underlies a succession. Therefore, typical species of sea-ice communities are expected (see Chapter 1.3). The species composition is also subjected to interspecific and seasonal conditions, which will be explained later. This seasonal change mainly explains the development from 94% bacillariophyta at the southernmost station to only 7% diatoms at 060920D and 060920F, as shown in Figure 3.2. Consequently, the influence of various factors on the sympagic communities does not allow an interpretation only due to its spatial distribution.

The temporal distribution is expected to follow seasonal variations. Following conclusions of (Günther and Dieckmann, 2001), the northernmost samples are presumed to include more photosynthetic active and silicon-dependent species, because they were taken earliest in the season. Again, the opposite was found, which excludes the three weeks difference between the stations from the main influencing factors.

Light conditions, which vary extremely during the year, were comparably similar throughout the sampling period. Of more significant impact on the availability of light for sea-ice organisms is the thickness of the snow- and ice-cover. As shown in Table 3.1, the ice cover of the Antarctic stations varies between 75 cm and 125 cm, the Arctic stations had only a 50 to 65 cm layer.
The snow layer on the ice, which also decreases the light intensities within the ice, reaches from 2 to 34 cm. To analyse, if there is a correlation between the snow- and ice thickness and the biodiversity, the Shannon index was calculated (see Chapter 3.3). In Figure 3.8, this biodiversity index is plotted over the snow layer (b) as well as over the total thickness (a), consistent of the snow- and ice layer. A regression of the determined distribution of data in (a) gives a slowly dropping slope, whereas (b) leads to a rising regression curve. The resulting assumptions of these plots are not consistent as expected for two light-dimmishing parameters. The thickness of the snow-cover does not influence the biodiversity whereas the total thickness leads to a slightly decreasing index, as indicated by the slope of the regression curve. A plot of the Shannon diversity over the sea-ice thickness alone (see Figure 3.9) shows the most significant decrease of the regression curve. This might be due to a varying snow-layer because of wind and changing temperatures, whereas the

ice-layer is much more stable. This confirms, that a thicker ice cover, which decreases the light intensity, supports the succession process, whereas the snow layer is not of clear importance. Besides, a thicker ice layer is usually caused by a longer freezing period or colder temperatures, which also increases succession and thus decreases biodiversity.

The abiotic conditions are generally influenced by the temperature. The cooling process within the ice causes a decrease in the volume of the brine channels. The resulting removal of water from the brine causes higher salinities. To investigate the impact of the temperature, the biodiversity index is plotted against the measurements within the corresponding ice layer of the Arctic and Antarctic samples. In Figure 3.10a, the regression curve indicates a slowly decreasing biodiversity due to a temperature rise. These results dissent from the expected negative influence on the biodiversity, because only well adapted species are able to tolerate low temperatures. The findings can be explained by the influence of the water column on the investigated bottom-ice layer. The temperature within this layer, as shown in Figure 3.1, is always almost at the freezing point due to the nearby water and hence, is relatively stabil. Therefore it can be concluded, that biodiversity of bottom ice communities is independent of the local air temperature at a certain time.
The temperature-dependent brine salinity of the Antarctic samples is shown in Figure 3.10b. The regression curve clearly indicates rising biodiversity indices due to higher salinities. This can be explained by the necessity for organisms to be well adapted to highly varying salinities by storing osmolytes such as DMSP inside the cell.

The most important limiting factor for phytoplankton, as claimed by Gosselin et al. (1990), are nutrients. The measured concentrations of dissolved inorganic nitrogen (DIN) and carbon (DIC), dissolved inorganic phosphorus (DIP) as well as silicate are listed in Table 3.2.
Silicate is essential for organisms, for instance diatoms, which incorporate it into their frustules. The maximum biomass of this often dominant class of sea-ice organisms is often controlled by silicate, but also the species composition is influenced as assumed by Günther and Dieckmann (2001).
A common tool to estimate the role of the various nutrients is the comparison of Si:N:P-ratios. This extended version of the so-called Redfield ratio (Redfield, 1934) is for diatoms proposed to be 15:16:1. The ratios of the Antarctic samples, which are also shown in Table 3.2, differ significantly from the Redfield ratio. The possible range of the C:N:P ration was also discussed by Geider and La Roche (2002). They finally emphasized the plasticity of the elemental composition depending on the irradiance, microbe reactions and the species composition, which adapt their biochemical compositions and nutrient-reserve

pools to the available nutrients. The Redfield ratio was also called an "average value" by Arrigo (2005), which is influenced by the growth strategy of the dominating organisms. Diatoms, for instance, have a higher N:P requirement than *Phaeocystis* and very low N:P ratios are used by N_2 fixers. This complex self-regulating process makes significant conclusions about the actual condition of marine or sea-ice organisms difficult.

Nevertheless, the results of this work will be compared concerning their nutrient composition. The complexicity of the interaction between biogeochemical, biotic and abiotic factors are very complex, especially at this low number of samples that is why this discussion is only speculative.
The station 060923 has a very high silicate fraction, which allowed the silicate-dependent organisms further growth but also might indicate nitrogen or even phosphorus limitation and hence, affect the maximum abundance of all phytoplankton species.
The other diatom-dominated stations 061002 and 061008 show much lower Si:N:P-ratios, but also low N:P-ratios. Low silicate values, maybe due to biological uptake, allow non-silicon-dependent organisms to compete increasingly successfully.
The N:P values of 2 or 3.5 are clearly below the expected Redfield ratio of 16 and the conclusion for these stations as well as for the station 060920, is therefore nitrogen and silicate limitation, also supported by the low measured absolute values. The nitrogen limitation is especially significant at the station 060920, even though similar low values were measured in Antarctic sea ice before (Fiala et al., 2006) but were not identified as the main limiting factor.
In case of nutrient limitation, the two northernmost stations, 060920D and 060920F were expected to consist of a high ratio of heterotrophic organisms, which do not depend on dissolved nutrients. This assumption can be confirmed by the results of this thesis (see Figure 3.2) but also by a chlorophyll *a* value, which is lower than at the stations 061002 and 061008. Similar low values were described by Gosselin et al. (1997) and Werner et al. (2007) for Arctic sea ice and by Günther and Dieckmann (2001) for Antarctic land-fast ice, which was explained by local conditions and processes during ice formation.
The very low chlorophyll *a* value for the silicate-rich sample 060923 ($2.7\,\mu g\ l^{-1}$) disagrees clearly with the high fraction of photosynthetic organisms as found in this thesis (see Figure 3.2). This discrepancy can be explained by the temperature, which was lowest at this station. At very low mean temperatures, the density of the ice decreases resulting in smaller brine pockets and less available space for sympagic communities. A calculated concentration of chlorophyll *a* per area would allow a more significant comparison. Without information about chlorophyll *a* values per area or the size of the brine pockets, two other possibilities have to take into account. The unexpected

low chlorophyll *a* per litre can be either explained by an overestimation of the bacillariophyta-fraction due to methodical mistakes (see Chapter 4.3.3) or by sea ice containing almost only dead bacillariophyta cells, which can also be detected by 18S PCR. It can therefore be suggested, that the nitrogen and phosphorus concentrations limited the photosynthetic active organisms, whereas heterotrophic species or nitrogen fixers developed increasingly resulting in a species composition as observed at station 060920.
In contrast, the stations 061002 and 061008 consist of high fractions of autotrophic organisms, but are also, compared to the Redfield ratio, nutrient limited. Even the wider range of measured N:P ratios, mentioned by Geider and La Roche (2002), leads to the conclusion of limited nitrogen. Therefore, growth conditions for diatoms are assumed to be unfavourable leading to an increased fraction of N_2 fixers and heterotrophic organisms.

In a spatial point of view, a nutrient depletion from south to north becomes apparent. This confirms the suggestion, that the sea ice at the southernmost stations is the youngest one and is therefore not nutrient depleted. Following the ocean currents in the Weddel Sea, sea ice drifts to the western part of the Weddel Sea and is then pushed to the north. Hence, organisms in the ice of station 060920 and 060923 differ from the other stations due to the lack of nutrients but also because of succession.
In case of the stations 060920D, 060920F and 060923, which are supposed to be formed at the same time due to its present position, the impact of abiotic factors on the species composition is obvious. The comparison of the thickness of the ice layer could lead to the assumption, that the stations 060920 are younger than 060923. But on the other hand, the lower nutrient supply of the stations 060920D and 060920F and the species composition would result in the opposite conclusion. A possible explanation has to be linked to the ice-formation (Fiala et al., 2006; Stewart and Fritsen, 2004) and later impacts on the ice structure and its abiotic conditions. This can be confirmed by the observed structure of the investigated part of the ice cores (see Figure 3.1). Whereas the ice structure of station 060920 was defined to be granular, which means fast growing, all other stations consist of a columnar ice structure. As explained in Figure 1.2, the formation of granular ice under rough conditions leads to the inclusion of higher amounts of organic and anorganic material (Gerhard Dieckmann, pers.comm.). These favourable conditions may have caused a rapid phytoplankton growth and hence, a fast depletion of nutrients, especially silicate and nitrogen. The resulting limitation of phytoplankton growth at the stations 060920D and 060920F allowed increased development of non-nitrogen dependent organisms, as shown in Figure 3.2.
Due to the slower ice formation at station 060923, a nutrient depletion and resulting change in biogeochemical processes did not occur yet, and hence,

species composition was still dominated by photosynthetically active algae. The succession may have also slowed down because of low temperatures and low-light conditions due to a thicker ice layer. This presumed influence of the temperature contradicts the conclusions of Chapter 4.3.2. Even though it was showed there, that the air temperature during sampling does not influence the biodiversity, the mean temperature trend controls other abiotic factors and therefore also the species composition. The mean temperature of at least several days has to be observed to reason on the biodiversity.
An additional reason for higher nutrient concentrations at station 060923 can be flooding events due to a thicker snow layer. The weight of the snow layer push the ice under the water surface and therefore causes the input of nutrient-rich water which replenish the nutrient concentrations in the upper sea ice (Günther and Dieckmann, 2001). Also, leads in the ice can cause higher amounts of biomass due to higher irradiance and the interaction with the open water. Larger openings and leads have to be considered in case of station 060923, because the ice floes were already broken by RV Polarstern.
A comparison between the two southernmost stations 061002 and 061008 shows very similar abiotic conditions except the brine salinity, which is significantly higher at station 061008. Increased brine salinities in the bottom-ice layer generally indicate smaller volumes of the brine pockets and channels or higher gravity drainage from upper ice layers. The volume of the brine pockets limits the maximal size of organisms within the ice. Hence, the much higher fraction of metazoa in sample 061002 compared to station 061008 maybe enabled even multicellular organisms to penetrate into the bottom ice layers to graze on protozoans.
Another possible explanation for the difference in metazoan abundance bases on the used methods. During the 18S PCR, all appropriate DNA fragments, which were fixed on filters, are amplified. If a multicellular organism was situated in the sample and not removed from the filter for physiological identifications, each single cell of this organism could be expressed by a sequenced clone. Finally, this would cause a high number of clones belonging to only one individuum. A quantification for multicellular organisms using 18S PCR is therefore not reasonable. The results in Figure 3.4 show two different CPGs, which means, that at least two organisms were included in station 061002.

The comparison of the Arctic and Antarctic results is difficult because of varying environmental conditions, but also sampling techniques and potential mistakes have to be taken into account. The climate conditions in the Arctic during the winter 2005 to 2006 varied from earlier years. The mean temperature in January was, for instance, about 10°C higher than ever measured. The comparability of these data therefore has to be questioned in

general.
Arctic sea ice origins from a shallow, semi-closed fjord system with a comparably calm water surface in contrast to the pack-ice on the open ocean in the Weddel Sea. Also the close coast of Svalbard is likely to influence the species composition within the fast-ice. A major difference between Arctic and Antarctic biodiversity can be found in the Metazoan composition (Spindler, 1994; Legendre et al., 1992). Also, some of the diatom species are not bipolar and also species abundance can vary (Horner et al., 1992).
A difference in Metazoan diversity between Arctic and Antarctic sea ice can be clearly seen in Figure 3.4. Whereas Antarctic samples only included copepoda-species, Arctic Metazoa were represented by Nematoda and Turbellaria. Also the composition of Dinoflagellate species (see Figure 3.3) differed distinctly since Suessiales and Syndiniales only occured in Arctic samples.
The investigated Arctic sea ice (fast-ice) was thinner and younger than at the Antarctic stations (pack-ice). The ice at the Arctic stations was not coloured due to algae growth as observed at the Antarctic stations.
The two Arctic samples were taken at the same position that is why the seasonal change in species compositon could be analyzed. The calculated biodiversity index of both stations is higher compared to the Antarctic results (see Table 3.5). This can probably be explained by the influence of the coast but also by a shorter influence of succession.
The low amount of diatoms in both Arctic samples is comparable with the northernmost Antarctic stations which were dominated by dinoflagellates due to nutrient depletion. Unfortunately these assumptions can not be proved because no oceanographic or nutrient measurements were done in the Arctic. Microscopic analyses of data, collected in March and April 2003 in the fast-ice of the western part of Svalbard were recently published by Werner et al. (2007). Results clearly show the dominance of diatoms in bottom communities, besides chrysophytes, cryptophytes and flagellates. Despite low light conditions at the bottom of the ice due to thicker ice layers and more extreme abiotic conditions, mainly photosynthetic active species were found in contrast to the findings in the fjord ice of Van Mijenfjorden. Besides nutrients, which can not be compared here, the generic ice-class and conditions during formation are supposed to be the main influencing factors. Rough water conditions on the open ocean cause mixing of the water column and allows therefore incorporation of non-motile cells into the ice. In contrast, in Van Mijenfjorden, cells sink during the dark period, if they are not attached to the ice during the autumn. The resulting extremely low concentration of cells in the water column combined with calm water in the fjord lead to only few incorporated cells during ice formation and hence, low numbers of large, non-motile cells in the bottom-ice communities.
Some of the species which were grouped to the CPG "other stramenopiles"

and "Fungi" still need to be identified. *Saccharomyces sp.* were also found in melt poles on Arctic sea ice (E. Helmke, pers.comm.), but additional fungi species are likely included.
The comparison between the results of the two dates in the beginning of the polar-day season are surprising. Instead of an increasing number of dinoflagellates, as observed in Antarctic samples, they are reduced and Metazoans become dominant instead. Again, caution is adviced to quantify these results. As discussed for station 061002, each cell of this multicellular organisms can be expressed by another sequence and therefore mislead the actual distribution of Metazoa. On the other hand, an increase of Metazoa after maximal primary production in spring is possible. To quantify the distribution, additional analyses are necessary.
The species composition can be compared to results by Schünemann and Werner (2005), who analysed distribution patterns of sympagic meiofauna in pack-ice in the Fram Strait. The opposite species composition was reported because Nematoda and Turbellaria were found in September whereas in March and April, Nauplii clearly dominated. The difference can be explained by the varying conditions during sea-ice formation. Additionally, an interaction between the two sampling sites can be supposed: Nematoda and Turbellaria overwinter in calm areas and return during summer to the open ocean to graze phytoplankton blooms.
A more significant conclusion about development in Arctic sea-ice biodiversity can be obtained by analysing more stations, each composed of larger clone libraries. The time period of sampling need to be longer and should last more than one year.During the expedition ANT XXIII/7 of RV Polarstern , the same ice stations were observed by Maike Kramer (IPOE Kiel, Germany) and Julia Hager (AWI Bremerhaven, Germany).
Microscopic analyses used by Julia Hager also show significant differences in the species composition and the number of empty frustules. A first rough investigation revealed at the northermost station 060920 very high numbers of empty frustules and indefinable material. Using electron microscopy, various dinoflagellates were identified. The found diatoms were dominated by centric species, which in total confirms the results of the molecular biological approach. In comparison, the southern station 061008 was clearly dominated by pennate diatoms, especially *Fragilariopsis* and *Pseudonitzschia*. But also, ciliats and dinoflagellates contribute to the higher biomass of this sample.

Metazoan distribution differed significantly between northernmost stations and station 061008 due to the influence of fast ice communities from the Larsen polynia (see Figure 4.1 nearby, as explained by Maike Kramer. As shown in Figure 3.4, the metazoan composition of the latter station also varied from the other stations investigated by 18S PCR. For pro-

tozoans, this interaction is not assumed because a migration is not known and a distinct variation between this station and the closest station further east.

The combination of molecular biology and microscopic techniques can give extensive information about the sea ice biodiversity: the complete identification of species can be done by DNA-based methods whereas the quality and quantity of the cells can be described by microscopic observations.

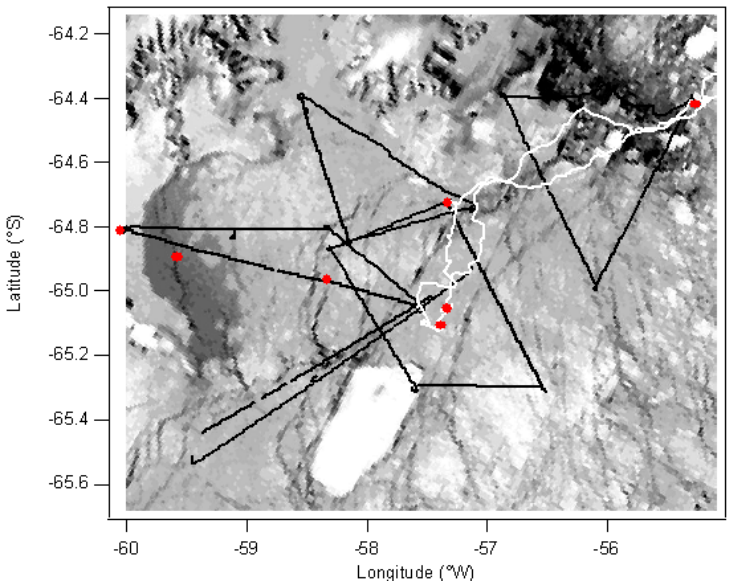


Figure 4.1: Map of the western Weddel Sea showing the Larsen Polynia (dark region in the west) and the southern part of the course plot of RV Polarstern (white line) (Nikolaus, 2006).

4.3.3 Limitations of the method

The intention of this work was the identification of the sea ice biodiversity, but compared to former studies, it based on sequence analysis. In contrast to microscopic techniques, also very small organisms or even deformed or destroyed ones can be recognized. An optimized application could allow a fast and easy monitoring of the entire ecosystem. But until now, there are some open questions that is why a combination with other methods is recommended.

Major difficulties with the interpretation of 18S PCR occur because of multiple copies of the 18S rDNA gene in many species. The number of copies per cell seems to be related to the genome size (Zhu et al., 2005). Protists (Acinas et al., 2004) and picoplankton (Zhu et al., 2005) have only a few copies whereas animal cells contained up to 19300 copies (Prokopowich et al., 2003). For microalgae, the range even varies between 1 and 25000 (Zhu et al., 2005)

and planktonic dinoflagellates mostly have about 1000 copies (Galluzzi et al., 2004) even though large species can also reach 25000.
The used universal primers are supposed to allow amplification of all eucaryotic 18S rDNA fragments. They were developed and tested almost 20 years ago (Medlin et al., 1988). And even though comparisons with other techniques only show one less represented class (Cryptophyta, Katja Metfies, pers.comm.) it is likely that the amplification efficiency varies. A combination of different primers and annealing temperatures would probably improve completeness.
18S PCR was used to analyze melted sea ice. This method gives sequence informations about all included 18S rDNA fragments, but it is not possible to distinguish between living and dead organisms, which can be important for the interpretation of the results. Also, the state of the organisms can not be described.
Incorrect conclusions can also occur because a high number of identical sequences are interpreted as a highly abundant class even though they origin from one single multicellular organism.

Conclusively, a quantification of the results gained only by 18S PCR is not possible. The method needs to be improved e.g. by excluding multicellular organisms and by the development of equations which calculate the number of OTUs depending on the copy number of the 18S rDNA gene of the identified species. This will only be possible, if more 18S rDNA sequences were generated. So far, a combination of different methods gives best results.

Chapter 5

Conclusion

The eukaryotic biodiversity of Arctic and Antarctic sea ice was investigated by sequencing of 18S clone libraries. Phylogenetic trees, which originate from two ice cores from Van Mijenfjorden on Svalbard, based on sequences of 91 and 174 clones respectively.
Antarctic ice cores were taken from 5 different locations in the Weddel Sea, which varied in their location, sampling-date, generic ice-class, age of the ice and abiotic conditions. The appropriate number of sequenced clones differed between 69 and 273, which were all gained by the same approach to exclude the influence of methodical differences on the investigated biodiversity. The resulting phylogenetic trees therefore show the variation in species composition caused by environmental factors.
Biotic and abiotic factors were measured on RV Polarstern for the Antarctic samples and were finally related to the observed eukaryotic biodiversity. Main influencing factors, which caused a significant change in species composition, were assumed to be abiotic conditions during sea-ice formation and ageing. Depending on the efficiency of the incorporation process, the number of cells and the concentration of nutrients within the ice varies. The composition of organisms therefore ranges from a clear dominance of bacillariophyta in relatively young and nutrient richer samples to almost 90% dinoflagellates in older and nutrient depleted samples.
The conditions within the ice are additionally influenced by extreme air temperatures, resulting in higher salinities, less space and maybe interspecific competition. This means, an increase of successtion, caused by extreme environmental conditions, influences the biodiversity of sea ice. Changing temperatures can also control the grazing pressure because larger brine channels at higher temperatures enable metazoans to penetrate into the ice. This distinct variation in metazoan abundance was found in Arctic and Antarctic samples, but can also have methodical reasons.
The observed class of organisms as well as the main distribution is in accord with results of microscopic investigations of the same stations in Antarctica and

former expeditions in the Arctic.
The boundaries of the used method clearly lead to the conclusion, that a combination of different methods is necessary to obtain reliable and significant results. The abundance of multiple copies of the 18S rDNA gen, for instance, makes a quantification difficult. A combination of different primers, which are tested for the actual number of known eukaryotic species, would help to ensure, that all eukaryotes are included in the results. Also, the design of new species- and class-specific primers would lead to a more complex picture of the biodiversity. The used method can detect unknown species which show low similarity to the sequences in the database. Those sequences with very low similarity-values were also part of the investigated samples. The origin of these sequences needs to be observed by full-length sequencing of the 18S rDNA gen.
The used protocol and the facilities for high-throughput analysis make a higher-scaled observation of the sea ice biodiversity possible. A comparison between different samples and an estimation of the impact of changing environmental conditions on the biodiversity of sea ice is possible, even though the method needs to be improved.

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Appendix A

Protocols

The medium was sterilized for 20 min at 121°C at a pressure of 2 bar.

1liter LB medium (liquid):

10 g	Tryptone	(Sigma-Aldrich, Munich, Germany)
5 g	yeast extract	(Omnilab, Bremen, Germany)
10 g	NaCl	(Sigma-Aldrich, Munich, Germany)

1liter LB medium (solid):

10 g	Tryptone	(Sigma-Aldrich, Munich, Germany)
5 g	yeast extract	(Omnilab, Bremen, Germany)
10 g	NaCl	(Sigma-Aldrich, Munich, Germany)
15 g	Agar	(Sigma-Aldrich, Munich, Germany)

Appendix B

Phylogenetic analysis

Table B.1: Phylogenetic position of the Antarctic station 060923 following BLAST search (best hit) and ARB; results are exemplary chosen from the complete list of OTUs

OTU	**CPG**	**BLAST result**	**coverage [%]**	**identity [%]**
G11-002C11	***Fragilariopsis sp.***	***Fragilariopsis cylindrus***	**100**	**98**
G11-003D08	***Fragilariopsis sp.***	***Nitzschia sp.***	**100**	**90**
G11-001D05	***Fragilariopsis sp.***	***Fragilariopsis cylindrus***	**100**	**95**
G11-005E08	***Fragilariopsis sp.***	***Entomoneis sp.*** ***Amphiprora sp.***	**100**	**98**
G11-004F08	***Fragilariopsis sp.***	***Fragilariopsis cylindrus***	**96**	**98**
G11-002B09	***Fragilariopsis sp.***	***Thalassiosira antarctica***	**100**	**99**
G11-002D08	***Fragilariopsis sp.***	***Fragilariopsis cylindrus***	**99**	**99**
G11-002A11	***Fragilariopsis sp.***	***Fragilariopsis cylindrus***	**100**	**100**
G11-004B12	***Fragilariopsis sp.***	***Fragilariopsis cylindrus***	**100**	**97**
G11-004H09	**pennate diatoms**	***Nitzschia sigma***	**100**	**97**
G11-001B07	**pennate diatoms**	**Plathelminthes**	**100**	**97**

OTU	**CPG**	**BLAST result**	**coverage [%]**	**identity [%]**
G11-002H09	**pennate diatoms**	***Fragilariopsis cylindrus***	**100**	**97**
G11-005B05	**pennate diatoms**	***Fragilariopsis cylindrus***	**94**	**96**
G11-003E04	**pennate diatoms**	***Haslea sp.***	**100**	**97**
G11-001A05	**centric diatoms**	***Amphora sp.***	**100**	**96**
G11-001H12	**centric diatoms**	***Haslea sp.***	**100**	**94**
G11-001E03	**centric diatoms**	***Stellarima sp.***	**99**	**95**
G11-005H08	**centric diatoms**	***Stellarima sp.***	**100**	**99**
G11-004H12	**centric diatoms**	***Stellarima sp.***	**100**	**97**
G11-004A12	**centric diatoms**	***Cylindrotheca sp.***	**100**	**96**
G11-004G01	**centric diatoms**	***Fragilariopsis cylindrus***	**100**	**97**
G11-001G10	**centric diatoms**	***Coscinodiscus sp.***	**100**	**99**
G11-003C08	**Cercozoa**	**unclassified** Cercozoa	**100**	**99**
G11-001C01	**Prorocentrales**	***Prorocentrum sp.***	**100**	**92**
G11-003B04	**Prorocentrales**	***Prorocentrum sp.***	**100**	**99**
G11-003D12	**Prorocentrales**	**uncultured** ***Prorocentrum***	**100**	**99**
G11-003D12	Prorocentrales	uncultured ***Prorocentrum***	100	99

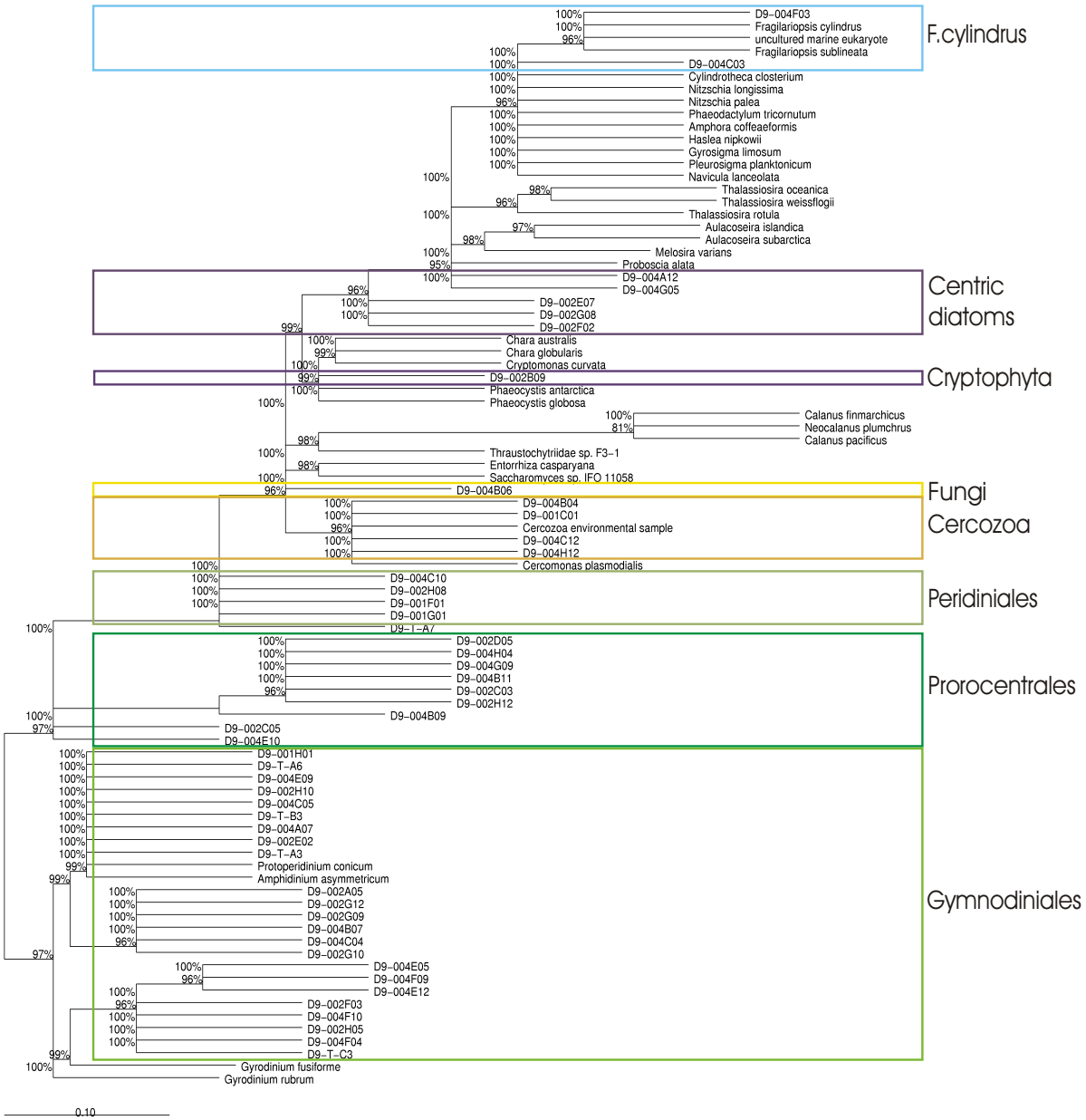

Figure B.1: Phylogenetic tree showing OTUs of station 060920D. The OTUs are combined to CPGs (based on ARB treeing and the BLAST search)

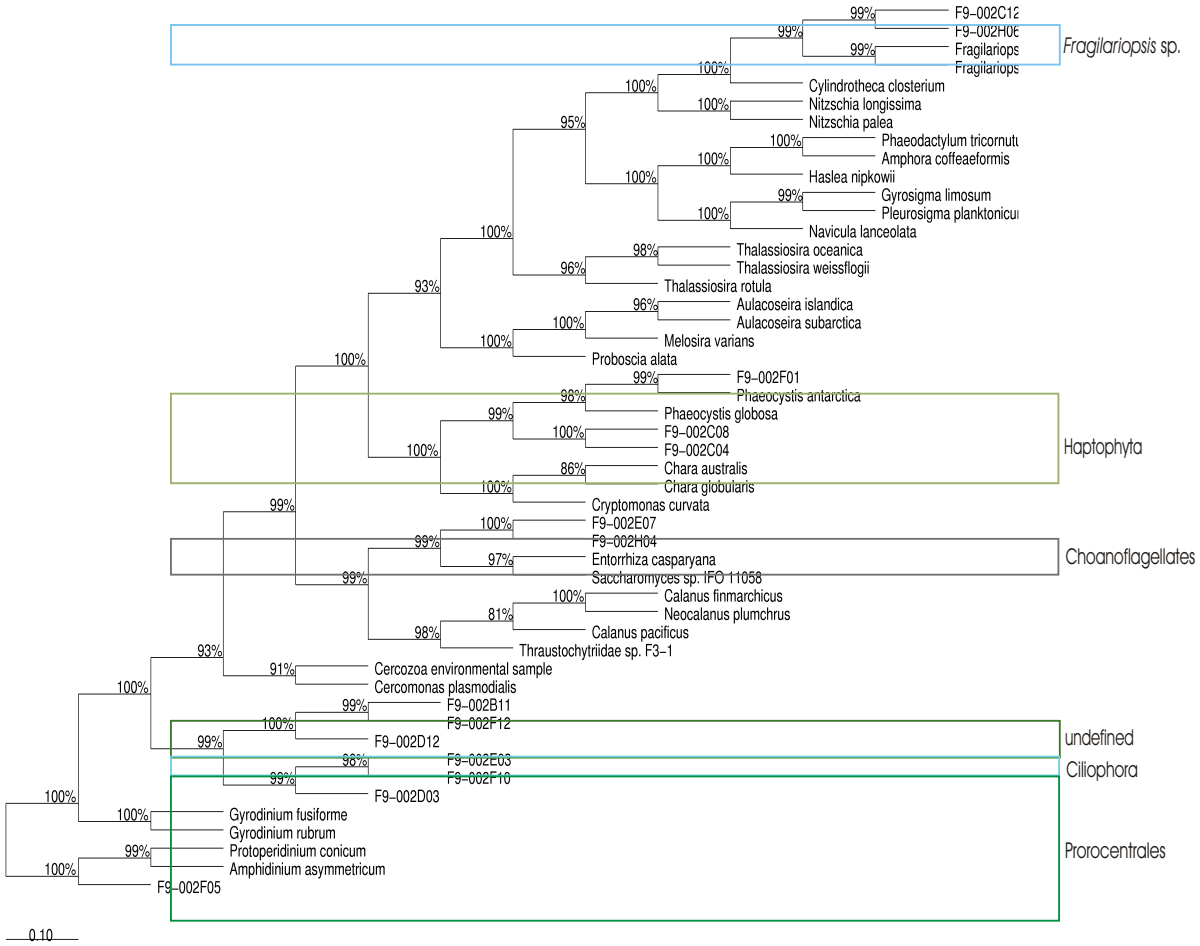

Figure B.2: Phylogenetic tree showing OTUs of station 060920F. The OTUs are combined to CPGs (based on ARB treeing and the BLAST search)

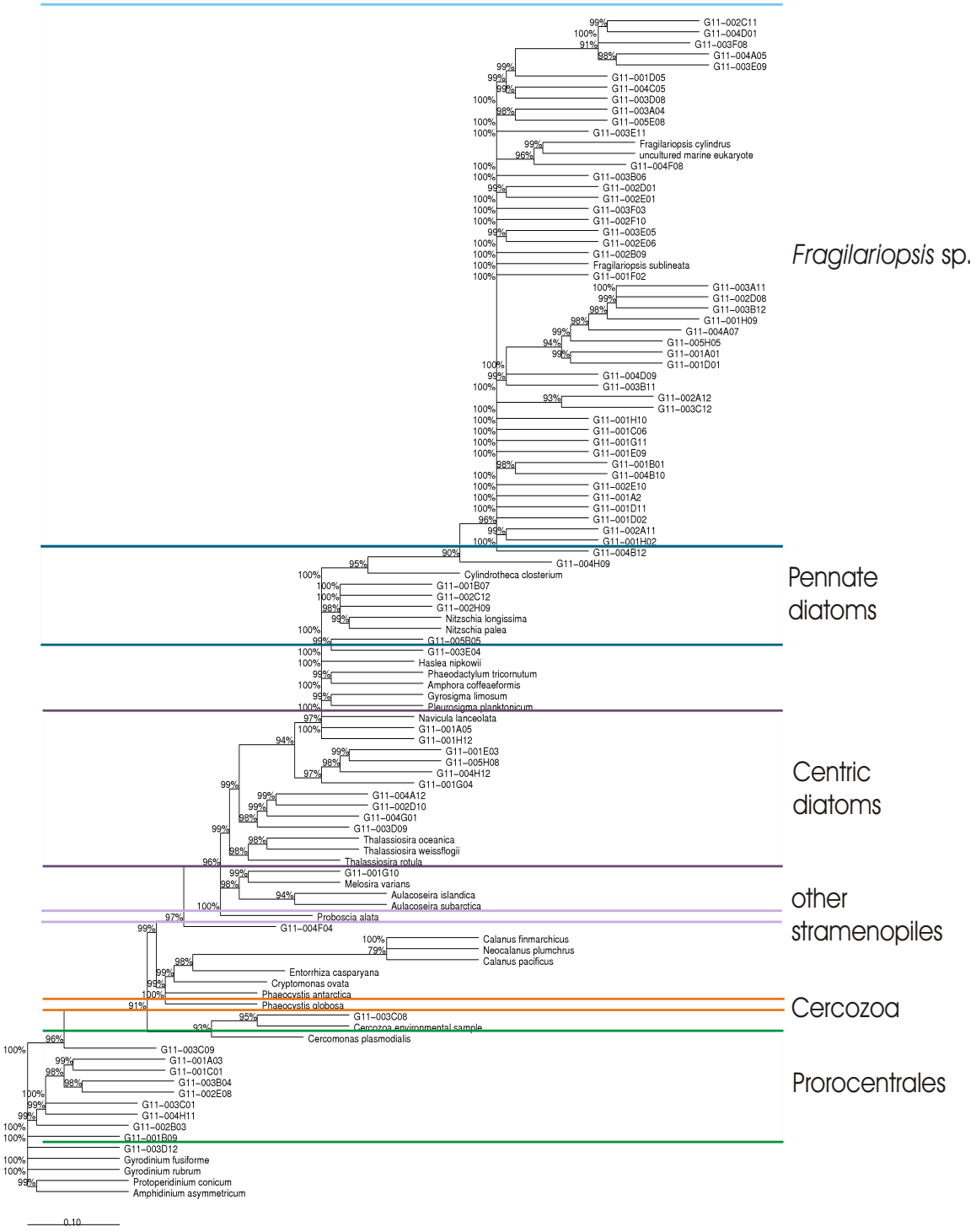

Figure B.3: Phylogenetic tree showing OTUs of station 060923. The OTUs are combined to CPGs (based on ARB treeing and the BLAST search)

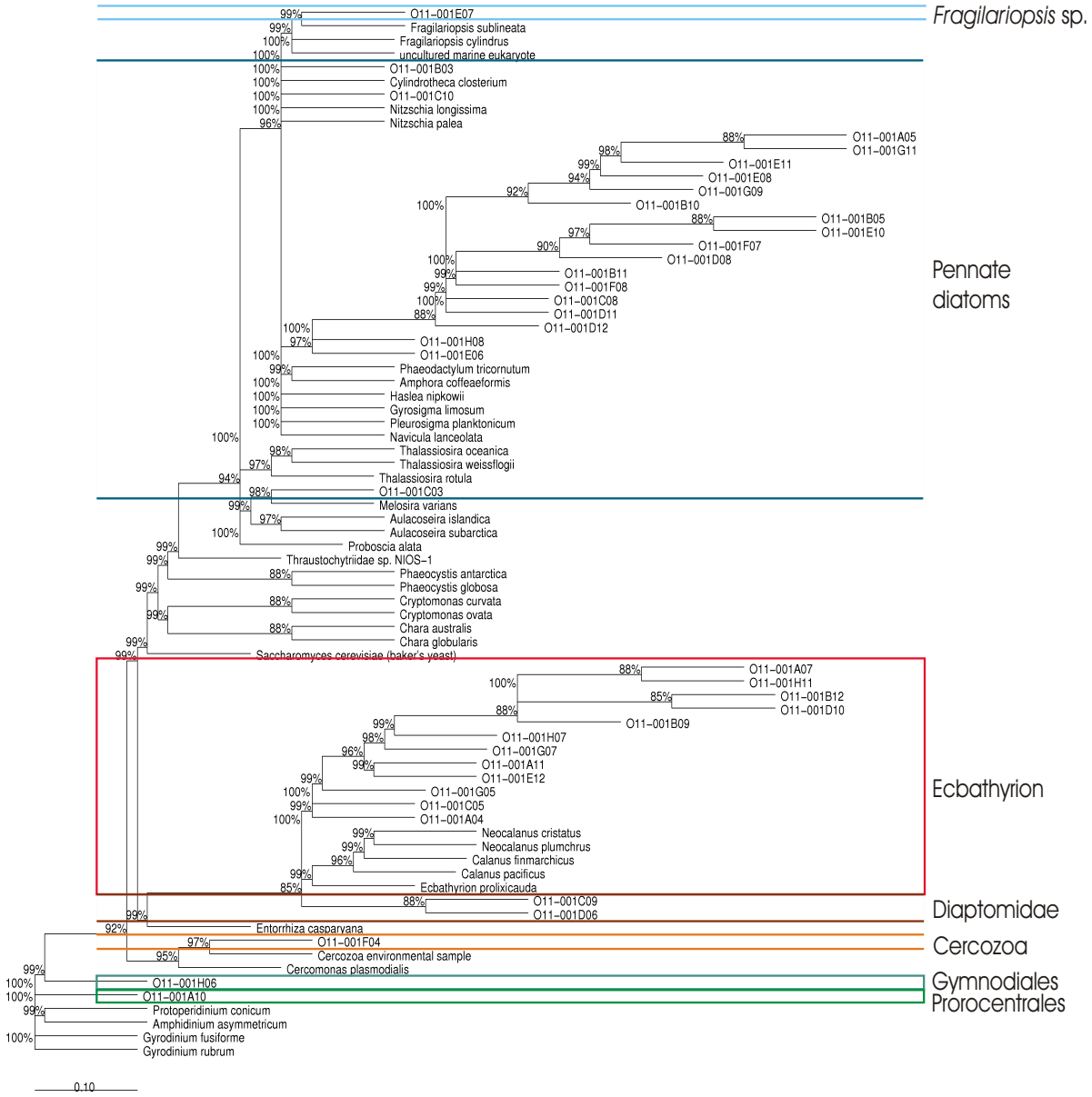

Figure B.4: Phylogenetic tree showing OTUs of station 061002. The OTUs are combined to CPGs (based on ARB treeing and the BLAST search)

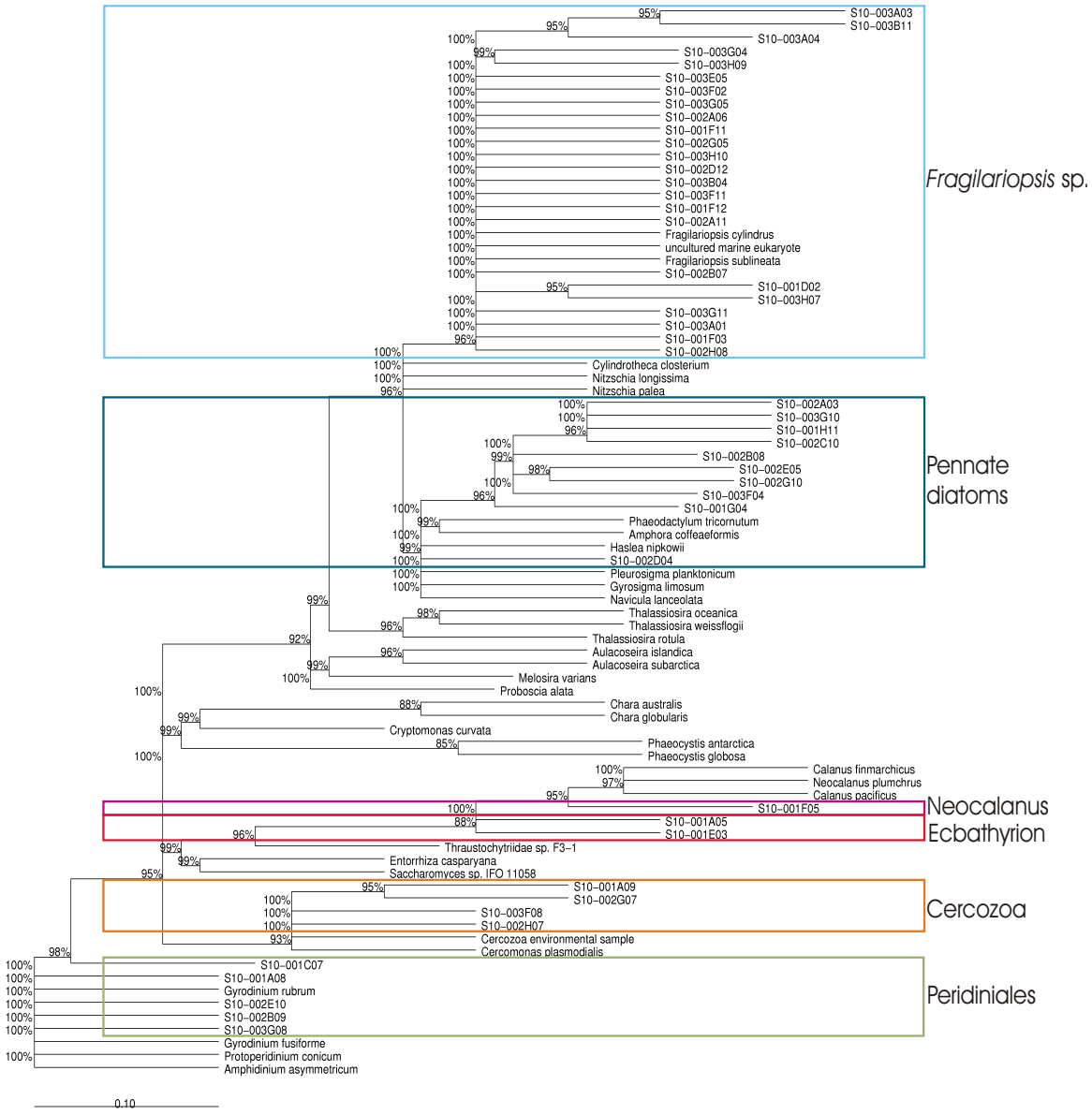

Figure B.5: Phylogenetic tree showing OTUs of station 061008. The OTUs are combined to CPGs (based on ARB treeing and the BLAST search)

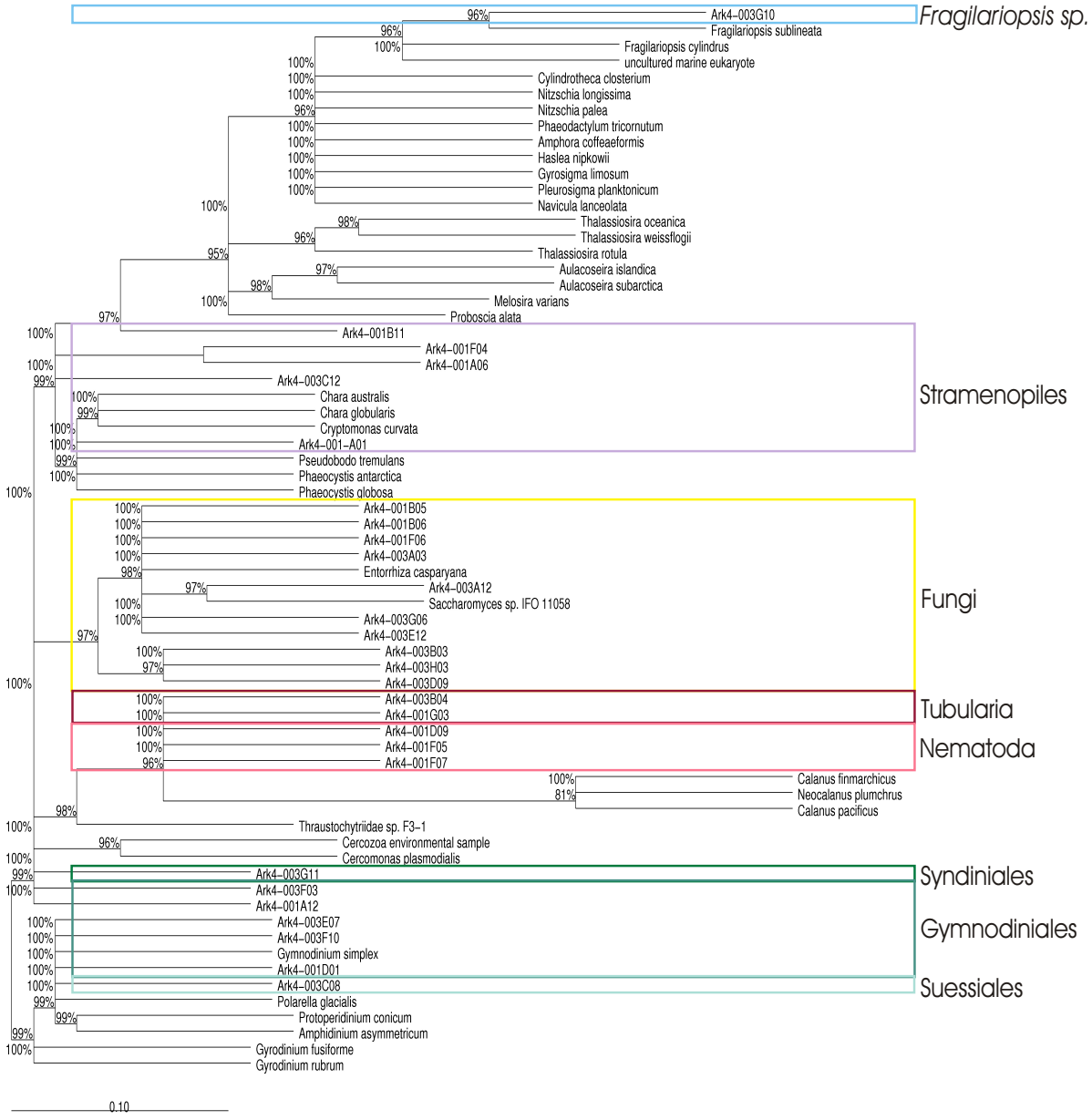

Figure B.6: Phylogenetic tree showing OTUs of station 060331. The OTUs are combined to CPGs (based on ARB treeing and the BLAST search)

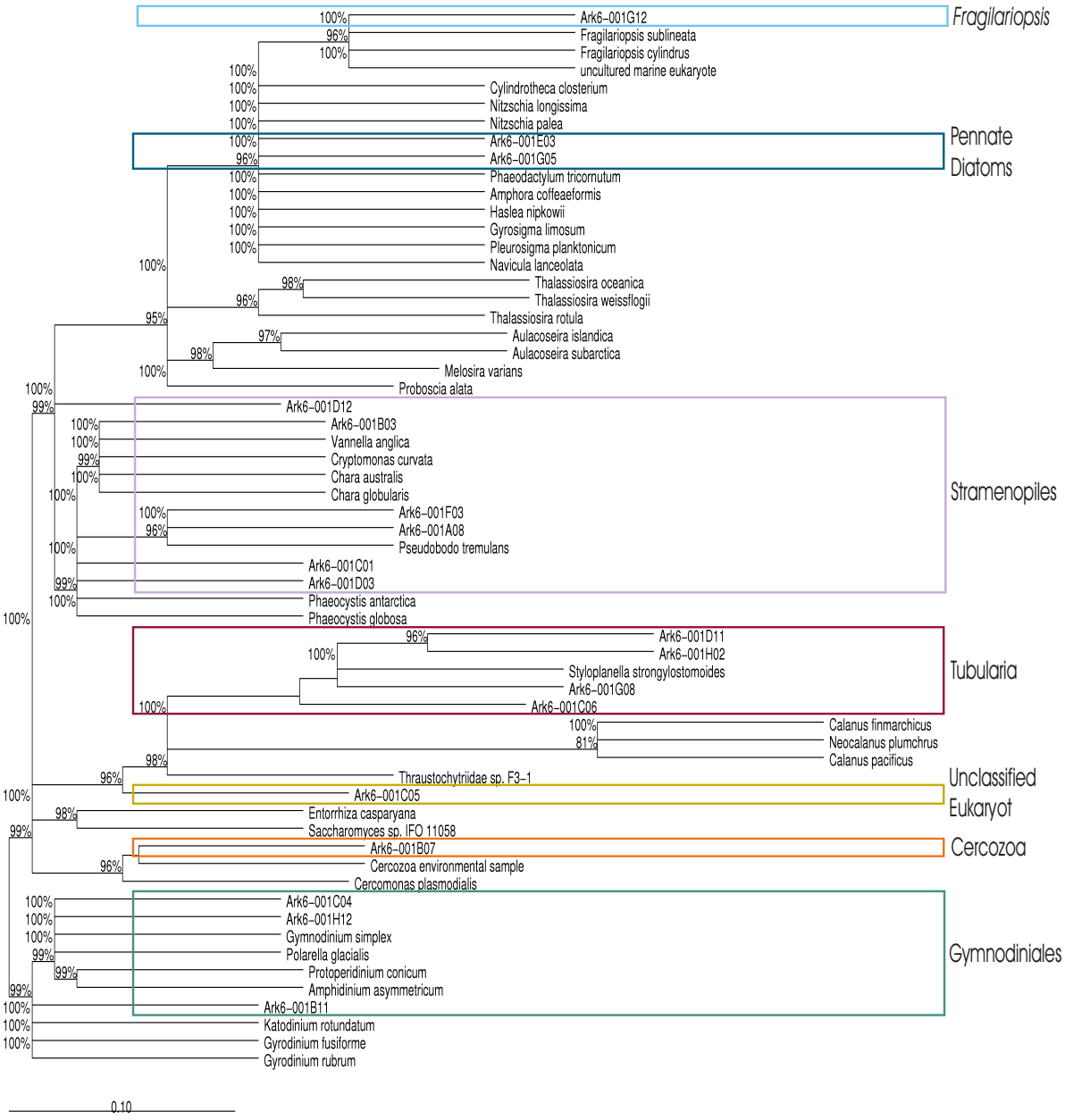

Figure B.7: Phylogenetic tree showing OTUs of station 060421. The OTUs are combined to CPGs (based on ARB treeing and the BLAST search)

Vielen lieben Dank

Ich möchte mich herzlich bei Prof. Dr. Michael Wettern bedanken, der mir diese Arbeit ermöglicht und mich während der Diplomarbeit, aber auch bereits während des Studiums, mit vielen Ratschlägen und großem Interesse unterstützt hat.

Bei Prof. Dr. Ulrich Bathmann möchte ich mich herzlich dafür bedanken, dass er mir die Durchführung der Arbeit am AWI ermöglicht und die Begutachtung übernommen hat.

Ein ganz besonderer Dank geht an Andreas Krell, der mir bei meiner täglichen Arbeit durch viele Tips und ermutigende Worte sehr geholfen hat.

Gerhard Dieckmann und Klaus Valentin möchte ich ganz herzlich dafür danken, dass sie in mir das Interesse für dieses Thema geweckt haben und jederzeit für Fragen zur Verfügung standen.

Für die liebevolle Aufnahme in die Meereis-Gruppe möchte ich Erika Allhusen, Christiane Uhlig, Maddalena Bayer, Jessi Kegel, Nikolai Hoch, Adnan Erdogan und Jan Strauß danken, die mich nicht nur bei der Arbeit durch ihre Aufmerksamkeit und Hilfsbereitschaft unterstützt haben, sondern auch meine Zeit in Bremerhaven abwechslungsreich und schön gemacht haben.

Die Durchführung der Probennahme auf Spitzbergen wurde vom University Centre on Svalbard (UNIS) logistisch unterstützt. Dafür, aber auch für die herzliche Aufnahme in seine Arbeitsgruppe möchte ich Prof. Jorgen Berge danken.

Diese Arbeit sowie mein gesamtes Studium wäre ohne die Unterstützung meiner Eltern nicht möglich gewesen. Ich möchte ihnen und meiner Schwester Juliane daher besonders lieb dafür danken, dass sie an mich geglaubt haben und immer für mich da waren.

Bei meinen Freunden in Braunschweig, Bremerhaven und dem Rest der Welt möchte ich mich ganz besonders dafür bedanken, dass sie mir immer bei kleinen und großen Problemen zur Seite standen, viel Verständnis in stressigen Zeiten gezeigt haben und ganz besonders für die schöne Zeit.

Ein besonders lieber Dank gilt auch meinem Freund Daniel, der mich trotz räumlicher Trennung bei allem liebevoll und geduldig unterstützt hat und mir immer ein wichtiger Halt war.

Erklärung:

Hiermit versichere ich, dass diese Diplomarbeit selbständig und nur unter Zuhilfenahme der angegebenen Quellen und Hilfsmittel angefertigt wurde.

Braunschweig, 27.09.2007 Susann Haase